The Relationship Between Genes Associated With The Pain Pathways And The Development Of Chronic Shoulder Pain And Disability In South African Breast Cancer Survivors

by **Firzana Firfirey**

This Thesis Is Presented For The Degree Of

Doctor Of Philosophy

February 2024

Supervisor: **A/Prof Delva Shamley**

Co-Supervisor: **Prof Alison V. September**

Division Of Physiological Sciences, Department of Human Biology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa

DECLARATION

Doctoral Degrees Board

University of Cape Town

Private Bag X3, Rondebosch

7701 South Africa

Tel: (021) 650-2202

Fax: (021) 650-4913

PhD THESIS TITLE: The Relationship Between Genes Associated With The Pain Pathways And The Development Of Chronic Shoulder Pain And Disability In South African Breast Cancer Survivors

DECLARATIONS:

I, Firzana Firfirey, hereby that I (a) grant the University of Cape Town free licence to reproduce the above thesis in whole or in part, for the purpose of research, and (b) declare that:

(i) the above thesis is my own unaided work, both in concept and execution, and that apart from the normal guidance from my supervisor, I have received no assistance except as stated below:

I gratefully received guidance and assistance with some of the more complex statistical analyses included in this thesis from Trevor Mafu, and genotyping from Dr Nancy Laguette as included in the acknowledgments.

(ii) neither the substance nor any part of the above thesis has been submitted in the past, or is being, or is to be submitted for a degree at this University or at any other university, except as stated below: N/A

I am now presenting the thesis for examination for the degree of PhD.

Candidate's signature Signed by candidate Date: 12 February 2024

ACKNOWLEDGEMENTS

Today, my heart brims with overwhelming gratitude as I pause to extend my deepest appreciation to each person who has steadfastly stood by my side, showering me with unwavering love, support, and encouragement. Your presence in my life is a cherished blessing, and I am forever indebted for your invaluable companionship along every step of my journey.

I am immensely grateful to **Professors D. Shamley and A.V. September** for their boundless guidance, support, and mentorship, which have been indispensable in my personal and professional development. Their patience and compassion have left an indelible mark on my life's path. To the staff of HPALS, Prof M Collins, Aunty Ayesha, Neezaam, Trevino, Lesa and everyone who has always been eager to assist in anything, even if just to explain a simple process while making toast, **a heartfelt thank you!!**

A huge thank you must be given to all the staff at Groote Schuur hospital, especially the front of house in LE33, Ms Cassandra, and to all the participants who eagerly contributed to this endeavour, as well as to the funders (NRF, UCT) of this project, I am eternally grateful.

I would like to thank my colleagues in the GenMS (UCT) research group for every cup of coffee, for every laugh, and for creating a space that is supportive in every way. A special thanks to my dear Dr Nancy Laguette, a friend and a colleague always ready to troubleshoot in research and life.

To my dearest Jamiela, Yusrah, and Hawwa shukran for your unwavering support, laughter, and companionship. Your friendship has been a source of solace and joy, and I am grateful for the countless memories we've shared and the ones yet to come. **Thank you for being you!!!**

To my extended family **Gaironesa, Zainonesa, Mustapha, Nazneen**, and all my nieces and nephews, **Najumunisa, Aqilah, Mahir, Ismaa-eel, Zafirah, Humaam, Umr, Taybah, Zakir and Hilaal**. Shukran for all your support. Your presence has brought immeasurable joy to my life. Thank you for every uplifting moment, every shared laughter, and every gesture of kindness.

A special thank you must be for my FIL, **Azmudien Firfirey**, and MIL **Shaheedah Firfirey** who, when the hours ran long made sure my children were occupied or fed and entertained.

To my siblings, **my boeta M Reza, my amie Shakeel, Tietie Aaliyah, Aziza my twin, Gamieda, Abdus-Samad, Shaabirah, and my beloved Wafiqah (Shaabs and Faabs),** thank you for the lifelong bond we share. Your presence in my life is a constant source of comfort and strength. I cherish the countless memories we've created together and your unwavering support through every moment of life's journey. Words are few to express how instrumental you guys have been and I'd run out of paper. So, let's just meet up at Larkspur (wink wink).

My dear children, **Khidr, and Sawda**, you are the **LIGHT OF MY LIFE, MY GREATEST JOY, AND MY MOST SIGNIFICANT ACHIEVEMENTS**. Witnessing your growth and flourishing fills my heart with immense pride and happiness. **Your love and laughter fuel my soul, and I am endlessly grateful for the privilege of being your parent.**

To my beloved husband, **Taariq**, you are my **ROCK, MY CONFIDANT, AND MY GREATEST BLESSING.** Your love, patience, and understanding illuminate my world every single day. Thank you for standing by my side through thick and thin, for believing in me when I doubted myself, and for always being my strongest supporter.

To my beloved Dad and Mum**, Sulayman October**, and **Wasiela October**. I am profoundly thankful for having you. You have been a pillar of strength from the very beginning. Your unconditional love, **guidance, and sacrifices have shaped me** into the person I am today. I am endlessly grateful for your unwavering support in every endeavour I undertake.

“Like a star in the night sky, you illuminate my world with your presence, guiding me through the darkness and shining brightly with your warmth and light. Your significance in my life is as profound as the stars in the universe, for you bring beauty, inspiration, and endless wonder. Just as the stars hold the cosmos together, you hold the pieces of my heart, binding them with your love and importance. You are not just a star; you are **my guiding light**, my constant source of strength, and the **most precious constellation** in my universe.”

Finally, I extend my heartfelt gratitude to my Creator for blessing me with a life filled with countless blessings and opportunities.

Dedicated

To my Parents, Sulayman, and Wasiela October

To my husband, Taariq Firfirey, my dearest son Khidr, and my beloved daughter Sawda

&

To the young Ms Firzana October, a dreamer who never imagined such heights

TABLE OF CONTENTS

LIST OF SCIENTIFIC OUTPUTS FROM THE THESIS

I confirm that I have been granted permission by my supervisors' A/Prof D Shamley and Prof AV. September, to include sections of the following publications (listed below) in my PhD thesis, and where co-authorships are involved, my co-authors have agreed that I may include the publications.

Firzana Firfirey : Signed by candidate

ARTICLES IN INTERNATIONAL PEER-REVIEWED JOURNALS:

1. **Firfirey F,** September AV, Shamley D. *ABCB1* and *OPRM1* single-nucleotide polymorphisms collectively modulate chronic shoulder pain and dysfunction in South African breast cancer survivors. *Pharmacogenomics* 23(9), 513-530 (2022) [1]
2. **Firfirey F,** Shamley D, September AV. Polymorphisms in *COMT* and *OPRM1* Collectively Contribute to Chronic Shoulder Pain and Disability in South African Breast Cancer Survivors. *Genes* 14(1), 9 (2023) [2]

PRESENTATIONS AT NATIONAL AND INTERNATIONAL CONGRESSES

NATIONAL CONGRESSES

I. **Firfirey, F,** Brookstein L, Radebe Z, Stewart N, Worthington Smith H, September AV, Shamley D, "Chronic pain in South African breast cancer survivors and opioid use", 17th Biennial Congress of the Southern African Society for Human Genetics (SASHG) Ubuntu conference, 13-16th August 2017, Durban, South Africa (Poster Presentation)

II. **Firfirey, F,** September, AV, Shamley D, *ABCB1* and *OPRM1* polymorphisms collectively modulate chronic shoulder pain and disability in a South African breast cancer survivor's population. Southern African Society of Human Genetics (SASHG)

Young Researchers Online Symposium (YROS), 25- 26 August 2021-Virtual congress (Oral Presentation)

III. **Firfirey, F,** September, AV, Shamley D, *ABCB1* and *OPRM1* polymorphisms collectively modulate chronic shoulder pain and disability in a South African breast cancer survivor's population., African Association of Physiological Sciences (AAPS) and the Physiological Society of Southern Africa (PSSA), 12-15 September 2021-Virtual Congress (Oral Presentation)

INTERNATIONAL CONGRESSES

I. **Firfirey, F**, Brookstein, L, Radebe, Z, Stewart, N, Worthington Smith, H, September, AV, Shamley, D, "Chronic pain in South African breast cancer survivors and opioid use", SIMPAR-ISURA conference March 29th-April 1, 2017, Florence, Italy (Poster Presentation)

II. **Firfirey, F,** Shamley, D, September, AV. "SNPs in *COMT* and *OPRM1* Collectively Contribute to Chronic Shoulder Pain and Disability in South African Breast Cancer Survivors", 14th International Congress of Human Genetics (ICHG), Cape Town, South Africa, 22-26 February 2023, (Poster Presentation).

ABBREVIATIONS

Abbreviation	Full Name
ΔG°_{37}	Change in Gibbs Free Energy at 37 degrees Celsius
2D	2 Dimensional
A	Adenine
ABCB1	ATP Binding Cassette subfamily B member 1
ABCC2	ATP Binding Cassette subfamily C member 2
ABCC3	ATP Binding Cassette subfamily C member 3
Aδ	A-delta fibres
AD plot	Allelic Discrimination plot
AI	Aromatase inhibitors
AIC	Akaike information criterion score
Ala	Alanine
ALND	Axillary lymph node dissection
ANS	Autonomic nervous system
AP1	Activator protein 1
Asn	Asparagine
Asp	Aspartic acid
ATP	Adenosine triphosphate
AWS	Axillary web syndrome
BBB	Blood brain barrier
BC	Breast cancer
BCRL	Breast cancer related lymphedema
BCS	Breast cancer survivors
BCT	Breast conserving therapy
BMI	Body mass index
Bp	Base pairs
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
C	Cytosine
°C	Degrees Celsius
Ca^{+2}	Calcium Ions
cAMP	cyclic Adenosine Monophosphate
CI	Confidence interval
CNR1	Cannabinoid receptor 1
CNS	Central Nervous System
COMT	Catechol-O-methyltransferase
CONCORD	Consolidated Criteria for Reporting Qualitative Research
CS	Central Sensitisation
CT	Chemotherapy
CYP2B6	Cytochrome P450 2B6

CYP2D6	Cytochrome P450 2D6
CYP2D7	Cytochrome P450 2D7
CYP3A4	Cytochrome P450 3A4
DA	Dopamine
DASH	Disabilities of the Arm, Shoulder, and Hand
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
***d*H_2O**	distilled Water
DME	Drug metabolising enzyme
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DOR	Delta opioid receptor
DRG	Dorsal root ganglia
EBRT	External Beam Radiation Therapy
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EP	Epinephrine
ER	Norepinephrine
ESR1	Estrogen receptor 1
FAM	6-carboxyfluorscein
FATHMM	Functional Analysis through Hidden Markov Models
FM	Fibromyalgia
G	Guanine
GenMS	Genomics solutions to Musculoskeletal injuries
GCH1	GTP cyclohydrolase 1
GIT	Gastrointestinal tract
Gln	Glutamine
GLOBOCON	Global Burden of Disease and Injury Control
Gly	Glycine
GPCR	G-protein coupled receptors
GSH	Groote Schuur Hospital
GWAS	Genome-wide association study
HCl	Hydrochloric acid
HER-2	Human Epidermal Growth Factor Receptor 2
HIFU	High Intensity Focussed Ultrasound
His	Histidine
HPA	Hypothalamic pituitary axis
HPALS	Health through Physical Activity, Lifestyle and Sport Research Centre
HREC	Human Research Ethics Committee
Hrs	Hours
HRT	Hormone replacement therapy
HS	Haploscore
HT	Hormone therapy

HUB	Human biology
HWE	Hardy–Weinberg equilibrium
IASP	International Association for the Study of Pain
IDC	Invasive ductal carcinoma
Ile	Iso-Leucine
IM	Intramuscular
IQR	Inter quartile range
IT	Intrathecally
IV	Intravenous
Kb	Kilobase pairs
KCl	Potassium chloride
kDa	Kilo Dalton
kg/m^2	Kilograms by squared meter
KOR	Kappa- opioid receptor
LD	Linkage Disequilibrium
Leu	Leucine
M	Moles
M3G	Morphine-3-glucuronide
M6G	Morphine-3-Glucuronide
MAF	Minor Allele Frequency
MAT1A	Methionine adenosyltransferase 1A
Mb	Mega base pairs
MB-*COMT*	Membrane-bound *COMT*
MEF2A	Myocyte enhancer factor 2
Met	Methionine
mg	Milligram
MgCl^2	Magnesium chloride
miRNA	MicroRNA
ml	Millilitre
mM	Millimoles
MOR	Mu Opioid Receptor
MOR-1	Mu Opioid Receptor 1
MOR-1X	Mu Opioid Receptor 1X
MRI	Magnetic resonances imaging
mRNA	Messenger Ribonucleic Acid
MT	Methyltransferases
MTX	Mastectomy
NaCl	Sodium chloride
NaClO^4	Sodium perchlorate
NBD	Nucleotide binding domain
NCBI	National Centre for Biotechnology Information
NCI	National Cancer Institute
NCR	National cancer registry
Neo CT	Neo adjuvant chemotherapy

NF-κB	Nuclear Factor κB
ng	Nanogram
NP	Neuropathic pain
NTCs	Negative controls
OGFR	Opioid Growth Factor Receptor
OMIM	Online Mendelian Inheritance in Man
OPRD1	Opioid Receptor Delta 1
OPRK1	Opioid Receptor Kappa 1
OPRL1	Opioid Receptor like 1
OPRM1	Mu Opioid Receptor 1
OR	Odds Ratio
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PD	Pharmacodynamics
PG	Pharmacogenetics
P-gp	P Glycoprotein
PK	Pharmacokinetics
pmol	Picomoles
PNS	Peripheral Nervous System
PO	Oral administration (per os)
POA	Post-operative analgesia
PP2	Protein Phosphatase 2
PR	Progesterone Receptor
PROMS	Patient reported outcome measure
PWMs	Position Weight Matrices
QC	Quality Control
QOL	Quality of Life
RFA	Radiofrequency Ablation
RFLP	Restriction fragment length polymorphism
ROI	Region of interest
ROM	Range of motion
rpms	Revolutions per Minute
RT	Radiation Therapy
SA	South Africa
SAM	*S*-adenosyle-1-methionine
SC	subcutaneously
SCN9A	Sodium Voltage gated channel alpha subunit 9
S-*COMT*	Soluble *COMT*
SDS	Sodium Dodecyl Sulphate
Ser	Serine
SERDs	Selective oestrogen receptor degraders
SERMs	Selective oestrogen receptor modulators
SES	Socioeconomic Status

SFOLD	Software for Statistical Folding of Nucleic Acids and Studies of Regulatory RNAs
SIFT	Sorting Intolerant from Tolerant
SLCO1B1	Solute Carrier Organic Anion Transporter Family Member 1B1
SLNB	Sentinel Lymph Node Biopsy
SNP	Single Nucleotide Polymorphism
SNS	Sympathetic nervous system
SPADI	Shoulder Pain and Disability Index
SST	Somatostatin
STREGA	STrengthening the REporting of Genetic Association Studies
STROBE	STrengthening the Reporting of OBservational studies in Epidemiology
T	Thymine
TE	Tris- EDTA buffer
TF	Transcription factor
Thr	Threonine
TM	Transmembrane
TMD	Transmembrane domain
TNBC	Triple-negative breast cancer
TP53	Tumor Protein 53
UCLA	University of California, Los Angeles
UCT	University of Cape Town
UGT1A1	UDP Glucuronosyltransferase 1A1
UGT2B7	UDP Glucuronosyltransferase 2B7
µl	Microliter
US	Ultra-sound
UTR	Untranslated Region
Val	Valine
VIC	2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein
WHO	World Health Organization
WLE	Wide Local Excision
Yrs.	Years
ZOR	Zeta opioid receptor

LIST OF FIGURES

LIST OF TABLES

LIST OF SUPPLEMENTARY TABLES

THESIS ABSTRACT

Background

A growing challenge in South Africa (SA) healthcare is the frequency of chronic shoulder pain and disability among breast cancer survivors (BCS). Compounding this issue is that a significant number of BCS in South Africa are low-income individuals, exacerbating the economic burden associated with managing chronic pain and disability. These sequelae can last for as much as 6 years post-surgery and thereby negatively impact the overall quality of life. The current standard treatment in SA for acute and chronic pain include opioids and opioid derivatives. Several risk factors have been highlighted to increase susceptibility to developing the sequelae which include severe acute post-operative pain and genetics. Polymorphisms within genes functioning within the opioid signalling and pain pathways have been implicated in variability in opioid use and the development of chronic musculoskeletal pain and disability conditions. The SA population has a diverse genetic background and an increasing BCS cohort that are of mixed ancestry. This thesis therefore sought to identify potential genetic contributors to variability in pain response and to understand the development of chronic pain and disability in SA BCS of mixed ancestry background.

The study **aimed** to (i) assess **chronic shoulder pain and disability symptoms** using self-reported outcome measures in a unique SA BCS cohort; (ii) investigate **non-genetic and genetic risk factors** associated with chronic pain and disability; (iii) explore the **genetic variability in key genes** within the opioid signalling and pain pathways and (iv) characterise the **potential biological and functional networks** related to the opioid signalling and pain pathways.

The objectives included :

i. Describing the prevalence of chronic pain and disability in BCS using the **Shoulder Pain and Disability Index** (**SPADI**) in the SA cohort of mixed ancestry

ii. Examine the genetic association between eight prioritised single nucleotide polymorphisms in three candidate genes: (I) **ATP-Binding Cassette- Subfamily B, Member 1 Gene (*ABCB1*);** (rs1045642 G>A; rs1128503 G>A), (II) **Opioid Receptor, Mu 1 Gene (*OPRM1*)**; (rs1799971 A>G; rs540825 T>A) and (III) **Catechol-O-Methyl Transferase Gene (*COMT*)**; (rs6269 A>G; rs4633 C>T; rs4818 C>G; rs4680 G>A) and the prevalence of chronic pain and disability.

iii. Evaluate the **gene-gene interaction** and association between prioritised SNPs for ***ABCB1*** **(rs1045642 G>A; rs1128503 G>A),** ***OPRM1*** **(rs1799971 A>G; rs540825 T>A)** and ***COMT*** **(rs6269 A>G; rs4633 C>T; rs4818 C>G; rs4680 G>A)**, and the prevalence of chronic pain and disability in the SA BCS.

iv. Conduct **bioinformatic** analyses to comprehensively examine the functional effects of the prioritised SNPs, identify **associated networks** and **identify potential protein network partners** relative to the opioid signalling and pain pathway.

Methods

A cross-sectional retrospective study was followed (**Chapter 2**), that enrolled two hundred and fifty-two SA BCS. This study was a sub-study of a larger project approved by the Human Research Ethics Committee of the Faculty of Health Sciences within the University of Cape Town (HREC: 312/2012, 125/2017). Qualifying participants were BCS that were diagnosed with unilateral breast cancer (>1yr before study), older than 18yrs, had no history of neck or shoulder pathology and self-identified as mixed ancestry. Patient-reported pain, disability, and combined symptoms associated with shoulder pathologies were evaluated using the Shoulder

Pain and Disability Index (SPADI). Participants scores were calculated and categorized into no-low (<30%) and moderate-high (≥30%) groups of pain, disability, and combined (pain and disability) symptoms. A convenience sampling analyses was conducted to profile the participants (n=13) clinical outcomes (pain, disability, anxiety, depression and affect) over a one-year course of BC treatment in the evaluation of their pain management. TaqMan SNP genotyping assays were used to genotype (N=252) all participants for candidate genes *ABCB1* (rs1045642 G>A), *OPRM1* (rs1799971 A>G), and *COMT* (rs4680 G>A) . Statistical analysis was performed to describe the relationship between a convenient sample's clinical variables and total drug doses. Moreover, analyses were performed to examine differences in outcome measure scores between the three time points, T1 (pre-operative), T2 (3-month post-operative), and T3 (1-year post-operative). Evaluation of the individual genotype, and allele frequencies of each SNPs for each gene between groups were also examined. Haplotype frequencies were statistically inferred for all SNPs within each gene and evaluated between groups. As a proxy for gene-gene interaction, inferred allele–allele combination frequencies were evaluated using the individual genotype data for each SNP. Nonparametric and parametric statistical tests were employed where appropriate, with statistical significance accepted at $p<0.05$. Furthermore, bioinformatic analyses were also conducted to examine the associated networks between the gene set relative to the opioid signalling and pain pathway.

Results

In the exploratory sample analyses, trends of increasing pain, disability, anxiety, and depression over a 1-year course of BC treatment, despite pain management were observed. The analyses also highlight patterns of increasing negative affect and decreasing positive affect, consistent with reports in the literature. In genetic association study (**Chapter 3**), the main novel findings included:

Independently, a significant association was noted for the ***ABCB1*** **rs1045642** single-nucleotide polymorphism (SNPs), with the **A/A genotype** and **A allele** associated with **reduced** likelihood of reporting moderate-high **disability** [**(p=0.028/OR:0.21/95%CI:0.05-0.93)** and **(p=0.015/OR:0.52/95%CI:0.29-0.89)**]. The same association was noted in the **combined** (pain and disability); [**(p=0.011/OR:0.25/95%CI:0.07-0.89)** and (**p=0.003/OR:0.48/95%CI:0.28-0.80**)] category. The ***COMT*** **rs4680 A/A genotype** and **A allele** was also significantly associated with **increased** likelihood of reporting moderate-high **pain** [(**p=0.024/OR:3.23/95%CI:1.33-7.81)** and (**p=0.035/OR:1.58/95%CI:1.03-2.43**)]. Once more the same association was noted in the **combined** (pain and disability); (**p=0.015/OR:3.81/95%CI:1.47-9.85)** and (**p=0.017/OR:1.71/95%/CI:1.07-2.71**) category.

For the ***ABCB1*** **(rs1128503 G>A-rs1045642 G>A)** haplotype analysis, the inferred **G-A (p=0.029, OR: 0.00, 95% CI: 0.00-0.00) haplotype** was significantly associated with **reduced** likelihoods of reporting moderate-high **disability**. In addition, the inferred **A-A (p=0.029, OR:0.63, 95% CI: 0.37-1.06) haplotype** was also significantly associated with **reduced** likelihood of reporting moderate-high **combined** (pain and disability). The ***OPRM1*** **(rs1799971 A>G – rs540825 T>A)** inferred **G-T (p=0.019, OR:0.33, 95% CI: 0.14-0.75) haplotype** was significantly associated with **reduced** likelihoods of reporting moderate-high **pain**. Inferred haplotype analysis of five *COMT* haplotypes noted significant associations for H2-H5, the most notable associations being for the **rs6269 A>G -rs4680 G>A** genetic interval. This analysis revealed the **G-G (p=0.026, OR: 0.67, 95% CI: 0.38-1.18)** and **A-A (p=0.007, OR: 2.09, 95% CI: 0.89-4.88) haplotypes** were associated with **reduced** and **increased** likelihood of reporting moderate-high **pain**, respectively.

Gene–gene interaction analyses demonstrated significant associations between the ***ABCB1*** **(rs1045642 G>A) –** ***OPRM1*** **(rs1799971 A>G – rs540825 T>A)** and the ***ABCB1*** **(rs1045642**

G>A) – *OPRM1* (rs1799971 A>G). The inferred **A-A-T (p=0.029, OR: 0.58, 95% CI: 0.18-1.45**) and **A-A** (**p=0.008, OR: 0.44, 95% CI:0.24-0.80**) allele-allele combinations were associated with **reduced** likelihoods of reporting moderated-high **combined** (pain and disability). ***ABCB1* (rs1045642 G>A) – *OPRM1* (rs540825 T>A)** combination analyses demonstrated that the **A-T (p=0.019, OR: 0.62, 95% CI: 0.33-1.16/p=0.014, OR:0.62, 95% CI:0.35-1.10)** combination was associated with **reduced** likelihood of reporting moderate-high **disability/combined (pain and disability) symptoms**. While the alternate **G-A (p=0.021, OR: 1.57, 95% CI: 0.30-3.10/p=0.030, OR: 1.50, 95% CI: 0.78-2.86)** combination was associated with **increased** likelihood of reporting moderate-high **disability/combined (pain and disability) symptoms**. The ***OPRM1* (rs1799971-rs540825) - *COMT* (rs4680)** combination analyses demonstrated that the **A-T-A** (**p=0.008, OR: 1.36, 95% CI: 0.77-2.41**) and **G-T-G** (**p=0.004, OR: 0.00, 95% CI: 0.00-0.00**) were associated with **increased**, and **reduced** likelihoods of reporting moderate-high **pain**. Similarly, the ***OPRM1* (rs1799971 A>G)-*COMT* (rs4680 G>A)** allele-allele combinations **A-A** (**p=0.004, OR: 1.35, 95% CI: 0.85-2.15**), and **G-G** (**p=0.010, OR: 0.23, 95% CI: 0.05-1.03**) combinations were associated **increased** and **reduced** likelihoods of reporting moderate-high **pain** and **combined** (pain and disability). The ***OPRM1* (rs540825 T>A) - *COMT* (rs4680 G>A) A-A (p=0.012, OR: 1.89, 95% CI: 0.81-4.38)** allele-allele combination was associated **increased** likelihoods of reporting moderate-high **combined** (pain and disability). Analyses of the ***ABCB1* (rs1128503 - rs1045642) - *COMT* (rs4680)** combination demonstrated that the **A-A-G** (**p=0.006, OR:0.68, 95% CI: 0.27-1.71**) were significantly associated with **reduced** likelihood of reporting **combined** (pain and disability). For the 2-SNP pairing, the ***ABCB1-COMT* (rs4680 G>A**) G-A allele combinations were associated with **increased** likelihoods of reporting moderate-high **pain** (**p=0.005, OR: 2.08, 95% CI: 1.12-3.84**), **disability** (**p=0.018, OR: 1.16, 95% CI: 0.62-2.15)**, and **combined** (**p=0.008, OR: 1.94, 95% CI: 1.02-3.69)** groups, **p<0.05**. In addition, the alternate combination **A-G,** were significantly associated with **reduced** likelihoods of

reporting moderate-high **disability** (**p=0.003, OR: 0.38, 95% CI: 0.15-0.98)** and **combined (pain and disability)**;**(p=0.002, OR: 0.53, 95% CI: 0.24-1.20)**, **p<0.05**. The three-way gene-gene interactional analyses (*ABCB1* rs1045642- *OPRM1* rs1799971- *COMT* rs4680), demonstrated that the **G-A-A** [in **pain** (**p<0.001**) and **combined** pain and disability (**p<0.001)]** allele-allele combination was associated with increased likelihoods of reporting moderate-high scores. While the **A-A-G** [in **disability** (**p=0.008**) and **combined** pain and disability (**p=0.006**)] and **G-G-G** [**pain** (**p=0.019**) only] allele-allele combinations were associated with reduced likelihoods of reporting moderate-high scores. Bioinformatic analyses supported the analyses by highlighting the functional and biological gene-associated networks.

Conclusion

This thesis concluded with findings that demonstrated the correlation between the frequency of chronic shoulder pain and disability in SA BCS, and genetic variations with the *ABCB1, OPRM1,* and *COMT* genes. The findings highlighted a correlation between the *ABCB1* SNP, rs1045642 G>A, and the prevalence of disability, indicating movement-related pain. Despite the absence of an independent association for the functional variant rs1799971 A>G, and rs540825 T>A, for *OPRM1*, haplotype analyses showed a correlation, supporting the relationship of pain treated with opioids being impacted by this gene. In addition to the independent association observed for *COMT* rs4680 G>A, gene interactions were observed highlighting the role of collective modulation in pain and disability in the present cohort. Additionally, in-silico analyses revealed strong relationships between the genes and important pathways and mechanisms, further strengthening and supporting the hypotheses presented in the aims of this study. The clinical implications of this study aimed to assist in the understanding of the pain mechanisms and opioid pathways towards the development of novel and innovative therapeutics for pain, in personalized medicine.

1 CHAPTER ONE: LITERATURE REVIEW

1.1 INTRODUCTION AND SCOPE OF THESIS

An estimated 40% of breast cancer survivors (BCS) develop chronic shoulder pain and disability following treatment for breast cancer (BC); [3]. This highly complex, multifactorial disease afflicts 1 in 8 South African (SA) women. Modern-day medicine has however made significant advances, which include a multitude of treatment options that are often prescribed in various combinations [4-6]. The surgical removal of malignant tissues, and adjuvant therapies like chemical and radiation therapy, are among the widely prescribed treatments [4-6]. In parallel to these varying treatment options, emerging data has shown an increase in survivors reporting treatment-related side effects such as tissue scarring, impaired shoulder function and more importantly pain [7-13].

Pain is a complex and highly subjective experience that can be classified into acute pain, which typically resolves within 3 months, and chronic pain which exceeds these 3 months as reported in BCS [9]. Chronic pain is multifactorial and can reduce an individual's quality of life, giving rise to an array of other challenges [13-15]. It is often accompanied by fluctuations in mood with heightened levels of anxiety and, the onset of depression which together create a self-enforcing cycle that exacerbates the experience of pain [16-18]. There is a vast amount of literature that describes several risk factors that can impact susceptibility, including age, surgical parameters, prescribed adjuvant therapies as well as prior pain symptoms and treatment thereof [14, 15, 19, 20]. From a biological and physiological perspective, genetics represents another important risk factor [13, 21-27]. It is a fundamental aspect that is inherent in each of the above-mentioned factors, and without a doubt, is vital for gaining insight into the mechanisms that underlie pain pathways. Returning to the topic of chronic pain, ongoing research reports that opioids and

opioid derivatives remain the preferred drug of choice for both acute and chronic pain control, particularly in low to middle income countries such as SA [28-31]. Despite the wide variety of opioid options, BCS still experience a heightened sensitivity to stimulus, pain, and movement-related pain symptoms and therefore, experience poorly managed pain control [32].

The genetic component has therefore been explored to explain the variability in the individual responses to pain management. One such example includes evidence which has shown that the genetic profile of an individual can influence drug metabolism and thereby modulate pain signal pathways which can create potential acute or continuous pain states in the case of chronic pain and inflammation [33]. Several genes have been implicated to contribute to the variable response to opioids, specifically concerning the different phases in drug pharmacology [34]. More specifically, genetic association studies have highlighted functional variants within key genes and linked them to interindividual variability in pain responses and opioid signalling. Clinically, understanding the complexity of genetics associated with opioid signalling and pain management has great potential for the tailoring of precise drugs for effective pain management [35]. The results have the potential to reduce the common burden of chronic pain and its impact on the overall quality of life of a significant proportion of BCS. Therefore, from a treatment management perspective, it is important to understand genetic diversity in populations and to recognize heterogenous cohorts when designing effective treatment strategies. South Africa has a large mixed-ancestry population, and of interest, is the population which are often marginalised and residing in economically challenged geographical regions. Identification of factors influencing both the clinical and economic aspects associated with chronic pain following BCS treatment is therefore pertinent. Bringing about the present thesis with the accompanying objectives.

This thesis was focused on breast cancer and its survivors, and **Chapter 1** includes a focused literature review. It provides a brief overview of breast cancer, the current medical management, and survivorship with particular attention to chronic pain and disability as latent sequelae (**Section 1.2**). This chapter also reviews the epidemiology, treatment protocols, and risk factors associated with chronic pain conditions **(Section 1.3**). Candidate genes within the opioid signalling and pain pathways previously associated with musculoskeletal pain in genetically homogeneous populations are described (**Sections 1.4 and 1.5**). **Chapter 2** is the methods section in which the SA BCS cohort (**Sections 2.2 and 2.3**) is presented, and a validated tool (**Section 2.4**) was used to describe the prevalence of musculoskeletal pain and disability within the cohort. The sampling and testing methods used to evaluate the candidate genes in a genetic association study are also presented (**Sections 2.5- 2.7**). In addition, initial data analyses were also conducted that aimed to describe the clinical profile of SA BCS during the first year period of treatment for BC (**Section 2.4.1**). **Chapter 3** forms the results section of the thesis where the clinical factors and genetic factors correlated with pain and disability symptoms observed within SA BCS are reported and the discussion of the main findings is presented in **Chapter 4**. The preliminary results and accompanying discussion are also presented in **Sections 3.1** and **4.2**, which describe the clinical profile for SA BCS concerning the clinical outcomes and opioid prescription within the first year of BC treatment. A summary of the novel findings, together with the study limitations and potential future directions are presented in **Chapter 5**.

1.2 OVERVIEW OF BREAST CANCER SURVIVORS (BCS)

1.2.1 Epidemiology of Breast Cancer

Worldwide, breast cancer (BC) is the most prevalent form of cancer observed in women, and in 2020 was reported globally to be diagnosed in 2.3 million women, and the cause of death in 685 000 individuals (World Health Organisation (WHO), [Breast cancer (who. int), (https://www.who.int/news-room/fact-sheets/detail/breast-cancer)]. Accounting for 24.5% of all cancers worldwide, followed by colorectal at 9.4%, lung at 8.4%, and cervical cancer at 6.5% in the GLOBOCAN (Global Cancer Incidence, Mortality and Prevalence) 2020 reports (www.uicc.org). In South Africa (SA), BC (23.22%) is the most prevalent of all cancers among women, followed by cervical cancer (15.85%), as recorded by the National Cancer Registry (NCR) in the latest statistics update for 2019 (NICD The National Institute For Communicable Diseases). The global WHO age-standardized BC incidence and mortality rates in females for 2020 were reported to be 52.6% and 16%. In SA, according to GLOBOCAN 2020 reports, BC had an incidence and mortality rate of 14.3%, and 8.2 %, respectively.

The classification of breast cancers which are complex diseases, has demonstrated notable success in new innovative approaches, with the most contemporary methods as seen in **Figure 1.1**, being the traditional histopathological, and molecular classifications [36]. Histological classification examines the point of origin of the tumour, anatomically. Tumours arising from connective tissues are characterised as sarcomas (~ 1% of BC); [36, 37]. On the other hand, tumours originating from epithelial-based cells are categorized as carcinomas, further divided into *in situ* and invasive carcinomas (>80% of BC); [36, 38].

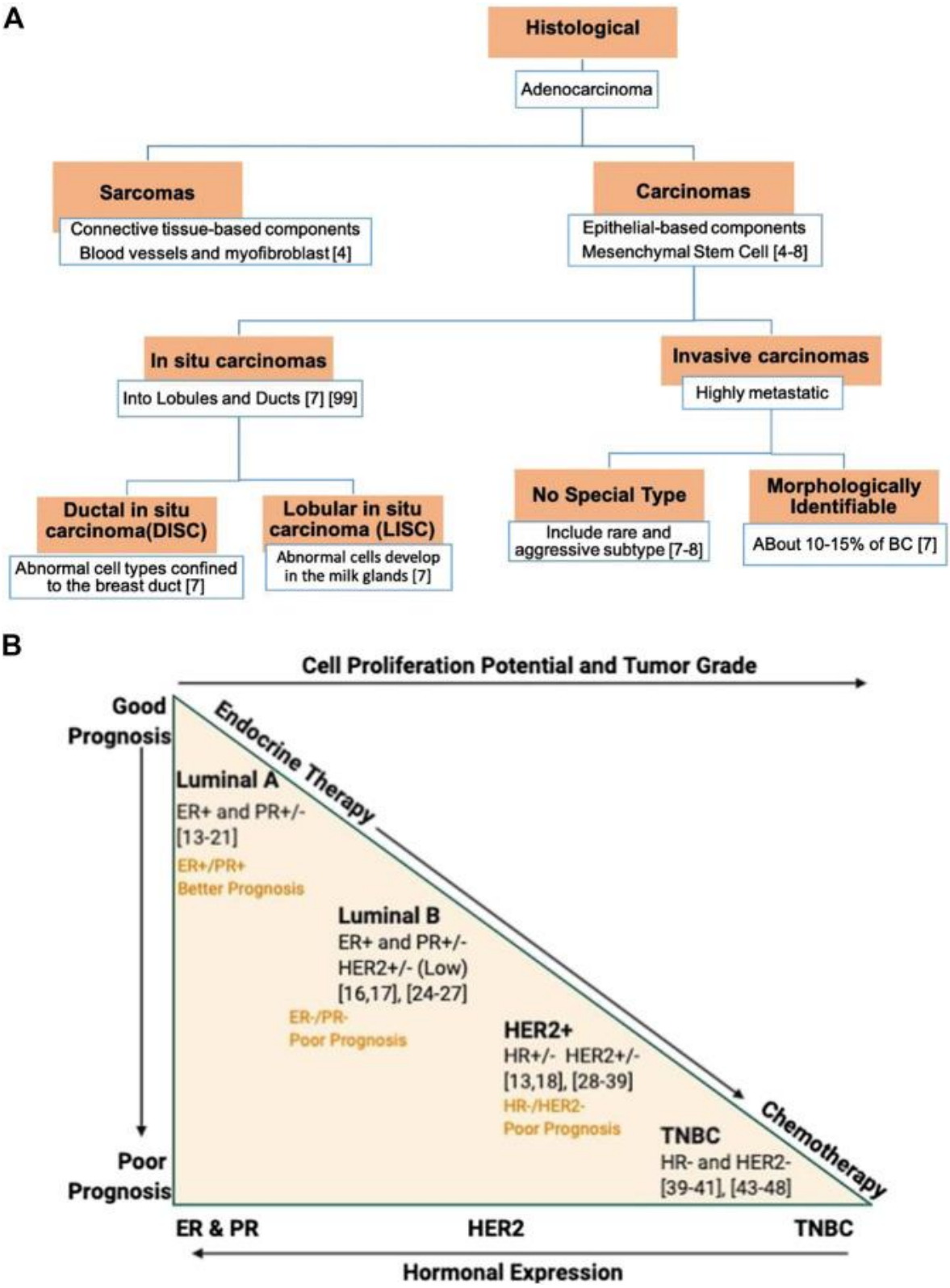


Figure 1.1: Modern-day classification of breast cancer. Shown here are the A) Histological approach that screens tissue samples for identification, and B) Molecular approach used to screen complex breast cancer diseases, obtained from Zubair *et al.* (2020)[36]. Abbreviations: ER+/-, oestrogen receptor; PR+/-, progesterone receptor; HER2+, human epidermal growth factor 2; TNBC, triple negative breast cancer.

Molecular classification has been advancing over time which uses the screening of biological and genetic markers, assisting in the understanding of the severe heterogeneity observed in many BC cases [36]. There are three distinctive biological markers/receptors currently being screened for, these are oestrogen, progesterone, and human epidermal growth factor receptor-2 (HER2); [39] , resulting in the identification of the five subtypes, it) luminal A, ii) luminal B, iii) HER2-positive luminal B, iv) non-luminal HER2-positive, and v) triple-negative subtype [39].

Breast Cancer Risk Factors

According to the Cancer Association of South Africa (CANSA), one in seven women is at risk of developing BC, which is a multifactorial disease. (www.cansa.org.za). The literature describes several un-modifiable and modifiable risk factors that contribute to BC susceptibility in women [40, 41]. Gender, age, reproductive/hormonal status, and genetics are factors that cannot be modified (**Figure 1.2**). Factors that can be modified to reduce the risk of developing BC are lifestyle behaviours, exposures to environmental carcinogens, and an extent socioeconomic status (**Figure 1.2**).

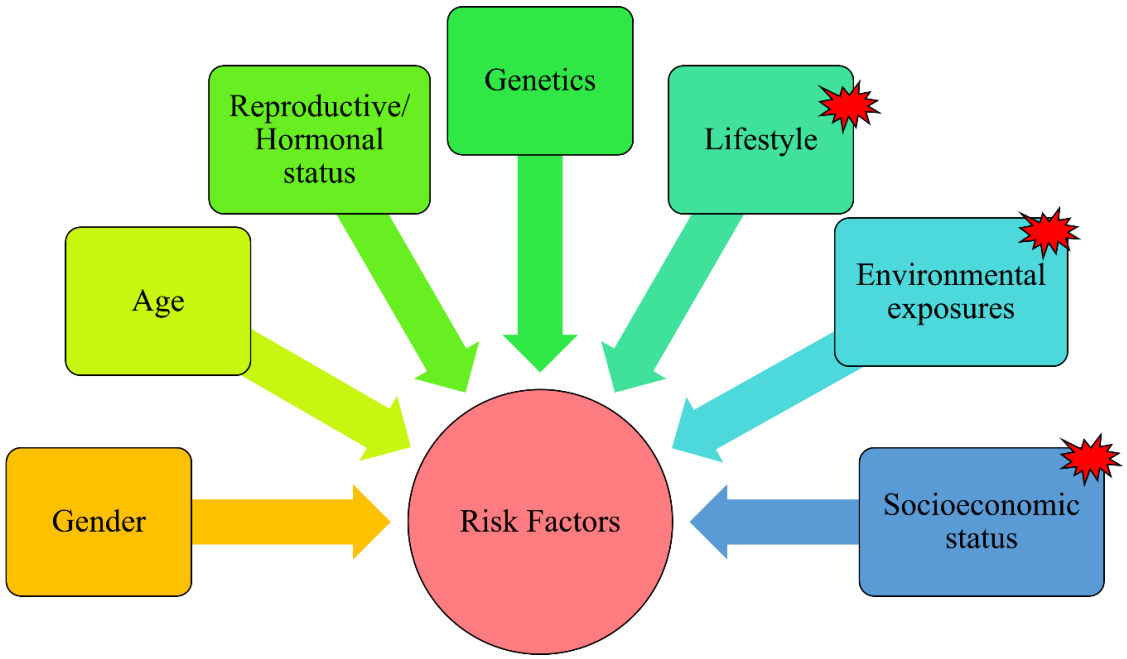


Figure 1.2: Risk factors for breast cancer. Many risk factors for BC are either non-modifiable and cannot be changed intrinsically like gender, age, reproductive status, and genetics. Modifiable risk (starred) factors like lifestyle, environmental exposures and socioeconomic status can be changed to an extent and reduce the potential risk of developing breast cancer.

GENDER AND AGE

One of the most unique characteristics of BC is that it is predominantly observed in women, and rarely observed in men accounting for <1% of all cancer cases reported for men [41, 42]. BC in men is mostly observed in older individuals who have hormonal imbalances, familial history and/or have been exposed to external influences [41, 43-45]. BC incidence has also been found to be highly correlated with increasing age crowning at menopausal age and subsequently plateaus [41, 44, 46]. In the last NCR report for SA, the BC incidence rate increased significantly with increasing age groups and with a slight variation within the different SA ethnicities (www.cansa.org.za); (**Figure 1.3**). SA is a multicultural nation that is home to four different ethnic groups: black, coloured/mixed ancestry, white, and Indian/Asian (South Africa: population, by ethnic groups 2022 | Statista).

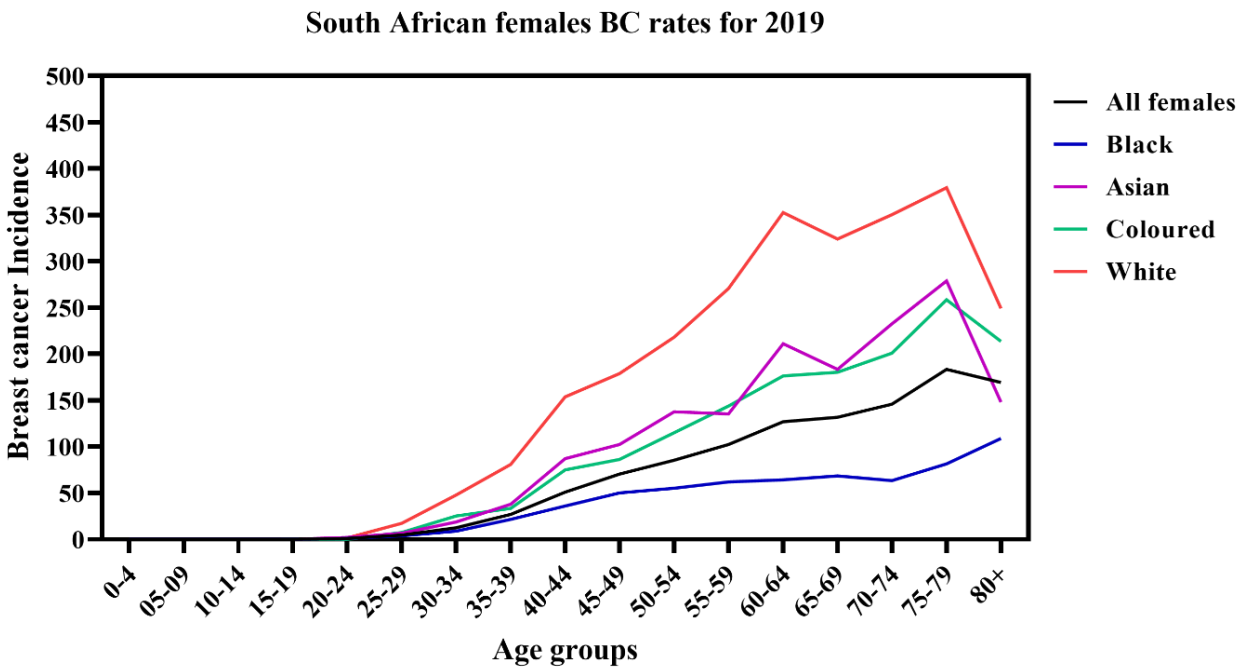


Figure 1.3: South African breast cancer incidence rate in 2019. Shown here are the stratified BC incidence rates by age groups in South African females, per 100,000 for the year 2019, a modified version of the NRC statistics report for 2019 at https://www.nicd.ac.za/centres/national-cancer-registry/.

In particular, the SA population self-identifying as "coloured", also known as mixed ancestry, is a unique ethnic population known for its complex and diverse genetic ancestry. The genetic profile of the mixed ancestry South African population is the admixture of some or several of the populations immigrated in and out of the European, Asian, and African continents, including the indigenous Southern African populations [47]. Analyses of admixed populations are a powerful and useful method to identify potential genetic factors that otherwise may elude conventional methods in homogenous populations for multifactorial diseases [47, 48]. However, studies reporting genetic variability for mixed ancestry cohorts are limited, most notably in BCS in Sub-Saharan Africa [49].

REPRODUCTIVE AND HORMONAL

BC is a hormonally driven illness, and therefore several reproductive and hormonal factors have been implicated to contribute to its risk and susceptibility [50]. The following crucial reproductive phases are reported to have an impact on the risk of developing BC:

i. Age at the time of menarche/menstruation
ii. Parity/pregnancies
iii. Breastfeeding and breast health
iv. Menopause
v. Hormonal therapy

There are reports that a 1-year delay for menarche can reduce BC risk by 5%, whereas early menarche (< 12 years of age) can double the risk [40, 41, 44, 51-53]. There are, however, studies which have not noted such observations [54, 55]. Parity is also significantly associated with BC risk, particularly the age at first and last pregnancy as well as the number of successful pregnancies [56-59]. One case-control analysis reported that women who had their first pregnancy at >30 yrs. old were six times more likely to develop BC [53]. The study highlighted the complexities of multiple BC risk factors, in particular the role of socioeconomic status and cultural differences influencing behaviours such as family planning and the pursuit of higher education. Reports have consistently shown a link between BC risk and first pregnancy after > 30 years of age, which makes 30 years of age the benchmark for assessing BC risk [41, 53, 58-64].

BC risk may also further be impacted by the health conditions and complications associated with pregnancies including breast-feeding health and habits [62, 65-70]. Breast-feeding for periods exceeding twelve months can reduce BC risk [71]. An analysis showed that breastfeeding could

reduce risk by 10%, as undifferentiated breast tissues in non-breastfeeding women tend to be highly sensitive to cancer-causing agents [71, 72]. In addition, it has been theorised that parity coupled with specific breastfeeding patterns may influence the degree of risk for hormone receptor-negative breast cancer [69, 73]. In SA women, exclusive breastfeeding is reportedly as low as 32%, and the risk for BC is reduced by only 4.3% for every 12 months of breastfeeding [74]. In addition, it is noteworthy that SA black mothers are more likely to breastfeed compared to white mothers, a trend that could potentially explain the disparities in the incidence rates observed between the two groups[75]. Data for BC risk concerning breastfeeding and receptor status in Sub-Saharan Africa is, however, mixed, and very limited [6, 76]. In this instance, breastfeeding can be classified as a modifiable factor and may benefit preventative protocols in addition to providing other health benefits to both the mother and infant [77]. Breast health is a also risk factor, and individuals presenting with malformations, or benign disorders are at a greater lifetime risk [78, 79]. Benign disorders have been associated with oestrogen receptor (ER) positive and negative BC cases [41, 80]. Still, some reports have yielded conflicting results. For instance, one study (n=1520) reported that postmenopausal women with a history of benign disorders had a reduced risk for BC [81, 82].

The delay in the menopause phase of life has also been consistently associated with BC risk [40, 83, 84]. It is reported that younger women (<44 yrs.) have a 34% reduction in BC risk, compared to older women (>54 yrs.); [85]. Moreover, a 1-year delay in the development of menopause (> 50 years of age), could increase BC risk by 3% [44, 51, 52]. The use of hormone therapy (HT) or hormone replacement therapy (HRT), for which there are different categories of use, has also been implicated in the risk of developing BC [86-89]. Several different categories of hormone therapy could be considered throughout a woman's lifetime depending on the individual's health needs and circumstances.

These are as summarised in **Table 1.1** and include:

i. Cancer hormone therapy
ii. Contraceptives
iii. Corticosteroids
iv. Hormone replacement therapy
v. Endometriosis hormone
vi. Fertility therapy
vii. Gender-affirming hormone therapy
viii. Thyroid hormone replacement therapy

Table 1.1: Different types of hormone replacement therapies.

Category	Primary indication	Participants' Country of Origin	Studies reporting associations for BC risk
Cancer hormone therapy	Used to treat types of cancers		
Contraceptives	Used to manage menstruation and associated issues such as irregular or heavy bleeding	Europe North America	Collaborative Group on Hormonal Factors in Breast (1996)[90]
		Denmark	Morch *et al.* (2017)[91]
		Southeast Asia	Nindrea *et al.* (2017)[92]
Corticosteroids	Management of the symptoms of autoimmune disorders and inflammatory conditions		
Hormone replacement therapy	Used to treat perimenopausal and menopausal women	United States Canada Western Europe	Ross *et al.* (2000)[93]
		United Kingdom	Beral and Million Women Study (2003)[86]
Endometriosis hormone	Management of endometriosis and associated issues	Korea, China, Sweden, Finland, America, United Kingdom, Denmark, Germany, Australia, Puerto Rico	Ye *et al.* (2022)[94]
Fertility therapy	Used to induce ovulation and to facilitate conception in women who have difficulty conceiving		
Gender-affirming hormone therapy	Used to facilitate the transitioning from male hormonal signatures to female signatures		
Thyroid hormone replacement therapy	Used to treat an underactive or overactive thyroid, restoring normal function		

Both negative and neutral findings concerning contraceptive use and BC risk have been noted in the literature [95, 96]. Women who use current oral contraceptives, primarily oestrogen and progesterone-based, may be at a 20% greater risk, which could increase with continued usage [88, 91, 97-99]. In addition, early contraceptive use (< 20 years), combined with genetic factors or receptor status, may further exacerbate the risk for BC [97, 98]. Similarly, the use of HRT for menopausal symptoms is associated with an increased risk of BC, which is increased with longer usage, particularly with combined oestrogen-progesterone HRTs [93, 98, 100, 101]. HRT in menopausal women is used to treat hot flashes, night sweats, mood changes and other symptoms, and is associated with a 1.6 relative risk for BC [44, 86]. Furthermore, clinical reports revealed that the discontinuation of contraceptive use for 5-10 years, could reduce the risk years [41, 102].

GENETICS

An individual's genetic makeup contributes strongly to the risk of developing BC, as reported in studies investigating familial history [103, 104]. Numerous reports have consistently linked an increased BC risk when a first-degree relative (i.e., mother, sister, daughter) has been previously diagnosed [103, 104]. A positive familial history of BC has been reported in nearly 20% of BC cases, and where having >2 relatives diagnosed before the age of 50 years can increase the risk 11 fold [41, 103, 105].

Hereditary BC is a term used to describe individuals who may have, i) a positive familial history of BC, ii) present with early onset disease or iii) triple-negative BC (TNBC) all of which could be attributed to genetic mutations in vital genes that regulate DNA repair and cell growth. Genetic mutations are pivotal in disease development and progression, accounting for 10% of all BC cases [105-108]. High-risk genes like breast cancer gene 1 (*BRCA1*), breast cancer gene 2

(*BRCA2),* and tumour protein p53 *(TP53),* contribute to nearly 20% of hereditary BC cases [105, 109]. Carriers of *BRCA1* (60%) and *BRCA2* (45%) have a lifetime risk for developing BC, with a 70% probability of developing BC before the age of 80 years [110]. In addition, carriers may also be at an increased risk of developing other cancer types like ovarian and colon cancer [105-108, 111, 112]. The exact mechanisms of these genes are still being explored and recent research has shown that they play a vital role in various processes [113].

Several comorbid conditions can influence risk for developing BC like previous cancers, autoimmune diseases, diabetes or hypertension, that are intertwined with other factors such as genetics and/or lifestyle patterns [94, 114-117]. A diagnosis of a comorbidity such as diabetes mellitus type II, can increase risk by 27%, and when corrected for body mass index (BMI) can be reduced to 16% [118]. A review screening fifty-three studies (n=38 000 women), found 10%-20% of BC women have diabetes [119, 120]. In SA, reports have found that more than 40% of women had comorbidities before a BC diagnosis [121-124]. Furthermore, reports highlighting that chronic metabolic conditions like obesity or diabetes are associated with risk for developing BC, hypothesize that awareness of lifestyle changes may potentially counter the risk [125].

LIFESTYLE FACTORS

The modifiable risk factors such as lifestyle, environmental exposures and socioeconomic status have all been implicated in the risk of developing BC [41]. BMI and obesity are lifestyle factors that are associated with BC risk [80, 126-129]. Although the association was noted as weak, a meta-analysis of twelve cohorts (n=22,728,674) reported a 2% increase in BC risk, for every 5kg/m^2 unit increase in BMI (**Figure 1.4**); [130]. Interestingly, the analysis also reported that a higher BMI was associated with a reduced risk in premenopausal women [130].

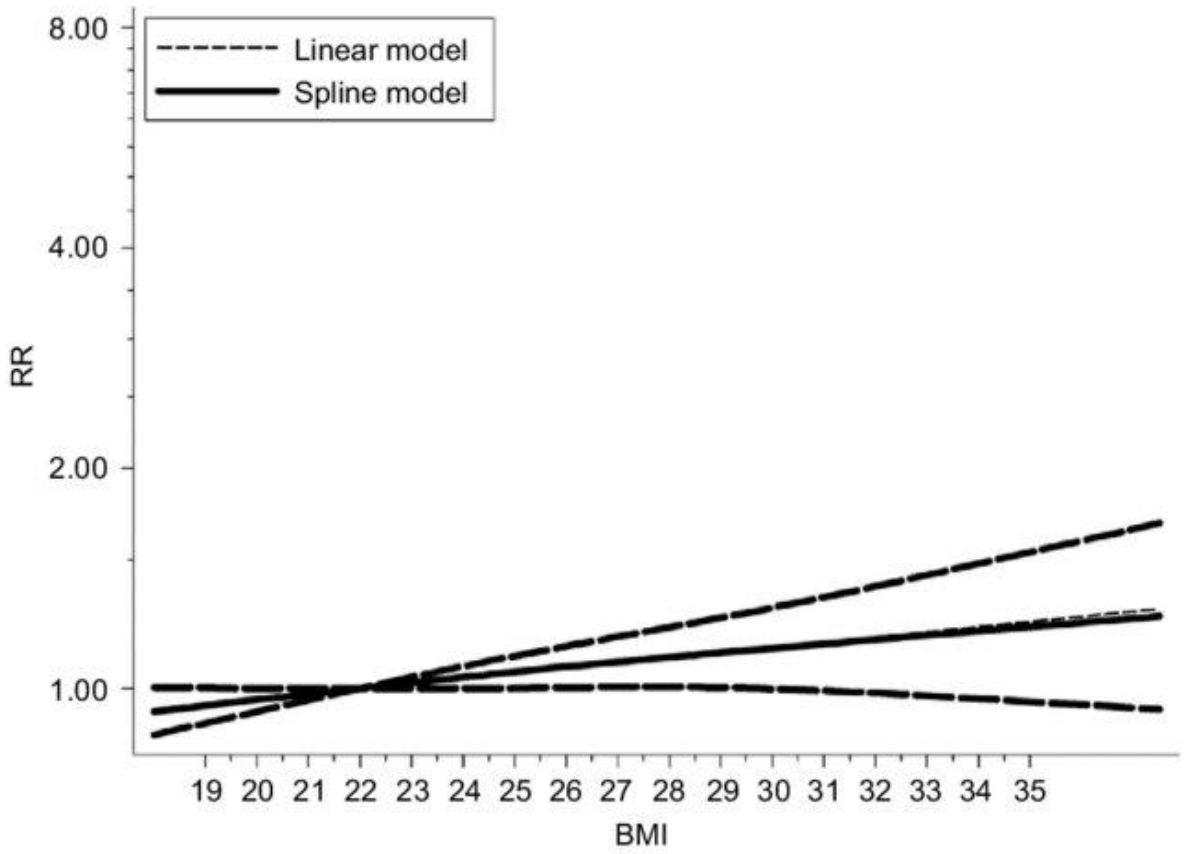


Figure 1.4: The relationship between body mass index and risk for breast cancer. A dose-response meta-analysis for 12 cohorts depicting the linear and nonlinear relationship between body mass index (BMI) and relative risk (RR) for developing breast cancer obtained from Liu *et al.* (2018)[130].

Many other reports that have examined BMI, have reported similar associations [46, 131-133]. Along with dietary intake, physical activity as well as substance misuse, reporting associations between these lifestyle habits and risk for developing BC [134-143]. Smokers, including passive smokers with an early start, who smoked before their first full-term pregnancy or have a history of smoking, have a 10%-15% increased risk for BC [144-146]. For alcohol use, several reports showed that moderate-high alcohol consumption is associated with a 30%-50% increased risk [147-152]. In SA, the burden of obesity is increasing, with data showing a significant increase among adolescent females between 1998 and 2016 [153]. In addition, reports also found that specific dietary intake within SA women, compounded by factors such as hormone status, can influence the risk of developing BC [143, 154, 155]. It has been shown that BC risk is reduced following weight loss, particularly sustained weight loss in postmenopausal women, also highlighting the role of physical activity [156].

Physical activity can reduce BC risk by 25%-30%, and the risk may differ based on factors like activity type, duration, BMI, ethnicity and more [157]. It is hypothesised that physical activity acts as a protective measure against BC development through hormonal mechanisms [158, 159 160]. Moreover, active women have lower BMI, and delayed and irregular menarche, reducing the risk for BC, compared to their inactive counterparts [161-163]. Sleep patterns have also been implicated in BC risk, where a lack of sleep (<7 hours/day with difficulty for > 4 nights/week) was associated with BC risk, particularly in multiparous and postmenopausal women [164-166]. In a multi-ethnic cohort (N= 74,481), reports found short (<6hrs) and long (>9hrs) sleepers with a high BMI to be at 35% and 50% increased risk for BC [167].

ENVIRONMENTAL EXPOSURE

There are debates about external environmental factors contributing to BC risk which revolves around substances like chlorinated hydrocarbon pesticides, solvents, and polychlorinated biphenyls. Earlier reports suggested that exposure to these substances may influence risk for BC [168]. Subsequently, BC risk has been linked to exposure to organochlorine-based pesticides, particularly pesticides containing dichlorodiphenyltrichloroethane (DDT) and Dichlorodiphenyldichloroethylene (DDE), however conclusive evidence regarding their risk remains uncertain [169-173]. A well-known environmental risk is exposure to ionizing radiation with high doses found to increase BC development [174-177]. Women frequently exposed for medical purposes, such as tuberculosis screening or prior carcinomas, are 2-3 times more likely to develop BC [41, 178-180]. The potential mechanisms of radiation-induced BC include gene mutations, disruption of gene expression, or the induction of oncogenic viruses [181].

SOCIOECONOMIC STATUS

It is reported that women who live in high socioeconomic status (SES) communities or, who are of the higher socioeconomic status class, are at an increased risk for BC [182, 183]. The research found women of higher SES are likely to experience early menarche, low parity, and often are late of age for their first birth, causing distinct reproductive patterns that influence risk [183-186]. In Sub-Saharan Africa, medical resources are scarce and trained professionals which is a significant challenge that is further compounded by the high number of women in need of care [6]. In addition, a lower SES is often associated with limited access to healthcare resources, including early detection and treatment, as well as other factors such as poor nutrition and living conditions that can contribute to an increased risk of BC [6, 187].

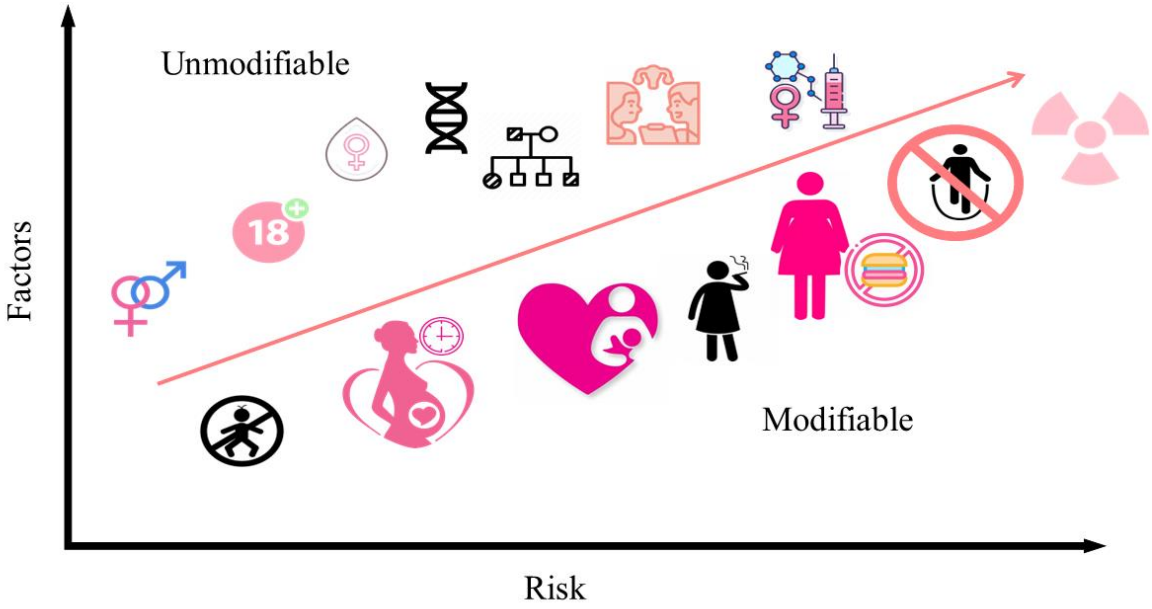


Figure 1.5: Modifiable and unmodifiable life factors associated with the risk of developing breast cancer in women.

In summary, an individual's gender, age, reproductive history, and genetic background can significantly increase their risk for BC, a risk that can be further influenced by lifestyle choices and external exposures (**Figure 1.5**). Therefore, gaining a thorough understanding of these factors is essential for effective BC management and treatment.

1.2.2 Current Medical Management of Breast Cancer

Management of BC has been successful largely due to the combined efforts of early screening, and the multidisciplinary approach of various treatments including newer surgical techniques and radiation therapies [188, 189]. Present-day treatments employed can be surgical and non-surgical adjuvant, depending on the stage and severity of the diagnosis.

There are two main forms of surgical approaches used to treat breast cancer patients that are guided by the stage, tumour type, and to an extent, the patient preference. These are a mastectomy (MTX), and a wide local excision (WLE), also referred to as breast-conserving therapy (BCT); [5, 190, 191]. A MTX can be total/simple which is the complete removal of breast tissues only [192]. However, a modified radical mastectomy has also been described, which involves the removal of breast tissues including the nipple, skin, axillary nodes, and in some cases a portion of the pectoralis major tissue [192]. A WLE which may also be described as a partial mastectomy, involves the removal of the tumour including a margin of healthy tissue, which is to ensure a complete removal [193]. Shown in **Figure 1.6** are the surgical comparisons noted between an MTX and WLE.

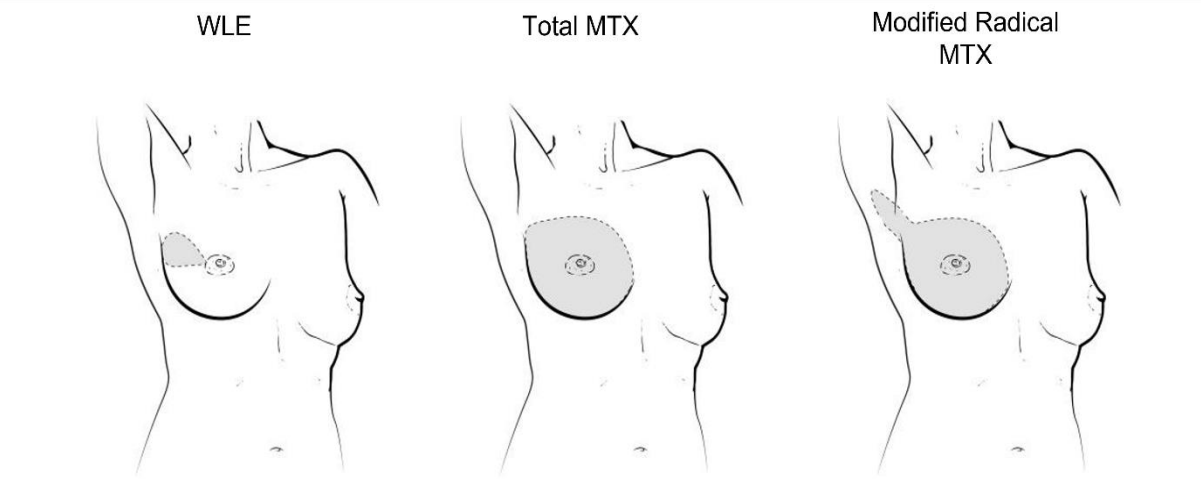


Figure 1.6: An illustration of the anatomy and different incision types for breast cancer surgery. The illustration shows the different surgical incisions applied for a i) Wide Local Excision (WLE), ii) total mastectomy (MTX) and iii) modified radical MTX, obtained and modified from Mastectomy | Baylor Medicine (bcm.edu).

Early studies theorized that selecting an MTX was associated with larger tumour sizes, metastatic lymph nodes and clinical stage [5, 194]. A retrospective study found 62.4% of BC patients had an MTX compared to 37.6% having a WLE, as a result of the advanced tumour stage at diagnosis [5]. However, the MTX vs WLE rates vary significantly due to disparities in population sampling and other factors such as age groups and as a result, any conclusions drawn from these studies remain inconclusive [195-197]. Additionally, MTX patients are counselled about the options of reconstructive surgery that can be immediate or delayed pending the success of the MTX, and the inclination of the patient and clinician [198, 199]. The choice for reconstructive surgery, however, remains variable as a systematic review screening twenty-eight studies revealed a significant disparity (4.9%-81.2%) in reconstruction surgery rates [200].

In SA (Soweto), total MTX was shown to be the most preferred surgical choice for patients in stages I, II, and advanced diseased states as opposed to a WLE [201, 202]. Given SA's low-middle income status, several concerns were described that patients need to consider [201]. Patients base their selection on the possibility of absolute and relative contraindications, socioeconomic difficulties, and more importantly, the need to keep therapy as simple and feasible as possible [201]. Whereas, the WLE treatment is commonly prescribed in combination with adjuvant therapies, and would therefore require additional consultations [192]. An outcome that will impact their employment and income, and this may also cause financial difficulties arising from the price of getting to these visits [192].

Non-surgical and Adjuvant Breast Cancer Treatments

There are a few non-surgical and adjuvant modalities that are employed to treat BC, that are non-invasive and improve survival rates, these are:

i. Ablation technology
ii. Radiation therapy
iii. Chemotherapy
iv. Immunotherapy
v. Hormone therapy

Advancements in cancer science have expanded treatment options, such as the non-invasive ablation techniques radiofrequency ablation (RFA), high-intensity focused ultrasound (HIFU), and cryoablation [203-205]. RFA is effective in treating various cancers, particularly when surgery isn't suitable, and uses an image-guided method of applying heat to kill solid tumours [205-215]. HIFU uses ultrasound to target deep-seated tumours, causing thermal necrosis [216, 217]. Whereas cryoablation uses cold temperatures to destroy cancer cells and stimulate an anti-tumour immune response, often used for BC patients who are ineligible for surgery [218-223].

Adjuvant therapies are additional non-surgical treatment options that are often prescribed in combination with surgery to treat cancer, including ionizing radiation therapy (RT), cytotoxic chemotherapy (CT) as well and endocrine therapy (Hormonal therapy, HT); [224]. Adjuvant RT is a well-known method that employs the use of subatomic particles (X-rays), like photons, electrons, carbon ions or neutrons to initiate apoptosis by creating mitotic destruction through cell division interferences [225, 226]. The therapy may be administered internally, known as brachytherapy, or externally (external beam), known as teletherapy [226]. The most used method is external beam radiation therapy of which there are several different technological approaches [227]. Amongst these, the more common approaches are three-dimensional conformal radiation therapy, intensity-modulated radiation therapy, volumetric modulated arc radiation therapy and even image-guided radiation therapy [227]. The basic mechanism of radiation therapy uses high-energy X-ray machines known as linear accelerators to direct the radiation energy at the tumour site [228].

The use of adjuvant RT is reportedly essential and highly effective in managing BC in curative and palliative cases, with RT treatment shown to have a 40% curative rate for >50% of BC cases treated [226, 229-233]. The treatment is guided by the clinician and BC diagnoses, is

fractionated over a period (typically 3-4 weeks) and is often prescribed in combination with BC surgery and/or other adjuvants [4, 234, 235]. Furthermore, the combination of adjuvant RT and surgical is associated with the prevention of local recurrence and improved survival, which is comparable to radical mastectomy in early-stage breast cancer patients [236-239].

Chemotherapy is the most common form of systemic cancer treatment, that treats BC through the blood circulation of cytotoxic chemical drugs [240, 241]. The most common route of administration is the intravenous route (IV) as it has a 100% absorption rate, however, it can also be administered orally (PO), intramuscularly (IM), subcutaneously (SC) and intrathecally (IT); [242]. In addition, chemotherapy is proven to be an effective neoadjuvant treatment and a core approach in metastatic BC cases [242-247]. Clinical trials and large cohort studies have shown that CT can reduce mortality risk between 7% and 33% depending on the cancer characteristics [248, 249]. Chemotherapy drug types are classified based on the mechanism of action (MoA), for which there a several classes [242, 250-257].

A promising advancement in oncology is the development of immunotherapy (IT). This significant addition uses the body's immune system to target and destroy cancer cells and prevent further invasion [258]. There are different forms of IT, though the most effective forms involve the use of monoclonal antibodies (mAb) and immune checkpoint inhibitors (ICI), shown to be most suitable in HER2+ and TNBC cases, respectively [258-260]. Studies have demonstrated that mAbs and ICIs, combined with adjuvant chemotherapy, improve clinical outcomes in both advanced and early-stage breast cancer [259, 260]. Immunotherapy is still regarded to be in its infancy and to date, only a select few drugs have been FDA-approved, these include:

i. Rastuzumab (Herceptin); Approval Date: 25 September 1998; Indications: HER2-positive breast cancer, both in metastatic and adjuvant settings.

ii. Atezolizumab (Tecentriq); Approval Date: 8th March 2019; Indications: First-line treatment for PD-L1 positive, locally advanced or metastatic TNBC, in combination with nab-paclitaxel (Abraxane).

iii. Pembrolizumab (Keytruda); Approval Date: 13 November 2020; Indications: TNBC, in combination with chemotherapy for patients with PD-L1 expressing tumours, and as monotherapy for previously treated PD-L1 positive TNBC.

Despite the promising clinical outcomes associated with immunotherapy, the success rates remain relatively low, mainly because ongoing clinical trials and explorations is still underway to fully understand the potential of IT [259, 261, 262]. Additionally, in some cases effective outcomes are not observed as some patients do not respond to therapy and others that may respond, can relapse due to resistance [259]. Of note, are epigenetic drugs (epidrugs) which holds the potential to enhance immunotherapy and chemotherapy, however, this line of therapy remains in early clinical trials and exploration [263].

Between 70% to 80% of premenopausal BC, cases are reported as hormone receptor-positive (HR+), and as a result, adjuvant hormone therapy (HT) is considered a mandatory approach [264, 265]. HT has been employed as a palliative course of treatment in metastatic cases, and curative in nonmetastatic cases as it modulates tumour growth, making significant contributions to the survival rates [264-267]. Presently, three types of HT are prescribed based on clinical parameters such as risk of recurrence, tumour characteristics, the hormone receptors oestrogen and progesterone, and menopausal status [264, 267]. Furthermore, HT types are

categorised by the mechanism of action, which may inhibit i) ovarium function, ii) oestrogen production or iii) oestrogen effects [264, 266, 267].

Inhibition of ovarian function may be done through RT, surgical removal of ovaries or chemical stimulation [265, 268]. The inhibition of oestrogen production can be done through the use of aromatase inhibitors (AI) which prevent the aromatase enzymatic conversion of androgen to oestrogen [268, 269]. AIs currently used can be steroidal inhibitors (type I) which are irreversible, or non-steroidal inhibitors (type II) that act as reversible competitive inhibitors of aromatase activity [269]. Whereas the inhibition of oestrogen effects relies on the use of selective oestrogen receptor modulators (SERMs) and selective oestrogen receptor degraders (SERDs); [268, 270-272]. The SERMs' (**Figure 1.7**) compete with oestrogen for oestrogen receptor binding, whereas SERDs produce an unstable protein complex, prompting the degradation of the receptor through proteosomes [271, 273]. There are several successful SERMs, and SERDs currently being employed, the most widespread being tamoxifen (SERMs), and fulvestrant (SERDs); [271].

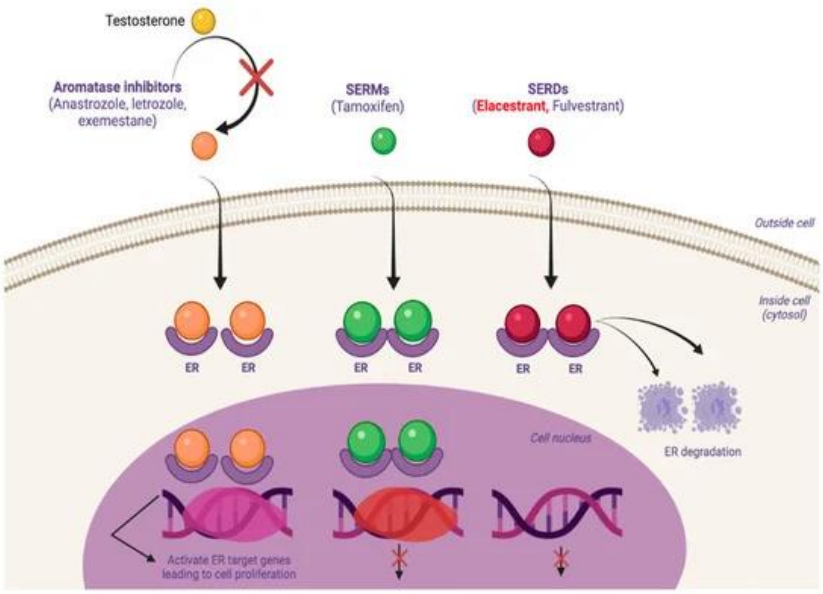


Figure 1.7: Mechanism of action for aromatase inhibitors. Selective oestrogen receptor modulators (SERMs) and selective oestrogen receptor degraders (SERDs) are endocrine therapies used in the treatment of hormone receptor-positive BC, obtained from Hernando *et al.* (2021)[271].

The continuous advances in medicine and BC treatment have significantly improved survival rates, with a BC diagnosis now often regarded as a chronic condition rather than a life-threatening disease [11]. Additionally, due to the availability of various BC treatment options, the number of survivors is increasing [274]. Two key components are crucial for ensuring survival, i) early detection and diagnosis, and ii) the implementation of the most effective treatment plan [11]. Reports indicate that a delay of more than three months in detection can reduce the 5-year survival rate by 12%, potentially leading to poor clinical outcomes and prognosis [275-277]. In this review, the study focus is on the essential aspects of survivorship and quality of life.

1.2.3 Survivorship Defined

Coined in the late 1980s, the term survivorship was used to describe the “time of life” in which an individual goes through a transition period following successful cancer treatment, from

acute cancer survival into an extended period of survival [278]. Accordingly, the National Cancer Institute (NCI), describes "survivorship" as the life quality following a cancer diagnosis, and the impact it has on various (physical, mental, emotional, social, and financial) components of life (https://www.cancer.gov/publications/dictionaries/cancer-terms/def/survivorship). The term is applied to include both individuals who are living with cancer, presenting with any type of cancer (early stages to advanced cases), and those who are free of cancer (post-treatment); (https://cancercontrol.cancer.gov/ocs/definitions).

In recent decades (2010-2017), the global rates of BC survivors at 1, 3, 5 and 10 years, have noted a significant increase in comparison to earlier years (1960-1969); [279]. Evaluation of these rates has been shown to vary considerably between high (>80%), middle (~60%), and low-income (<40%) countries [279, 280]. Also, the evaluation of 10-year survival rates has noted distinct disparities amongst the specific age groups; 46-50yrs (70%), <30yrs (60%), and >75yrs (59%); [279, 281]. For SA, following the most recent CONCORD surveillance reports, women diagnosed with BC between 2000 and 2014, noted an age-standardised 5-year net survival rate ranging between 40% and 53% [282, 283].

Despite the increasing survival rates and numerous benefits associated with various treatment approaches, BC survivors are known to experience long-term adverse effects that can significantly impact their quality of life (QoL); [284]. It is reported that as many as 90% of survivors endure long-term sequelae, due to the treatments they undergo for BC [18]. These findings have given rise to an emerging global field within cancer care and management, aiming to address the side effects on physical, mental, social, emotional, and overall health [285]. Therefore, it comes as no surprise that research efforts are now increasingly focused on survivorship and life after cancer.

1.2.4 Quality of Life (QOL) and Latent Effects of Medical Management

Survivorship studies report that survivors experience treatment-related sequelae that have a major impact on the quality of life (QoL); [286, 287]. Several psychological and physiological effects have been associated with surgery, chemotherapy, radiation, and others (**Figure 1.8**).

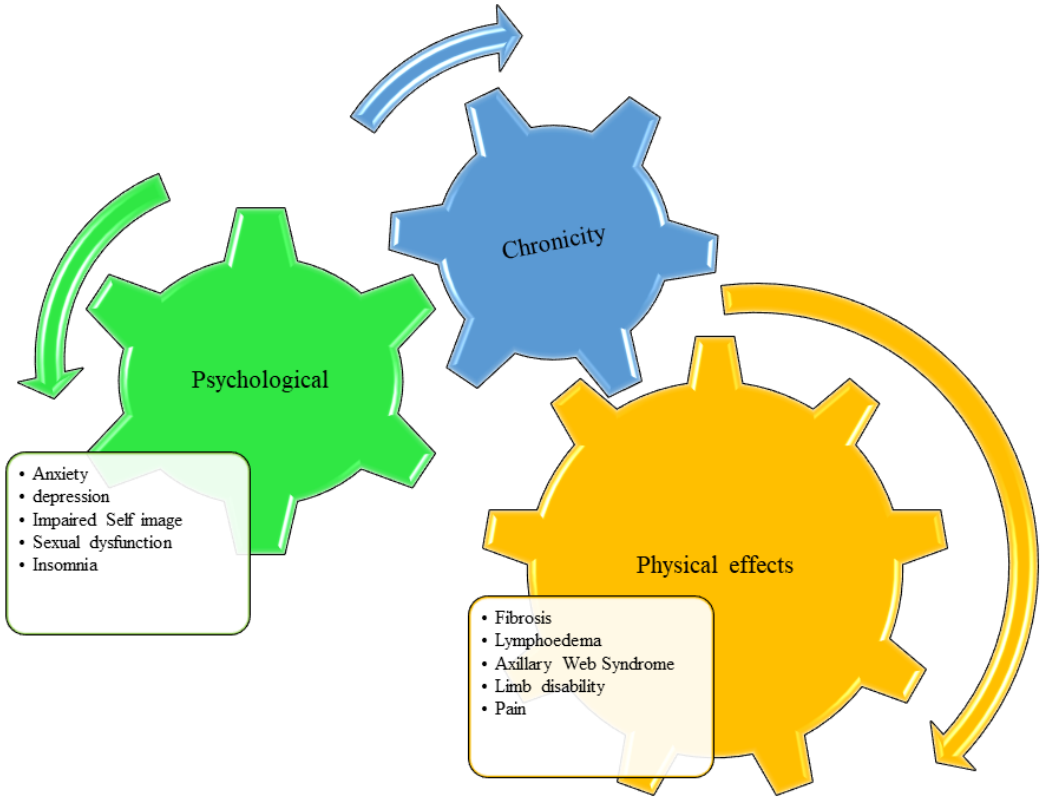


Figure 1.8: The most prevalent physical and psychological sequelae associated with breast cancer treatment in survivors.

Psychological Side Effects

Despite the advances made in treatment, women are not only faced with accepting their diagnosis, but also understanding the complexity of prognosis, reoccurrence, and side effects, all of which can lead to mental distress [288]. Recent data report that anxiety is the most frequently

observed psychological symptom experienced in BC [288-292]. According to systematic analysis, the prevalence of anxiety symptoms ranges from 18% to 33.3% in BC patients [291-293]. The immense anxiety and paralysis brought on by fear and the uncertainty of a future, are noted to have a considerable impact on the overall QOL [288, 290, 294]. In addition, BC patients may also experience depressive states distinguished by low moods (sadness), sleeping problems, and a loss of energy and enjoyment in life, all having a significant impact on QOL [290, 295]. Earlier studies reported that depending on the screening and study design, the prevalence of depression among BC patients ranged from 10% to 30% [290]. However, according to a recent systematic analysis incorporating seventeen studies, depression symptoms are estimated to range from 9.4% to 66.1% in BC patients and were hypothesized to increase one year after diagnosis [293].

Above all, the effects of BC treatments primarily impact a patient's body image and can have dire consequences on the sexual/physical functioning or how they engage socially. A systematic analysis has shown that sexual dysfunction is frequently observed in midlife BCS [296]. One study reported that 65% of BCS were sexually active, of which 52% reported physiological problems and a follow-up analysis reported that 26% and 19% of BCS still experience this side effect at five and ten years after, respectively [297]. Additionally, another co-related psychological effect of anxiety and depression, is insomnia, afflicting 40% of survivors [298]. Insomnia, the difficulty of falling or remaining asleep, is the most common sleep problem, and its prevalence in the BCS is more than double that of the general population [298, 299]. Consequently, these psychological effects can negatively impact social interactions and relationships, productivity at work, influence pain perception related to cancer, and thereby reduce QOL [298, 300, 301].

Physical Side Effects

As much as 90% of women experience physical side effects to the shoulder [302], which include:

i. Fibrosis
ii. Lymphoedema,
iii. Axillary web syndrome,
iv. Limited mobility
v. Pain phenotypes

FIBROSIS

Radiation therapy-induced (RT-induced) fibrosis of the tissue is permanent and therefore has been noted to be associated with an incidence ranging from 10% to 23% [303-305]. Fibrosis is a term used to describe the hardening and scarring of tissues, mainly due to excessive production of collagen and other extracellular matrix (ECM) proteins [306]. Several mechanisms have been proposed to underlie the development of fibrosis. These include chronic inflammation, oxidative stress, fibroblast and myofibroblast activation, excessive ECM deposition, dysregulated immune response, tissue remodelling imbalances, and aberrant signalling pathways [306, 307]. In addition, RT-induced fibrosis can lead to severe structural and functional alterations of the connective tissues that may spread to adjacent areas as well as the area affected [305]. These fibrotic features are also often associated with limb morbidity and pain, with the latter affecting roughly 40% to 45% of patients [305].

LYMPHOEDEMA

A recent study within Sub-Saharan Africa reported a 14% incidence of BC-related lymphoedema (BCRL); [308]. Although BCRL is most frequently noted in the arm (>90%), it can also affect the breast and trunk (<30%); [309]. Lymphoedema is the build-up of protein-rich fluids within the interstitial spaces boosting the volume of the limb, which indicates damage to healthy vasculature [310, 311]. An imbalance between lymphatic flow and the body's ability to circulate it properly is the main factor contributing to the growing limb volume as seen in **Figure 1.9** [312]. Surgery for BC frequently involves the excision of lymphatic nodes, which can lead to disruption of the lymphatic system, and cause a blockage thus resulting in limb swelling [312-314]. Lymphedema with its accompanying symptoms, swelling, tightness, stiffness, fatigue, weakness, numbness, pain and entire loss of mobility of the affected limbs, are shown to negatively impact the BCS's QoL [315].

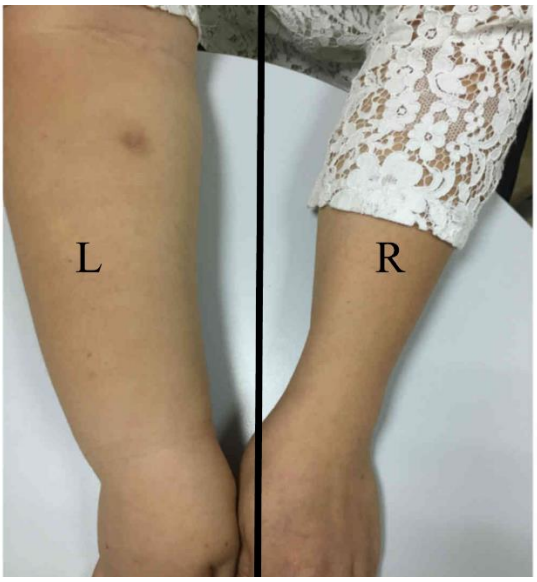


Figure 1.9: An example of breast cancer-related lymphoedema. Lymphedema occurs when the volume of the affected arm left (L) increases substantially compared to the unaffected arm right (R), leading to restricted mobility, and often causing pain. Adapted from He *et al.* (2020)[311].

AXILLARY WEB SYNDROME

The axillary web syndrome (AWS) is also a common complication of breast cancer treatment with >90% of BCS presenting with the side effect [18, 316-319]. The occurrence is characterised by the visible formation of distinct cords that are weblike as seen in **Figure 1.10** [320]. It is hypothesised that during breast cancer surgery, lymphatic fluids may coagulate in the presence of increased plasma thrombokinase levels [316, 320, 321]. AWS is described as causing painful, reduced and restrictive limb functionality [320].

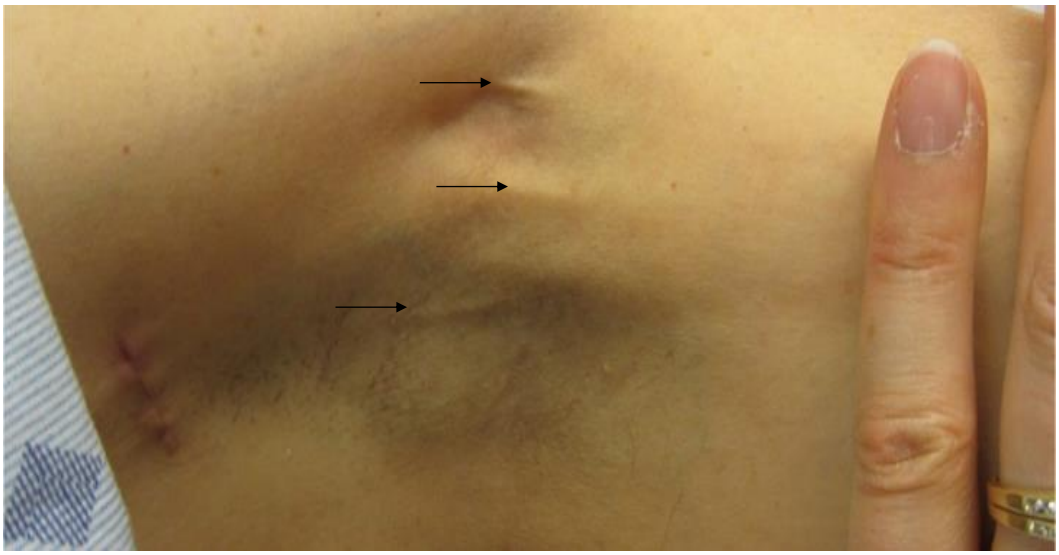


Figure 1.10: Axillary Web Syndrome in Breast Cancer Survivors. Depicted here are multiple cords in the mid-axilla (underarm) of a BCS obtained from Koehler *et al.* (2019)[317].

The evaluation of each of the discussed side effects, fibrosis, lymphedema, and AWS, is linked to shoulder pain and disability which leads to the side effects that are the focus of this investigation.

1.3 CHRONIC SHOULDER PAIN AND DYSFUNCTION

Shoulder pain and dysfunction remain the most prevalent side effect observed in BCS and are complementary symptoms to fibrosis, lymphedema, and AWS. Shoulder morbidity and persisting arm pain a long-term symptoms observed in >40% BCS [322, 323]. It is reported that BCS experience a limited range of motion (ROM) in the shoulder and diminished reaching abilities [9, 324-326]. Kinematic investigation of shoulder movements in BCS has highlighted maladaptive movements and scapula dysfunction like that of other shoulder conditions such as impingement syndrome [326]. In addition, these maladaptive patterns are reported to be bilateral because of compensation on the unaffected side of unilateral BC cases [9]. There are distinct pain categories with each presenting in different forms, and in BCS many of these forms have been reported including chronic pain, allodynia, paraesthesia, and phantom sensations [7, 327].

1.3.1 Epidemiology of Pain

The term pain is defined as "An unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" by the International Association for the Study of Pain (IASP); [328]. Chronic pain is described as pain that persists for a period beyond the recovery window of 3 months [329, 330].

Global prevalence for chronic shoulder pain is reported to be between <1% - 55%, and 10%-50%% for the general population, and BCS, respectively [331, 332]. SA statistics report a prevalence of 18.3% within the general population and women report pain 20% more frequently than their male counterparts [333]. To the best of this author's knowledge, this report presented the most current statistics. In Sub-Saharan African women, 43% of BCS report shoulder pain, in addition to stiffness (36%) and swelling (23%); [334]. It is reported that the

burden of shoulder pain, particularly in disadvantaged communities, is the third most prevalent musculoskeletal condition affecting individuals and negatively impacting QOL [331, 335, 336]. Additionally, chronic pain in the shoulder pain is regarded as the most prevalent anatomical site, followed by the back and stomach/abdomen [333].

The evaluation of chronic shoulder pain and dysfunction in the primary care setting requires an extensive knowledge of the anatomical site and employs different approaches which include the use of physical examination, medical history diagnostic assessments and imaging [337]. Clinically referred to as the glenohumeral joint, the shoulder is a ball-socket joint consisting of the joints, sternoclavicular, coracoclavicular, and acromioclavicular, as well as the scapulothoracic interface and subacromial space [338, 339]. The scapula and humerus attach to the clavicle and are supported through the aforementioned joints along with other anatomical structures like muscles, tendons, and ligaments that enable the joint to achieve a wide ROM [338, 339]. The shoulder relies on four key muscles namely, supraspinatus, infraspinatus, teres minor and major, which enable the wide range of motions, medial rotation and adduction, abduction, and lateral rotation, known as the rotator cuff muscle group (**Figure 1.11)**; [338, 340, 341].

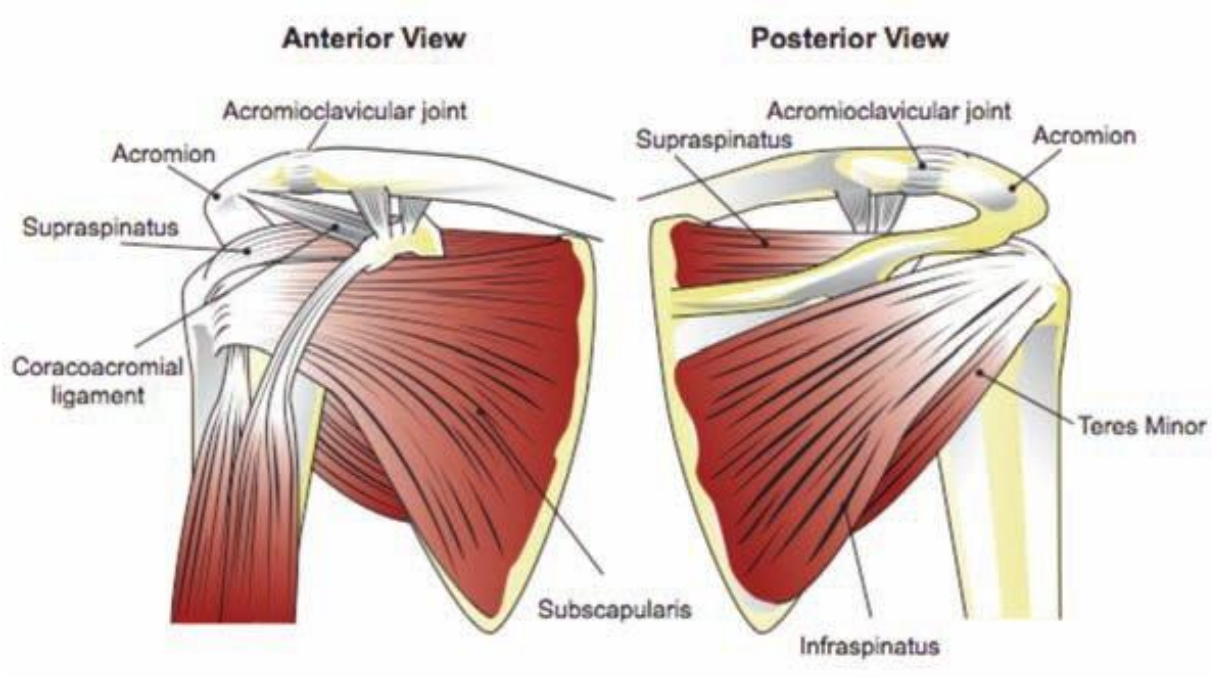


Figure 1.11: The anatomy of the shoulder joint. Depicting the complex joints and the rotator cuff muscle group (supraspinatus, infraspinatus, teres major and teres minor) in anterior and posterior view obtained from Boykin *et al.* (2010)[342].

Physical assessments involve the systematic inspection of the shoulder through palpation, range of motion, and limb strength and provocative tests, [337, 343]. Briefly, the inspection involves determining the stability of the shoulder joints and examining the main movements provided by the rotator cuff muscle group, including flexion, abduction, adduction, lateral and medial rotation, as well as extension [343-345]. To date, there are more than 180 types of physical examination tests used to evaluate shoulder pain and dysfunction [346]. A few of the factors which have broadly been implicated in the development and prevalence of shoulder conditions include age, occupation and recreational activities, familial conditions, comorbidities' autoimmune conditions and previous injuries [343, 345, 347-349].

Given the complex and subjective nature of pain, diagnostic tests or patient-reported outcome measures (PROMs) are used to evaluate shoulder conditions [350]. PROMs are often used in research and healthcare settings because they provide a consistent testing method for evaluating states of pain and dysfunction [351]. These PROMs can be unidimensional or multidimensions, with the former measuring 1-item measurement, which is simple, fast, and easily understandable, and the latter measuring more than 1-item and requiring some time [351, 352]. In addition, using PROMs allows for a better understanding between patient and doctor, which is key in electing the best treatment plan [353, 354].

Several different validated PROMs are available, and the selection of the appropriate PROMs is based on the requirements and expected outcomes evaluated. Examples of validated PROMS include the University Of California - Los Angeles Shoulder Scale (UCLA), the Simple Shoulder Test (SST), and the Shoulder Pain and Disability Index (SPADI); [355]. Imaging has also been used to identify and diagnose shoulder pathology, with ultrasound/ultrasonography (US) and magnetic resonance imaging (MRI) being the gold standard in diagnostic imaging [337, 356, 357]. It is reported that US and MRI are very useful imaging tools for the detection of rotator cuff tears and shoulder pain [358, 359]. A systematic analysis however found conflicting and low-grade evidence, motivating further investigations to fully comprehend the association between diagnostic imaging and shoulder pathologies [360].

1.3.2 Types of Pain

As a musculoskeletal condition, chronic shoulder pain and the associated disability in BCS can be divided into three types of pain, namely nociceptive, neuropathic, or pain stemming from central sensitization [332, 361-363]. As described previously, pain is defined as an “unpleasant

sensory and emotional experience associated with actual or potential damage", as in the case of nociceptive pain, which is described to be the result of an "active neural pathway caused by actual tissue damage or potentially tissue-damaging stimuli" [364]. The nociceptive pain pathway, which includes anatomical structures such as skin, viscera, and musculoskeletal tissues, is a complex interactive relay of neurotransmission between the peripheral (PNS) and central (CNS) nervous systems [365].

Forming part of the ascending pain pathway (**Figure 1.12**), noxious stimuli are detected by specialised sensory receptors known as nociceptors; generating signals through unmyelinated C fibres and small Aδ fibres and transmitting them towards the dorsal root ganglion (DRG); [366, 367]. Additionally, numerous tissues have nociceptors, which are activated by biological, electrical, thermal, mechanical, and chemical stimuli [368]. These include the epidermis, periosteum, joint capsule, ligaments, muscles, cornea of the eye, dental pulp and others [368]. Peripherally, activated nociceptive signals are transmitted via projection neurons found in the lamina I and V of the dorsal horn in the spinal cord, crossing the midline, and ascending via supraspinal tracts towards the CNS [367]. Depending on the tract (for example the spinothalamic tract), these signals can reach different brain processing centres like the anterior cingulate cortex and prefrontal cortex where pain is perceived [368, 369]. Nociceptive pain can be somatic or visceral with the former being localized, dull or intense, superficial or deep, constant and movement-related [370]. The latter, however, can be persistent, cramping, agonizing, referred, frequently deep, but poorly localized and typically accompanied by emesis [370, 371]. In BCS, nociceptive pain is thought to be caused by the activations of nociceptors in response to painful stimuli during breast cancer therapy, such as surgery, which is frequently accompanied by post-surgical movement-related or mechanical stimuli [372-374].

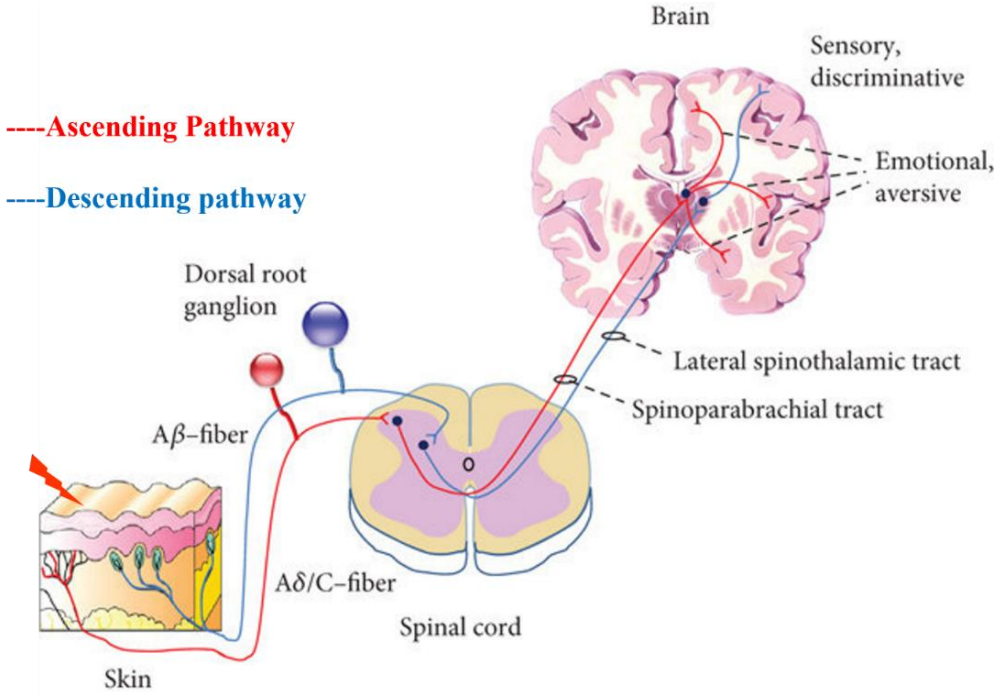


Figure 1.12: The pain pathway following noxious stimuli and nociceptive sensory detection. Adapted from Sun *et al.* (2020)[367].

Neuropathic pain (NP) is described as "pain initiated or caused by a primary lesion or dysfunction or transitory perturbation in the peripheral (PNS) or central nervous system (CNS); [375, 376]. It is often observed in clinical settings and characterised by symptoms of ongoing pain in sensory-impaired parts or states of hypersensitivity, like hyperalgesia, referred pain or recurrent stimulation, which may negatively impact QoL [376-379]. The mechanisms of neuropathic and nociceptive pain are however not mutually exclusive as studies indicate that pain-generating-lesions must include the activation of the nociceptive pathways [380]. The exact mechanism of neuropathic pain is highly complex and varies greatly. A proposed cause for neuropathic pain such as peripheral sensitization involves the repeated activation of peripheral nerves in response to noxious stimuli causing the activation of an inflammatory response [369]. The release of inflammatory chemicals like substance P, calcitonin-gene-related peptides and

others can result in the sensitization of nociceptors and reduction in the firing thresholds [369, 381-384].

Neuropathic pain symptoms, in contrast to nociceptive pain, are varied and may present as peripheral or central neuropathy like post-herpetic neuralgia, and post-stroke pain [60, 371, 385, 386]. It could be inflammatory as seen in cases of arthropathies, or musculoskeletal, like back pain [371, 387, 388]. It may also present as mechanical or compressive pain as described in cases of ilioinguinal neuralgia observing the compression of nerves [389]. Common descriptors used in cases presenting with neuropathic pain are pain that is burning, shooting, stabbing, numbing or tingling [371]. In the present population, studies looking at the effects of chemotherapy have found that neuropathic pain can be brought on by cancer-related symptoms or cancer therapy itself [363, 390].

Central sensitisation (CS) pain, also referred to as nociplastic pain, is another classification described in BCS and is far more complex as it engages both nociceptive and neuropathic mechanisms [391-393]. Following the most updated definition provided by the IASP, central sensitization is the "increased responsiveness of nociceptive neurons in the CNS to their normal or subthreshold afferent input". One key feature known about the nervous system is its plasticity and both systems, PNS and CNS can rapidly adapt to sensory changes [394]. It is theorised that the mechanism underlying CS is through the activation of the PNS and the sensitization of the nociceptive pathway, which can lead to long-term potentiation, ascending the spinal cord and may initiate maladaptive plasticity within the CNS giving rise to increased pain perception [394]. Ongoing research surrounding CS has been aimed at understanding the dynamic of CS in chronic pain conditions as both may present as hypersensitivity to stimuli with potential interaction as described in states of allodynia and hyperalgesia [394, 395]. Several

conditions reporting chronic pain have been regarded to fall under the spectrum of central sensitisation like fibromyalgia (FM), temporomandibular disorder (TMD) and irritable bowel syndrome (IBS); [396-400]. Therefore, understanding and precisely characterizing pain, as well as understanding its presentation in a clinical setting, can provide healthcare practitioners with vital insights into developing the most successful treatment regimens.

1.3.3 Current Pain Management Protocols

In the acute hospital setting, post-breast cancer surgery pain is usually managed with the use of opioid drugs [401]. The "gold standard" for relieving pain and the current most effective natural opiate available is morphine which is clinically used for the management of severe pain [402]. In the immediate postoperative period, it is the most effective and acceptable analgesic for the majority of patients since morphine acts directly on pain-modulating receptors known as opioid receptors found in the nervous systems [403, 404]. Opiates are however known for their negative side effects such as respiratory depression and addiction which is why lesser synthetic derivatives are developed and these can include tramadol and panadeine [405].

Tramadol is a centrally-acting analgesic with a binding affinity to the mu-opiate receptor [406]. It is a synthetic structurally related to codeine and morphine placing it in the same class of opiate drugs and equally prescribed for post-operative pain [407]. Panadeine on the other hand is a combination of codeine and paracetamol also known as acetaminophen. The combination is either 8mg codeine to 500mg paracetamol or 30mg codeine to 500mg paracetamol the latter described as Panadeine forte can be used for moderate pain [408, 409]. The specifics of their mechanisms and interactions within pain pathways are very complex and varied [410, 411]. Although both drugs follow distinct metabolic pathways, they both exert their effects on the

pain inhibition pathway, primarily through the central nervous system due to the presence of opioid components [412].

Regardless of the options available, tramadol appears to be the preferred opioid of choice for the treatment of various pain conditions including post-surgery pain [413-418]. The drug is also reported to be effective in the management of post-operative anxiety, depression, and movement-evoked pain associated with a caesarean [419, 420]. Ongoing clinical studies also indicate that tramadol in combination with paracetamol is a more effective analgesic treatment option for chronic pain, particularly in musculoskeletal conditions [421-423]. The literature, however, has acknowledged inconclusive reviews regarding the benefit of tramadol in multimodal treatment settings and that risks are notwithstanding [422, 424]. However, it is important to note that risk factors associated with the development of chronic shoulder pain and disability can influence the success of the current pain management protocol [425]. It is, therefore, of utmost importance to examine the most prominent risk factors and their influence on pain management protocols.

1.3.4 Risk Factors Associated with Chronic Shoulder Pain and Disability in BCS

The aetiology of chronic shoulder pain and disability in BCS is influenced by both intrinsic and extrinsic factors [19]. Following the systematic and meta-analysis of numerous studies, risk factors associated with chronic shoulder pain and disability in BCS, are summarised in **Table 1.2** [19, 426]. The most consistently associated risk factors include age, BMI, comorbidities, lifestyle (for example smoking and alcohol use), lymphedema, breast cancer surgery, axillary surgery, adjuvant therapies, acute pre-operative pain, acute post-operative pain, post-surgical complications, reconstructive surgery, education as well as genetics [19, 426-430].

The foremost predictors include auxiliary lymph node dissections (ALND), radiotherapy, pre-operative, and acute postoperative pain where 21%, 7%, 6% and 3% more patients develop persistent pain respectively [20]. It has also been highlighted that younger patients experiencing pre- and post-operative pain and subjected to a different combination of breast cancer treatments, have varied potential risks for developing chronic pain. A "significant predictive relationship" exists between the pain intensity of the acute post-operative period and the development of chronic long-term pain [431]. Inadequate pain control during this period is known to increase morbidity and thereby mortality [431, 432]. Therefore it seems critical that pain regulation during the acute post-operative period is a crucial component for long-term health [432]. Additionally, the psychological state of individuals is also known to play a role with studies reporting increasing levels of anxiety, depression, and negative affect (positive affect decreasing) especially in musculoskeletal pain conditions [433-437]. Particularly noteworthy across different time points, including the pre-operative period, at 3, 6 and 12 months, and continuing up to 24 months [433, 435, 436]. In BCS, however, the precise mechanism, or predictors of developing chronic pain post-operatively remains unclear. While it is evident that risk factors such as age and treatment type may contribute to the development of chronic shoulder pain and disability, evidence suggests that examining the genetic contribution has the potential to identify the key factors influencing pain responses [438-440]. Moreover, it can shed light on the mechanisms underlying inadequate pain management [438-440]. By focusing on the genetic component, a deeper understanding of pain pathways can be gained, enabling the tailoring of more effective pain management protocols.

Table 1.2: Summarized grading of gecommendation assessment, development and evaluations, evidence profile for predictors of persistent pain after breast cancer surgery, reproduced from [20].

Predictor (Patients/median follow-up)	Quality assessment	Relative effect (95% CI)		Anticipated absolute effect	
	Publication Bias (p values for Begg test /Egger test)	Overall		Baseline risk [b]	Risk Difference (95% CI)
Age (10-year decrement) 11 030/ 12 months	Undetected (p=0.8/p=0.8)	High	OR 1.36 (1.24-1.48)	30% for age 70yr[c]	7% more patients with 10 yr. decrement of age persistent pain
Radiotherapy (yes v no) 9468/ 23.5 months	Undetected (p=0.6/p= 0.2)	High	OR 1.35 (1.16-1.57)	30%	7% more patients with radiotherapy have persistent pain
Axillary lymph node dissection (yes v no) 7699/12 month	Undetected (p>0.9/p=0.5)	High	OR 2.41 (1.73-3.35)	30%	21% more patients with ALND have persistent pain
Acute postoperative pain: 10cm pain scale indicated by lower values. 1387/17.5 months	Uncertain only 5 studies	High	OR 1.16 (1.03-1.3)	30% for 1cm on a 10 cm scale[c]	3% more patients with per 1cm increment of acute pain on a 10cm pain scale having persistent pain
Preoperative pain (yes v no) [d] 2504/7.5 months	Uncertain only 8 studies	Moderate	OR 1.29 (1.01-1.64)	30%	6% more patients with preoperative pain have persistent pain
BMI (5point increment) 3178/12months	Uncertain only 8 studies	High	OR 1.11 (0.99-1.24)	30% for BMI 25kg/m^{2}[c]	2% more patients with per 5-point increment of BMI having persistent pain
Breast Surgery: (BCS v Mastectomy/modified radical mastectomy) 8566/17.5 months	Undetected (p=0.2 /p=0.8)	High	OR 1.08 (0.90-1.30)	30%	2% more patients with BCS have persistent pain
Chemotherapy (yes v no) 8481/12months	Undetected(p=0.6/p>0.9)	High	OR 1.12 (0.98-1.29)	30%	2% more patients with chemotherapy have persistent pain
Endocrine therapy (yes v no) 8312/27month	Undetected (p=0.3/p=0.2)	High	OR 1.07 (0.94-1.22)	30%	1% more patients with endocrine therapy have persistent pain

Note: BCS=Breast-Conserving surgery; BMI= Body Mass Index; CI= Confidence Interval; GRADE= Grading of Recommendations Assessment, Development and Evaluations; OR= Odds ratio; [a.] other assessments included risk for Bias, Inconsistency, Indirectness, and imprecision for which all showed no risk; [b.] Baseline risk based on a subpopulation of patients undergoing sentinel lymph node biopsy with lowest absolute risk for persistent pain in the study with the largest sample size amongst studies at low risk of bias; [c]. Reference groups for age, BMI and acute postoperative pain were obtained from the largest study; [d.] Serious Imprecision-Quality was rated down based on imprecision; the risk difference included a predefined threshold of 10% for modifiable factors.

1.3.5 Genetic Risk Factors in Analgesic Response

Present-day research is showing strong evidence for the role of genetics as a risk and contributory factor to interindividual variability to analgesic response, and in the development of chronic pain and disability in BCS. Analgesics can be administered in various ways including oral, sublingual, buccal, intranasal, rectal, intravenous, and subcutaneous for example. Additionally, the route of administration and the formulation of the analgesic are both determined and dependent on its pharmacokinetic (PK) and pharmacodynamics (PD) properties [441]. Therefore, to understand the mechanism of opioid metabolism and pain management in a clinical setting, it is imperative to examine the PK, PD, and pharmacogenetics/pharmacogenomics (PG) of these drugs (**Figure 1.13**).

A considerable amount of literature has examined the PK and PD of pharmaceuticals, but its usefulness is restricted to understanding drug-dose to dose-effects [442-444]. The investigation of PG, on the other hand, provides for the investigation of the genetic component that influences the PK and PD capability of prescribed medications. As a result, this holistic approach serves as a bridge that connects an individual's biology to the functionality of pharmacological therapy and response.

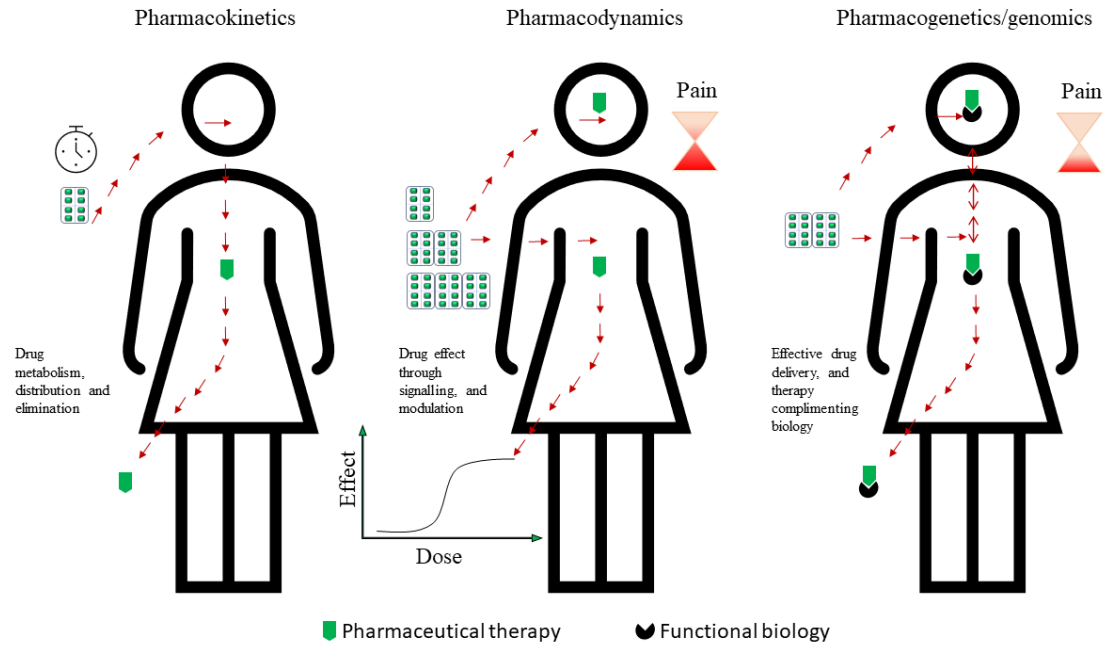


Figure 1.13: A schematic illustrating the pharmacokinetics (PK), pharmacodynamics (PD) and pharmacogenetic (PG) components that contribute to effective pain therapy. PK: studies describe how the body alters drugs, for example, the bioavailability of active and inactive metabolites and elimination thereof, whereas PD describes how the drug affects the body (e.g. pain relief vs minimal effect to no effect at all); [445, 446]. PG: describes how an individual's genes/genome influence the body's ability to modify, and the effects of those drugs [447].

Genetic association studies have identified several loci in which polymorphic changes can influence the PD and PK of analgesic drugs [448]. Polymorphisms that cause a significant change to the amino acid or structure of a protein are considered potential biomarkers/identifiers for the risk of reduced capacity for opioid metabolism. Amongst the most widely studied genes are *CYP2D6*, *UGT2B7, ABCB1*, *OPRM1, SCN9A* and *COMT* [401, 449-452]. Genetic variations like single nucleotide polymorphisms (SNPs) in these genes have been implicated and associated with inter-individual pain variability in various opioid drug metabolism and pain signalling studies with contrasting results across ethnically different cohorts [453].

1.4 OPIOID SIGNALLING PATHWAY GENES

Opioids remain the most widely used drug of choice for chronic pain and as a result, directly implicate the opioid receptor-dependent signalling and associated modulatory pathways to pain relief (**Figure 1.14**).

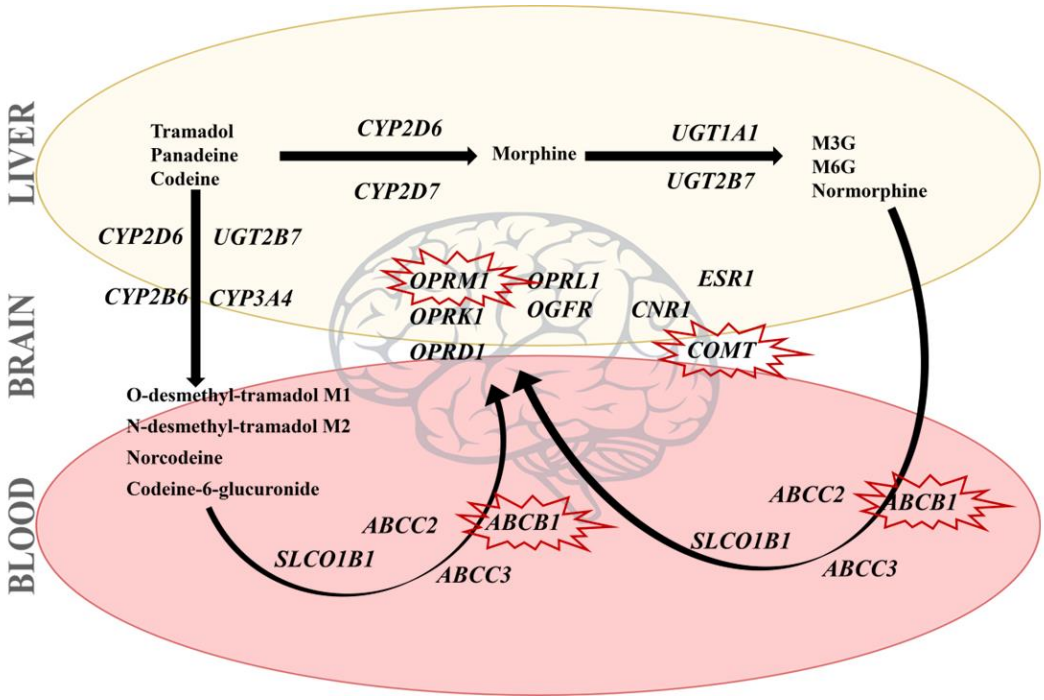


Figure 1.14: Metabolic pathway for opioid metabolism, distribution, and signalling. A simplified way to describe opioid metabolism can be seen above. Opioids are metabolised primarily in the liver and the metabolites (e.g. M3G and O-desmethyl-tramadol M1) are transported via blood to the brain for opioid receptor uptake. The starred genes are crucial for the conversion, delivery and acceptance of opioids which results in an anti-nociception effect. Opioid drugs like morphine, tramadol and Panadeine may be converted via different enzymes but are all agonists for the μ-opioid receptor placing these three drugs in one category of pain signalling. Abbreviations: *ABCB1* , ATP Binding Cassette Subfamily B Member 1; *ABCC2* , ATP Binding Cassette Subfamily C Member 2; *ABCC3* , ATP Binding Cassette Subfamily C Member 3; *CNR1* , Cannabinoid Receptor 1; *COMT* , Catechol-O-Methyltransferase; *CYP2B6* , Cytochrome P450 2B6; *CYP2D6* , Cytochrome P450 2D6; *CYP2D7* , Cytochrome P450 2D7; *CYP3A4* , Cytochrome P450 3A4; *ESR1* , Estrogen Receptor Alpha; M3G , Morphine-3-Glucuronide; M6G ,

Morphine-6-Glucuronide; *OGFR* , Opioid Growth Factor Receptor; *OPRD1* , Opioid Receptor Delta 1; *OPRL1* , Opioid Receptor-Like 1; *OPRM1* , Opioid Receptor Mu 1; *OPRK1* , Opioid Receptor Kappa 1; *SLCO1B1* , Solute Carrier Organic Anion Transporter Family Member 1; *UGT1A1* , UDP Glucuronosyltransferase 1A1; *UGT2B7* , UDP Glucuronosyltransferase 2B7.

The most basic pathway described for opioids details the metabolism of opioids, seen in **Figure 1.14**, in the liver implicating the *CYP2D6* and *UGT2B7* genes [446]. Opioids such as morphine, and tramadol primarily undergo extensive metabolism in the liver, however, metabolism may also occur in the blood-brain barrier (BBB) transport system or specific brain regions [446, 454]. The resulting opioid metabolites are transported via carrier and membrane proteins that facilitate the efflux and uptake of drug metabolites which include the Adenosine Triphosphate (ATP)-Binding Cassette (*ABC*) and the Solute carrier organic anion transporter (*SLC*) superfamilies [455]. The most prominent members responsible for opioid distribution noted in the literature include *ABCB1, ABCC2, ABCC3 and SLCO1B1* [455].

The *ABCB1* gene is an ATP-efflux transporter with broad specificity and is extremely relevant as it actively drives drugs out of the CNS and has been identified as an important component of the BBB [456, 457]. Solute carrier organic anion transporter family member 1 (*SLCO1B1)* is a multi-specific transporter for Na^+-independent uptake of bile acids and other organic anions [458]. Although evidence is weak for this gene, the family has been reported to be involved in the transportation of Morphine-6-Glucuronide metabolite across the BBB [459]. Moving forward in this pathway and transitioning to the inhibition of pain signals, involves the activation of G-coupled opioid receptors in the CNS, initiating the descending pain inhibitory pathway [460, 461].

There are five groups of opioid receptors, namely the mu receptor (MOR), kappa receptor (KOR), delta receptor (DOR), nociception receptor (NOR) and zeta receptor (ZOR);[462]. Except for ZOR, each opioid receptor is involved in the inhibition of ascending pain signals upon binding to an opioid agonist [462]. To date, evidence is strong and consistent in supporting the mu-receptor as the main opioid receptor for analgesic response, as the majority of opioids utilized in clinical settings act upon it [460, 461]. Furthermore, in this complex pain inhibitory pathway, the activation of opioid receptors is not an isolated process; it involves interactions with other biological systems, like the dopaminergic system [463, 464]. Through the engagement of other systems, genes like the cannabinoid receptor 1 (*CNR1*) and the Catechol-O-methyl transferase gene (*COMT*), can modulate pain perception**.** *CNR1* is co-localised with *OPRM1* and mediates binding sensitivity, whereas *COMT* is responsible for the bioavailability of catecholamines like dopamine [463, 465].

It has been shown that opioid binding activates a cascade of reactions that leads to the activation of the dopamine pathway seen in **Figure 1.15**, and pain relief which is linked to the reward and addition processes in the brain [466, 467]. This mechanism can have a feedback loop effect in which using opioids more frequently results in higher sensations of reward and ceasing use causes severe withdrawal, promoting continuous opioid use [466, 467]. Dopamine levels are controlled by *COMT* activity, and it is evident how this activity influences pain perception by controlling reward responses that are triggered by opioid-induced dopamine activation.

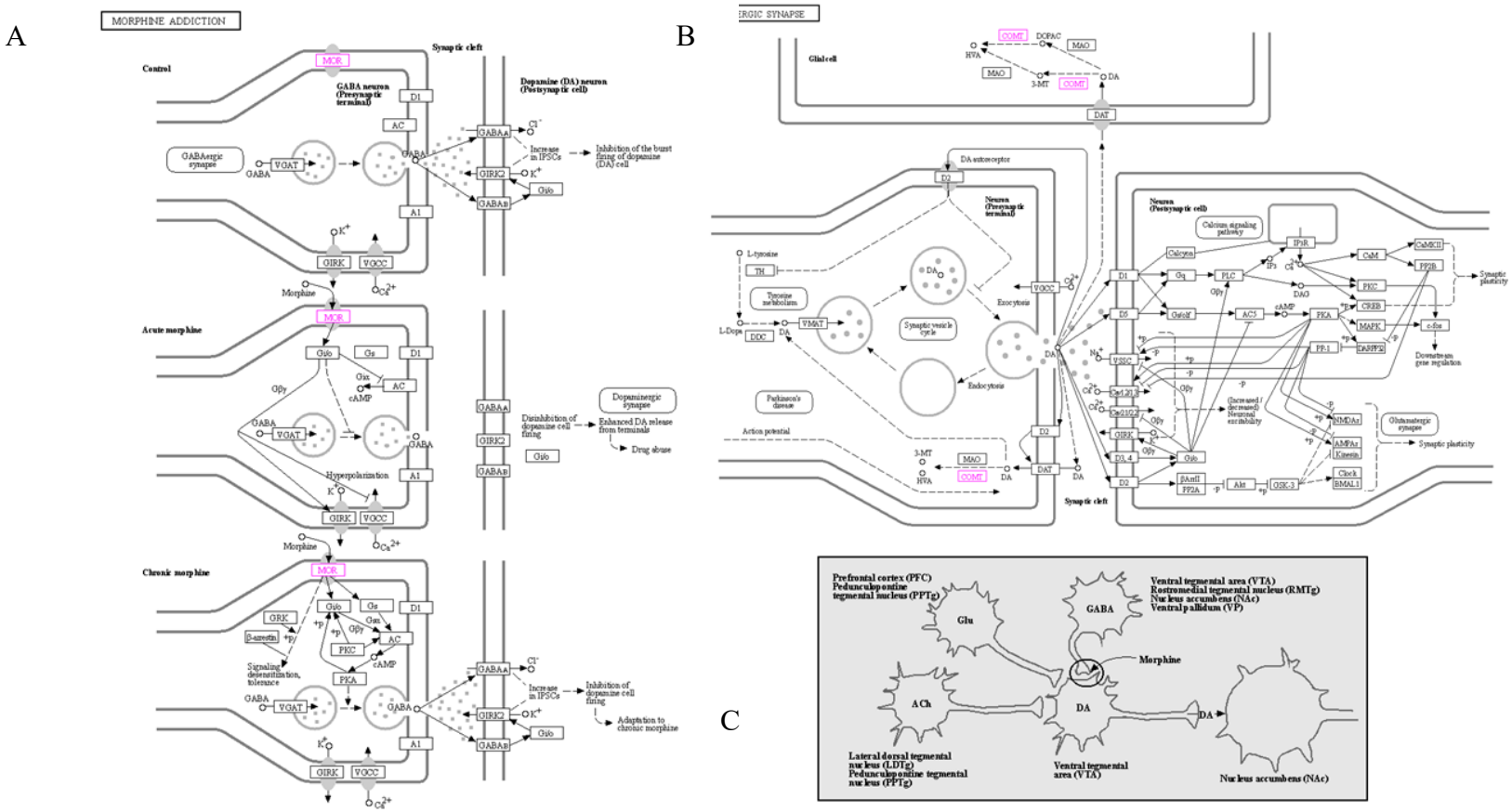


Figure 1.15: A snapshot visualization of the interacting biological systems implicated in the pain pathway. Briefly, the illustration shows the A) effects of opioid (morphine) binding at the synaptic left to *OPRM1* receptors and setting off a cascade of events that includes the release of GABA and inhibition of Dopamine, B) the life cycle of activation of dopamine including enzymatic catalysis by *COMT* and *MOA* enzymes, and C) anatomical location of downstream effects. The illustration was obtained from the KEGG Pathway Database (KEGG PATHWAY Database, accessed on 25 October 2023).

Three genes are functioning in the opioid signalling pathways which have consistently been implicated in chronic pain conditions and the primary focus of this thesis [464, 468-471]. These are:

- *ABCB1*: for the distribution and transportation of opioids to key brain regions,
- *OPRM1*: for receptor signalling at synapses, and
- *COMT:* for the indirect modulation of the inhibitory pathway.

1.4.1 The ATP-Binding Cassette- Subfamily B, Member 1 Gene (*ABCB1*)

The adenosine triphosphates (ATP)-binding cassette (ABC) family are known efflux transporters with broad substrate specificity that operate in an ATP-dependent manner [472, 473]. There are 48 putative ABC transporters identified in the human genome that are subdivided into seven classes (A-G) based on genomic organisation [474, 475]. ABC transporters are ubiquitous integral membrane proteins that have a basic structure of four core domains and are driven by ATP hydrolysis to translocate substrates in and out of cells [476]. The *ABCB1* gene is one of the many abundant ABC-subfamily B genes, a family of highly conserved membrane transporters that are crucial and actively participate in numerous physiological and cellular processes [477, 478]. It was the first transporter identified for ABC transporters, and since has become the most frequently investigated and well-described gene for this superfamily [479-481].

The *ABCB1* gene has been implicated in a variety of conditions due to its ubiquitous nature in therapeutic function and is associated with cancer [482-484], renal [485] autoimmune [486], mood [487], neurodegenerative [488, 489] studies and more [478]. The gene is highly polymorphic with more than fifty clinically relevant polymorphisms that have been associated with various outcomes like opioid addiction, drug resistance to chemotherapy as well as pain perception [469, 471, 475, 490-496]. Early expression studies in healthy volunteers found in exon 26, the rs1045642 G>A polymorphism was associated with differential expression of the P-gp protein in vivo [497]. It was reported that A/A genotype carriers exhibited lower P-gp levels compared to the A/G and G/G carriers. Several other polymorphisms have also been identified with high allele frequencies (>10%), particularly at exon/position 12/1236, and intron/position 6/-139, 17/+137, with the former corresponding to rs1128503 (G> 1236); [497]. Summarised in

Table 1.3 are three of the most widely studied *ABCB1* SNPs, which have been associated with altered protein activity and have been implicated in pain and opioid-related studies [498, 499].

Table 1.3: Single Nucleotide Polymorphisms (SNPs) in *ABCB1* associated with pain studies - Comprehensive summary of genetic variations with their respective rsIDs, genomic positions, and minor allele frequency.

Polymorphism	Alleles (5'-3')	MAF	Genomic Position	Amino acid change	Exon/Intron
rs1128503	G>A	A=0.4161	1236	Gly > Gly	Exon 12
rs2032582	G>T/A	A=0.2586	2677	Ala > Ser/Thr	Exon 21
rs1045642	G>A	A=0.3952	3435	Ile > Ile	Exon 26

MAF: Minor Allele Frequency as the 1000Genomes Project; Abbreviations: Ala, Alanine; Ser, Serine; Thr, Threonine; Gly, Glycine; Ile, Isoleucine

Characterisation of the rs1128503 and rs104562 SNPS found each SNP were located at a wobble position, specifically in the cytoplasmic loop flanking the first ATP-binding domain, and at the secondary intracellular ATP-binding domains, respectively [474, 500, 501]. Whereas the rs2032528 SNP is located in the cytoplasmic loop between transmembrane domains (TMD) 10 and 11 [501]. Populations studies have reported that minor allele frequencies for rs1128503 and rs1045642 were <30% in African or African American groups, [502, 503]. Whereas the major alleles in populations such as Asia, Malaysia, Japan, and Europe all reported frequencies of >60% [502, 503]. On the other hand, minor allele frequencies for the rs2032528 polymorphism were <10% in all populations for the A allele and ranged between 5% and 60% for the T allele [502]. It is hypothesised that these three polymorphisms are co-inherited and linkage disequilibrium (LD) analysis in varying populations found a strong linkage between rs1128503 C>T, rs2032582 G>T, and rs1045642 C>T.

Pairwise LD analyses between the SNPs rs1128503 and rs2032528 noted moderate to strong D' values of 0.6, 0.8 and 0.9 in populations of Polish (n=95), Caucasian with Chinese (n=107), and Jewish (n=98) groups [490, 504, 505]. Evaluation of the SNP pair, rs2032528-rs1045642 noted low to moderate D' values like 0.3, 0.6, 0.8 in the Polish, Caucasian-Chinese mix, and Chinese (n=292) and Jewish groups [487, 490, 504, 505]. Moreover, evaluation of the rs1128503-rs1045642 SNP pair noted D' values that were weak to moderate, ranging from 0.4 to 0.8 for all the populations noted above [490, 504, 505]. In contrast, one study conducted in the USA (n=698) reported no linkage between the rs1128503 and rs1045642 SNP pair [506]. In addition, evaluation of all three SNPs in a Bangladeshi (n=180) cohort, noted very weak LD patterns (D'<0.5); [507]. Still, the three SNPs, have consistently been examined in haplotype form, for instance, one study reported that the *ABCB1* (rs1128503 G>A- rs2032582 G>A- rs1045642 G>A) A-A-A haplotype was associated with greater opioid requirements in a Jewish cohort (n = 98); [490]. Whereas an Australian cohort (n=120) reported that wild-type haplotype carriers, G-G-G, required more opioids [508].

Many studies have placed focus on the rs1045642 loci due to the functional impairment associated with it. Reports have indicated that (rs2032582 C>T- rs1045642 C>T) C/T genotype carriers were associated with increased opioid addiction [509], while others did not [510, 511]. In cancer pain-related studies, reports have shown that rs1045642 T/T genotype carriers required more opioids for pain relief [512]. While examining the effects of fentanyl, another study reported that the rs1045642 SNP had no impact, and instead noted that rs1128503 T/T genotype carriers required fewer opioids [513]. Furthermore, postoperative studies have also shown that rs1045642 T/T genotype/allele carriers required fewer postoperative opioids and reported lower pain scores than the wild-type genotypes [469, 494, 496, 514-517]. However, inconsistencies amongst other post-operative studies have been reported [22, 518].

The *ABCB1* gene, also known as the multidrug resistance gene (*MDR1*), is located on chromosome 7q21.12 [NCBI, Genome browser, November 2021, Primary Assembly (GRCh38.p13)]; [499, 504, 519]. It consists of 28 exons spanning 120 kilobases and has two promoters, P1 and P2 [499, 520]. Translation of *ABCB1* begins in exon two, producing a protein-coding sequence made up of 27 exons, with the first 14 exons coding for the first half of the transmembrane domain and the remaining 13 exons for the second half. *ABCB1* transcription is regulated by various elements, including a GC-rich region, an initiator element (Inr), and other transcription regulatory elements [520, 521]. *ABCB1* transcribes into a 4872bp messenger RNA (mRNA) that includes the 5'untranslated region and translates into 1280 amino acids, the P-glycoprotein [478].

The P-glycoprotein (P-gp) protein consists of two homologous halves containing six transmembrane segments [transmembrane domains (TMD)] and one intracellular segment [nucleotide binding domains (NBD)] and each with an N- and C- termini that are located inside the cell. Three glycosylation sites can be found in the first loop connecting TMD segments one and two. The NBDs are ATP-utilizing regions that facilitate the opening and closing of the channel, and recent studies found two major substrate binding sites in TMD 5-6 and TMD 11-12 and reported the two NBDs interact via a flexible linker region [499, 522, 523]. The basic mechanisms of ATP-dependent transportation of substrates across the membrane involve the hydrolysis of ATP to ADP and free phosphate ions, activating the transportation of substrates across membranes. Additionally, P-gp is subject to post-translational modifications that are crucial for its stability, function, degradation, and activity. These modifications include glycosylation, phosphorylation, and ubiquitination [524]. Glycosylation occurs at the N-linked glycosylation regions, N91, N94, and N99, in the first extracellular loop of P-gp [523]. Studies revealed changes or mutations of these sites influence protein stability [525]. P-gp has multiple

phosphorylation sites, and particularly in the flexible linker region connecting the two TMD halves, eight consensus sites allow for phosphorylation by the enzyme's protein kinase A and/or C [526, 527]. Ubiquitination which aids stability, function and localisation, is important in protein degradation and can affect drug resistance in cancer treatment [528]. There is also a connection between glycosylation and ubiquitination, where inhibiting glycosylation can increase P-gp degradation through ubiquitination [528].

P-gp is a 170 kilodalton (kDa) single polypeptide chain that has been identified in a variety of epithelial tissues like liver tissue, kidneys, intestinal tissue, pancreas, adrenal glands, capillary endothelium of the blood-testis and blood-brain barrier, choroid plexus, placental trophoblast, and many others [474, 523, 529-533]. The physiological role described for P-gp is in maintaining cellular homeostasis, protecting against cell toxicity and the transport of various hormones, cytokines, and lipids [534, 535]. In the adrenal cortex and medulla, P-gp's play a role in either i) facilitating the transport of steroids or ii) serving as a protective mechanism in response to elevated steroid levels [500]. The protein interacts with three different types of substances, inducers, inhibitors, and substrates. Substrates are compounds that are transported across the membrane via P-gp activity, whereas inducers and inhibitors will promote or block P-gp transportation [536, 537]. Some of the substrates transported by P-gp include corticosteroids, cortisol, corticosterone, and aldosterone [478, 534, 538, 539]. In cancer research, the co-administration of inhibitors is suggested to potentially enhance the bioavailability of chemotherapy agents within cancer cells by inhibiting P-gp activity [540]. Although there are several other membrane transporters, P-gp remains one of the most significant transporters to protect the brain against harmful drugs/toxins [530]. In the context of this thesis, the function of P-gp in the BBB is especially important concerning the distribution of xenobiotics.

1.4.2 The Opioid Receptor, Mu 1 Gene (*OPRM1*)

The Mu-Opioid receptor is a member of the opioid receptors, a group of seven transmembrane spanning (7TM) Guanine protein-coupled receptors (GPCRs); [541]. This prominent superfamily of rhodopsin-like GPCRs is regarded as the most significant contributor in regulating neurotransmission, and hormonal action at the CNS level, as well as sensory perception and primary targets for opioid signalling [460, 462, 541, 542]. Since the discovery of these receptors in the 1970s, five types have been identified and summarised in **Table 1.4**, namely mu receptor (MOR), kappa receptor (KOR), delta receptor (DOR), nociception receptor (NOR) and zeta receptor (ZOR); [462, 541, 543]. In addition, sequence homology is highly conserved with MOR, KOR, DOR, and NOR being 60% identical to each other, mainly in the regions transcribing the 7TM and intracellular loops [461]. Although most of the receptors have roles in analgesia, it is the MOR receptor that is described as the primary target for exogenous opioids in the management of moderate to severe pain [544].

Table 1.4: Anatomical location and physiological roles of G protein-coupled receptors in the human body adapted from [462].

GCPR	Gene	Organ system	Physiological action
δ-receptor (DOR-delta)	*OPRD1*	Brain, CNS	Analgesia, Constipation
κ-receptor (KOR-kappa)	*OPRK1*	CNS, PNS	Analgesia, Diuresis, Dysphoria
μ-receptor (MOR-mu)	*OPRM1*	Brain, CNS, GIT, PNS, Spinal Cord	Analgesia, Constipation, Dependence, Euphoria, Miosis, Respiratory depression, vasodilation
nociception receptor (NOR)	*OPRL1* *	CNS	Analgesia, Hyperalgesia (concentration-dependent)
zeta receptor (ZOR)	*OGFR**	Brain, Heart, Kidney, Liver, muscle, pancreas	Regulator of cell development

*Opioid like receptors that are similar in structure; Abbreviations: DOR, delta-opioid receptor; OPRD1, Opioid Delta Receptor 1; CNS, Central Nervous System; KOR, Kappa opioid receptor; - Opioid Kappa Receptor 1 (OPRK1) - Central Nervous System (CNS), PNS, Peripheral Nervous System; MOR, mu-opioid receptor; *OPRM1*, Opioid Mu Receptor 1; GIT, Gastrointestinal Tract; NOR, nociception receptor; OPRL1, Opioid Receptor-Like 1; ZOR, zeta receptor; OGFR, Opioid Growth Factor Receptor

Molecular research identifying polymorphisms describes a mutation resulting in the loss of an N-linked glycosylation site of the receptor, specifically the functional substitution at position 118, which codes a missense change of (A>G) Asparagine (Asn) to Aspartic acid (Asp) in the main transcript MOR-1 [545-547]. Another functional variant rs540825, is reported to be responsible for the missense change of (A>T) Glutamine (Gln) to Histidine (His) that leads to changes in the C-terminus of the splice variant MOR-1X, potentially causing structural and functional protein modifications [548-551]. Pharmacological studies suggest that multiple μ-opioid receptor subtypes could explain the wide variety of sensitivity towards μ-opioids and their side effects as incomplete cross tolerance among μ-opioid receptors occurs and cannot be resigned

to just s single μ-opioid receptor [541, 551]. Additionally, it has been reported that the different isoforms of MOR-1 are altered by exogenous opioids, and MOR-1X specifically are found to facilitate "unique signal transduction with distinct functional consequences" when induced by morphine [552, 553]. Summarised in **Table 1.5** are two functional *OPRM1* SNPs, which have been associated with structural protein changes and have been implicated in pain and opioid-related studies [541, 552, 553]

Table 1.5: Single Nucleotide Polymorphisms (SNPs) in *OPRM1* associated with pain studies - Comprehensive summary of genetic variations with their respective rsids, genomic positions, and minor allele frequency.

Polymorphism Rs ID	Alleles (5'-3')	MAF	Genomic Position	Amino acid change	Exon/Intron
rs1799971	A>G	G=0.2234	118	Asn>Asp	Exon 1
rs540825	A>T	A=0.1178	1839	Gln>His	Intron 3

MAF: Minor Allele Frequency as the 1000Genomes Project; Abbreviations: Asn, Asparagine; Asp, Aspartic acid; Gln, Glutamine; His, Histidine

Genetic studies report a considerable amount of association between the *OPRM1* rs1799971 A>G variant and opioid sensitivity, morphine requirements, sufentanil dose, and postsurgical pain [470, 510, 554-556]. Whereas fewer studies have investigated the rs540825 A>T variant, which has been associated with fentanyl-induced emesis, pain, postoperative morphine consumption, and positive affect [551, 557, 558]. Globally, studies report varying MAF for rs1799971 G, ranging from 4% in African Americans to more than 40% in the Asian populations [512, 559]. It has been noted that European populations report roughly 16% for the minor allele [559]. Whereas reports from South Africa observed very low frequencies (<0.1%); [560].

Focussing on the functional rs1799971 SNP, studies conducted in European and Asian cohorts have shown that G/G allele and genotype carriers, report greater pain and require more therapeutics for pain relief postoperatively [24, 512, 561-563]. In addition, the associations were noted to be more prevalent in women than men [562, 564, 565]. Association studies have indicated a link between rs540825 and fentanyl-induced emesis, and response to antidepressants, however, data reports on postoperative pain are limited [551, 557, 566]. For example, linear regression analysis of seven *OPRM1* markers (rs1319339, rs7776341, rs1799971, rs563649, rs442075572, rs540825, rs4677830), reported that carriers of the haplotype H2 (TA**G**CC**T**G) required more post-operative analgesia (POA) than the reference haplotype H1, and carriers of the H5(CA**A**CT**A**AG) haplotype required less POA [557]. Which is in alignment and supports the above-mentioned studies [24, 512, 561-565]. However, a meta-analysis found rs1799971 was not associated with postoperative pain, mainly due to disparities in sampling, surgical settings and other factors [556]. For example, a Polish cohort (n=207) evaluating knee arthroplasty could not report any associations between rs1799971 and postoperative pain [567].

The MOR receptor is encoded by the Opioid Receptor Mu 1 (*OPRM1*) gene, found on the long arm of chromosome 6 bands q25.2 [NCBI, Genome browser, December 2021, Primary Assembly (GRCh38.p13)]; [568]. There are inconsistencies regarding the exon count for *OPRM1*, whereby Pasternak and Pan (2013)[569] reported 12 exons in comparison to Shabalina *et al.* (2009)[547] reporting 18 exons. According to the OMIM database (Updated to 06/04/2021 and reporting for the latter study), comparative alignment and characterisation of the gene shows the gene consists of 18 exons spanning ~200 kb, which include regulatory elements, promoters, and alternative exons, (5'-3' orientation exons -11, 1, T, 14, 13, 2, 3, R, Y, 16, X, 17, 5, 4, 18, 6, O/7, and 9); [547].

OPRM1 expression is complex and modulated by various factors that include dual promoters, epigenetic factors, and alternative splicing [569]. The human *OPRM1* gene is highly homologous and reported to have dual promoters, the absence of a TATA-box, and a complex TF-binding site [569, 570]. The two promoters described are a distal promoter (E11) located ~30kb upstream from exon 1 which is associated with exon 11, and a proximal (E1) promoter found ~1.5kb upstream and associated with exon 1 [548]. Post-transcriptional studies report several positive and negative transcription factors (TFs) for the *OPRM1* gene, including activator protein1 (AP1) and neuro-restrictive silencer element (NRSE); [570-581]. *OPRM1* expression is also subject to DNA methylation and histone modification, two fundamental epigenetic mechanisms described in mammalian gene expression [582]. DNA methylation involves the addition of a methyl group to the C5-position of a cytosine (form a 5-methylcytosine), thereby modulating expression through inhibition of TFs [583].

The assignment of methyl groups to the promoter region for *OPRM1* has shown to be a powerful epigenetic regulator, that correlates to reduced gene expression [582, 584, 585]. Additionally, there are several Cp-G sites (>20) described in the *OPRM1* promoter region creating CpG islands that are subject to epigenetic regulations by DNA methylation, and thus modulation of expression [585]. Different types of histone modifications are also described for *OPRM1* in literature, including histone acetylation, methylation, phosphorylation, and ubiquitination, all known to regulate DNA chromatin structure and transcription [586-591]. For example, one study found demethylation of the lysine-9 residue at the core histone H3, resulted in the repression of gene expression [461, 592, 593]. Through alterations in the histone complexes, this epigenetic mechanism directly influences gene expression for *OPRM1*.

The concept of one gene encoding one protein has been challenged, as over 95% of the human genome undergoes constitutive splicing with alternative splicing having shown associations between genes and diseases [594-605]. During transcription, precursor mRNA is transcribed, removing intronic regions and connecting exons to create mature mRNA, however, alternative splicing can lead to the removal or retention of exonic/intronic regions [605-607]. Many GPCRs, including *OPRM1*, undergo alternative splicing to maintain protein diversity, with *OPRM1*'s alternative splicing being unique compared to *OPRK1* and *OPRD1* [606, 608-610]. *OPRM1* has three distinct protein structures: full-length 7 transmembrane domain (TM) C-terminal variants, truncated 6TM variants, and truncated single TM variants, with multiple splicing variants within each subtype (**Figure 1.16**); [569, 605, 611]. Furthermore, *OPRM1*'s alternative splicing is highly conserved between humans and rodents [605].

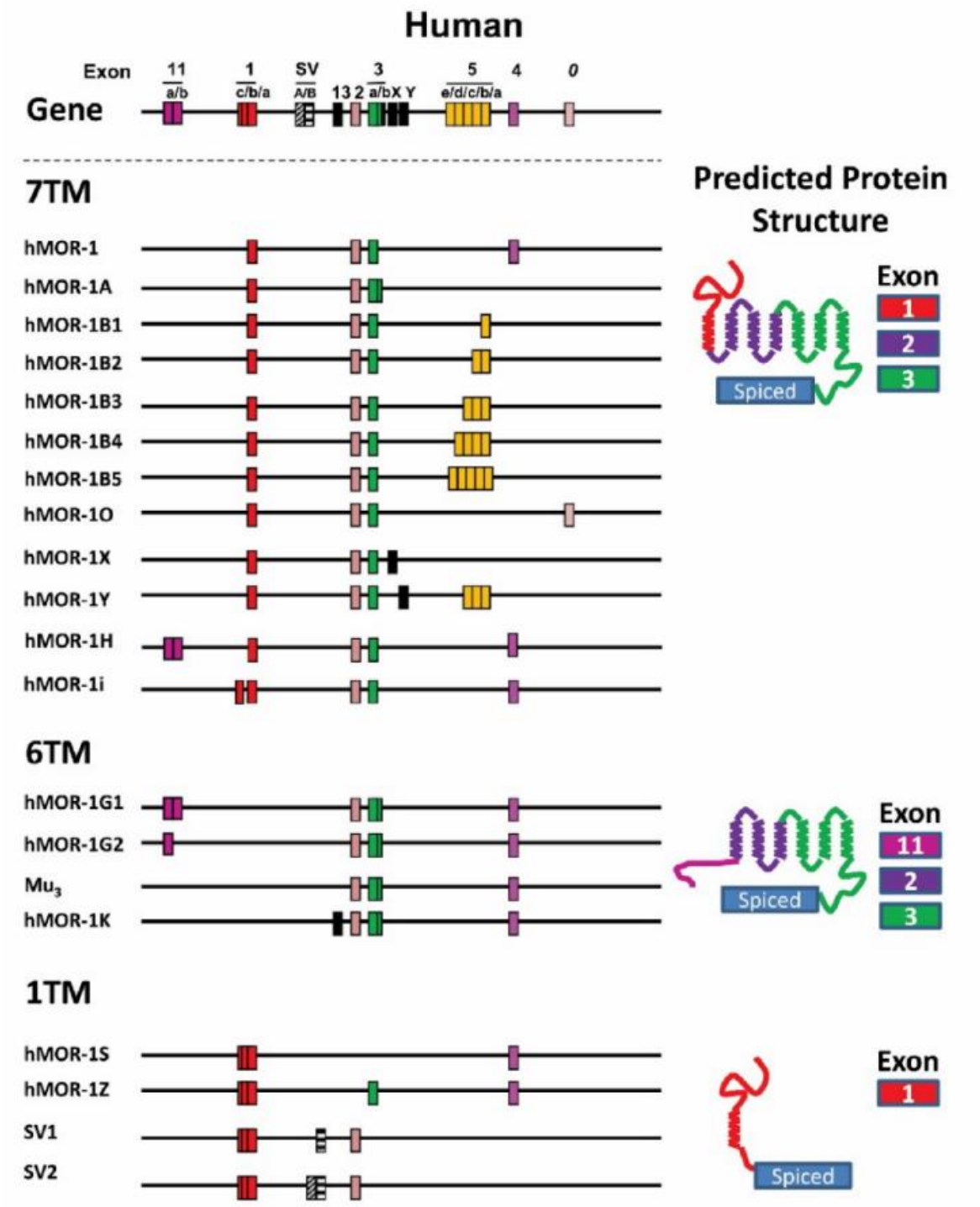


Figure 1.16: Three human MOR splicing variants obtained from Pasternak and Pan (2013)[569].

The 7TM C-terminal variant is the result of the 3` splicing of the *OPRM1* gene starting at exon three, each presenting with an N-terminus, a 7TM domain, three intracellular and extracellular loops, and a part of the intracellular C-terminus [605]. Up to twelve full-length variants have been isolated from humans, each presenting with different amino acid sequence lengths [569, 605, 612, 613]; (**Table 1.6**).

Table 1.6: Alternative protein sequences encoded by the different 7tm variants resulting from 3' splicing at exon 3 for *OPRM1*, adapted from [605].

Variant	Exons	Amino acid sequence
hMOR-1	4	LENLEAE*T*APLP
hMOR-1A	3b	VRSL
hMOR-1B1	5a	KIDLFQKS*S*LLNCE
hMOR-1B2	5ab	RERRQK*S*DW
hMOR-1B3	5abc	GPPAKFVADQLAG
hMOR-1B4	5abcd	S
hMOR-1B5	5abcde	VELNLDCHCENAKPWPLS*Y*NAG
hMOR-1O	O	PPLAVSMAQIFTRY*S* PP*T*HRE KTCNDYMKR
hMOR-1X	X	CLPIPSLSCWALEHGCLVVY PGPLQGPLVRYDL PAILHSSCLRGNTAPSPSGGAFLLS
hMOR-1Y	Y/5abc	IRDPISNLPRV*S*VF
hMOR-1i	4	LENLEAE*T*APLP

Predicted phosphorylation sites within the amino acid sequences are noted in green italics. Predicted phosphorylation codes are underlined and highlighted. Adapted from [605].

The 6TM and 1TM variants, on the other hand, are the result of early splicing and each generates four different variants [569]. Exon 11, which is around 30 kb upstream of exon 1 and under the control of its promoter region, is where splicing for the 6TM variants occurs [569]. By skipping exon 1, splicing generates 6TM variants with varied C-terminal ends [611]. While alternative splicing at exon 1, bypassing exon 2 results in frameshifts and varied amino acid sequence lengths of intracellular tails downstream of the single TM [569, 611]. Although these variants differ significantly in TM length and therefore functionality, these variants remain pharmacologically important [605, 611]. One study showed that 1TM variants have a distinct chaperoning role, increasing the expression of 7TM variants in the endoplasmic reticulum [614, 615].

Relating to the present work, the role of MOR associated with analgesia and pain modulation within the PNS and CNS is well described as the receptor is widely expressed throughout [616, 617]. The mu-opioid receptor (MOR-the primary isoform), a 45kDA protein, comprised of 400 amino acids is a surface receptor localised to the outer and inner membrane of a cell with the N- and C- terminal respectively [574, 618]. Opioid receptors are synthesised in the dorsal root ganglia (DRG), whereby the μ-, δ-, and κ-opioid mRNA and proteins are expressed within cell bodies of DRG neurons that are subsequently distributed peripherally and centrally to nerve terminals [616, 619]. In the PNS, opioid receptors are present in both neuronal and non-neuronal tissues of endocrine, immune, smooth muscles, and ectodermal cells and also in the enteric nervous system of the gastrointestinal tract (GIT); [460, 619-628]. In the brain, MOR expression under normal physiological conditions is primarily confined to the CNS, specifically the Hypothalamic Pituitary Adrenal axis (HPA); [461, 570]. Moreover, MOR splice variant expression profiles are region- and strain-specific, to brain regions such as the prefrontal cortex, thalamus, striatum, and others [605, 611, 629, 630].

MOR receptors are involved in a variety of physiological processes that include pain modulation, motility, mood, diuresis, thermoregulation and stress, as well as the modulating role in the respiratory, gastrointestinal and cardiovascular systems [631]. In instances of tissue damage, the ascending pathway transmits nociception signals, perceiving the sensation as pain. G-protein dissociation reduces internal cyclic adenosine monophosphate (cAMP), which, in turn, decreases substance P release through voltage-gated Ca^{2+} channel reduction, contributing to analgesia during exogenous opioid use [632]. Whereas endogenous opioids, distributed throughout the PNS and CNS, play a pivotal role in modulating nociception, mood, and cardiovascular functions via MOR activation. [620, 632-635].

Unlike, *ABCB1*, the MOR- receptor does not transport substrates but binds to a ligand that can be endogenous or exogenous that will activate a physiological response through various cellular changes [632]. Exogenous ligands may be an agonist or antagonist, with the former activating a reaction, and the latter having an inhibitory effect [632, 636]. There are four groups of endogenous ligands (**Figure 1.17**), also known as endogenous opioids, namely β-endorphins, enkephalins, dynorphins, and nociceptin/orphanin FQ [637]. Studies report each of these opioids is broadly expressed throughout neural circuits, including those relating to pain [637].

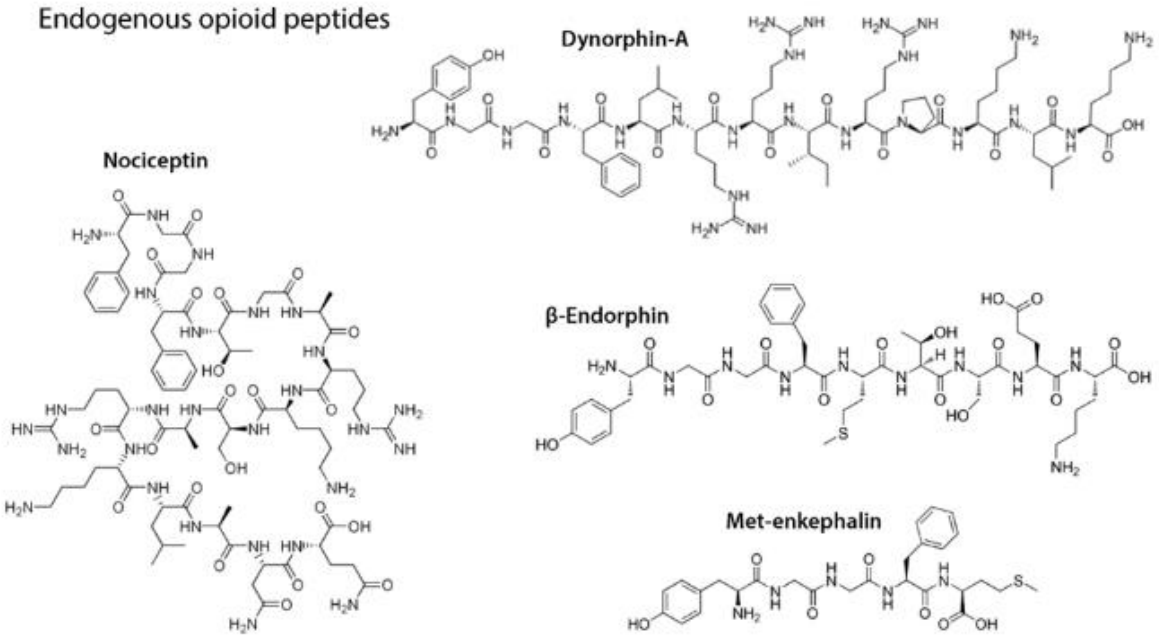


Figure 1.17: Chemical structure for the endogenous opioids, Dynorphin, Endorphin, Enkephalin and Nociceptin. Each opioid-containing the Tyr-Gly-Gly-Phe-Leu or Tyr-Gly-Gly-Phe-Met sequences at the N-terminus. Adapted from Corder *et al.* (2018)[637].

Pharmacological research has led to the synthesis of numerous agonists, that induce varying efficiencies through variable degrees of hyperpolarisation at the cellular level of MOR receptors, including tramadol, methadone, meperidine and fentanyl [632, 638-640]. These exogenous opioid compounds isolated from the poppy opium plant, have been used for centuries particularly in rituals to induce euphoria, before the isolation and identification of endogenous opioids [641, 642].

Amongst the many opioid compounds isolated from the opium plant is morphine, a compound that is heavily relied on for pain relief [569, 628, 643]. Furthermore, opioid antagonists are another class of compounds that elicit a counter-response to opioid agonists that are essential for mitigating opioid-induced side effects, including respiratory depression [644-646]. Several antagonists, such as naloxone, naltrexone, methylnaltrexone, and others have been developed, and while some may have dual action (agonist and antagonist), classifying them as partial or mixed antagonists [645, 647, 648]. In the context of this study, given its role in opioid signalling, *OPRM1* is vital and therefore may account for the variability in pain responses observed in chronic post-operative pain phenotypes.

1.4.3 The Catechol-O-Methyl Transferase Gene (*COMT*)

Methyltransferase (MTs) are enzymes that facilitate chemical reactions through methylation and are key in several biological processes like modulating gene expression, cell signalling, metabolism and others [649-652]. As one of the most significant conjugative enzyme groups, MTs promote O-methylation through the exchange of the methyl group, S-adenosyl-l-methionine (SAM), to a hydroxyl-containing substrate in a Mg^{2+} rich environment [653-656]. The Catechol-O-methyltransferase (*COMT*) gene translates to an enzyme that is responsible for the bioavailability of catecholamines and is one of the most widely studied O-methyltransferases [649].

Studies have shown the *COMT* polymorphisms listed in **Table 1.7**, are associated with chronic and/or persistent pain states [23, 26, 562, 657, 658]. The key source of variation in *COMT* activity, is the functional *COMT* rs4680 G>A polymorphism found on exon four at positions *S- COMT*[108], and *MB- COMT* [158] with a valine (*Val*) to methionine (*Met*) substitution [653, 659, 660]. This substitution is reported to cause a decrease in enzyme thermostability and activity due to the lesser hydrophobic *Met/Met* residues accounting for ~40% activity difference [653, 659, 661-663]. Three other polymorphisms, *COMT* rs6269 A>G (*intron 2*), rs4633 C>T (*His>His, exon 3*) and rs4818 C>G (*Leu >Leu, exon 4*), have also been investigated in haplotype studies with rs4680 G>A, with strong LD (D'> 0.94) reported between the four polymorphisms [21, 664].

Table 1.7: Single Nucleotide Polymorphisms (SNPs) in *COMT* associated with pain studies - a comprehensive summary of genetic variations with their respective rsIDs, genomic positions, and minor allele frequency.

Polymorphism rs ID	Alleles (5'-3')	MAF	Genomic Position	Amino acid change	Exon/Intron
rs6269	A>G	G=0.3568	-	-	Intron 2
rs4633	C>T	T=0.3716	62	His>His	Exon 3
rs4818	C>G	G=0.2969	86	Leu>Leu	Exon 4
rs4680	G>A	A=0.3692	158	Val>Met	Exon 4

MAF: Minor Allele Frequency as the 1000Genomes Project; Abbreviations: His, Histidine Leu, Leucine; Met, Methionine; Val, Valine

Many studies implicating the functional SNP rs4680 were observed in neurological and behavioural cohorts investigating Schizophrenia, Parkinson's, mood and affect [665-668]. Reporting that the Val/Val genotype was associated with increasing psychotic symptoms and poorer response to treatments, independently and in combination with the other SNPs listed. In the context of this thesis, pain perception, cancer pain, post-operative pain, and opioid-related pain studies have also implicated this variant although the data may vary by cohort. For example, reports showed the rs4680 A/A genotype and the minor allele haplotype for rs6269-rs4633-rs4848-rs4680, were associated with higher pain, delayed recovery, and increased postoperative analgesia, respectively [452, 669]. Furthermore, the studies had a consistent ethnic background with participants primarily being of Caucasian ethnicity [452, 669]. However, the cohorts differ in surgical settings where one study focused on a postoperative adenotonsillectomy, and the other examined the musculoskeletal condition of lower back disc herniation.

Interestingly, the opposite is reported for this variant in cancer pain. A population study from Italy (n=87) and China (n=146) reported that G/G genotype carriers were reporting greater perceived pain and required higher doses of opioids compared to the alternative genotypes [471, 670]. The disparity between cohorts highlights the importance of the characterisation of the pain pathways activated post-operatively compared to other pain phenotypes.

Focusing on post-operative and musculoskeletal pain cohorts, systematic analysis of the minor rs4680 A allele is reportedly associated with pain intensity in fibromyalgia and chronic widespread pain [671-673]. Reports have also shown that the rs4680 A/A genotype is associated with increasing POA post-3rd-Molar extractions [23]. Interestingly, a recent report showed that A allele carriers reported greater musculoskeletal pain response to electroacupuncture in BCS [674]. The mechanism proposed in this study suggests that acupuncture may activate the release of neurotransmitters that may inhibit pain signals through the dopaminergic and adrenergic systems having an impact on endogenous opioids acting on opioid receptors. However, negative or the absence of an association for the functional rs4689 SNP in experimental studies, has been suggested to be due to a larger genetic interval contributing to the variability in pain perception [671].

For rs6269 A>G, it has been reported that G/G genotype was more prevalent in conditioning pain modulation (Caucasian n=77) subjects, and protective against disability in lower back pain (European n=371); [675, 676]. The G/G genotype has also been associated with a reduced need for POA (Irish n=100) along with the G/G genotype for rs4818 C>G [23]. While the G/A genotype has been associated with postoperative pain and disability (Australian n=140); [677]. Another study reported the rs4633 C>T and rs4680 G>A, T/T and A/A genotypes were

associated with less morphine required post-operatively for a hysterectomy (Chinese n=755, Malay n=136, Indian n= 82); [678]. In the same cohort, the data also showed the rs4818 C>G G/G genotype was associated with increasing post-operative morphine and higher postoperative pain [678].

Defining one haploblock, these four polymorphisms have been used to characterize three major levels of pain sensitivity, low (LPS: GCGG), average (APS: ATCA) and high (HPS: ACCG) [679, 680]. Reports have shown that the LPS, APS and HPS haplotypes have altered secondary mRNA structures, and thereby different folding potential, and enzymatic activity [659]. These three haplotypes reportedly account for 11- to 25-fold differences in *COMT* activity and as a result may surpass the functional significance associated with individual polymorphisms. [21, 659, 664]. In particular, in chronic pain conditions, differences in pain sensitivity have been associated with specific haplotypes of the *COMT* gene variants [21, 659, 681-686]. For example, in a musculoskeletal pain study, independent associations were observed where the rs6269 G, rs4818 G and rs4680 A alleles were protective in a Spanish group [687]. In addition, the ACCG haplotype bearing the rs6269 A, rs4633 C and the rs4818-rs4680 G alleles were associated with more severe pain in FM (Korean n=832, Spanish n=57); [687, 688]. Another study examining opioid consumption found no significant association for any of the four independent SNPs, however, haplotype analysis revealed a signification association for the HSP haplotype [689]. It was noted that ACCG carriers received and required significantly more post-operative fentanyl for a radical gastrectomy (Chinese n=115); [689]. The studies described have shown reports of associations that are variable in their findings for each of the four SNPs, with disparities across the surgical settings and pain types. Additionally, these studies report limited to adequate cohort sizes, with very homogenous ethnic ancestries.

The *COMT* gene is located on chromosome 22 coordinated to the long arm q11.21, and is comprised of two and four, non-coding and coding exons, respectively [690]. The single gene encodes for two isoforms, a water-soluble (*S- COMT*) and a membrane-bound (*MB- COMT*) form, each transcribed and regulated by two distinct promotors, P1 and P2 respectively [653, 690-692]. Each isoform has its initiation start codon (ATG) within exon 3 and with the promoters P1 at exon 3, and P2 upstream of exon 1 [690]. The gene is 27.22kb in length and has several putative binding sites for the factors Sp1, AP-2, NF-D and Ets-1 [690, 693-697]. The isoforms, S-*COMT* and MB-*COMT* are transcribed to 1.3kb and 1.5kb in mRNA length, respectively [661, 690]. Although both transcripts are distributed through the body, S-*COMT* is highly expressed in the liver, kidney, heart, lung, intestinal tract, reproductive organs, gland, muscle, adipose tissue, skin and red blood cells [653, 661, 698]. MB-*COMT* on the other hand, are shown to be highly expressed in the brain and especially in regions relating to the dopaminergic and noradrenergic systems [653].

COMT gene expression, like other genes, is regulated post-translationally, and functional studies have reported multiple CpG sites within the *COMT* promoter region that can significantly impact expression [699-701]. Once such studies have shown that hypermethylation of these sites within MB-*COMT*, significantly correlated with hyperexpression of *COMT*, and further to this, have been implicated in endometrial cancer, breast cancer and nicotine disorders [699, 701, 702]. Although data is limited, *COMT* is also subject to phosphorylation proteome mapping studies reveal compartment-specific phosphorylation sites within S-*COMT*, particularly in the C-terminal [703, 704]. The author indicates that the expression of the soluble form may be modulated by the subcellar localization. Murine studies on the other hand, having the same phosphorylating site S261 as noted in humans, have reported altered levels of phosphorylated *COMT* in placental and foetal livers [704, 705]. The study highlights the effects of dysregulated *COMT* activity on the clearance of catecholamines.

The S-*COMT* isoform, the smallest of the two, encodes for a 221 amino acid protein with a 24.4kDa molecular mass and is limited to the cytosol and nuclei, sharing 80% sequence similarity to that of the rat [661, 706]. The MB-*COMT* encodes the longer sequence with 271 amino acids, weighing 30kDA, and is mainly associated with residing in the endoplasmic reticulum [661, 706]. The enzyme is expressed throughout the body in a variety of tissues like the mammillary and adrenal glands, in organs like livers, kidneys, and even in dental pulp [654, 698, 707-712]. In the brain, however, is where *COMT* expression is mostly studied, particularly in the prefrontal cortex [712, 713]. Reports indicate that the S-*COMT* enzyme is predominantly expressed in peripheral tissues, while expression of the MB-*COMT* enzyme predominates in brain tissues [653, 661, 692, 712]. Crystallization examinations report the *COMT* enzyme consists of eight alpha-helices and seven beta sheets, creating what is known as the shallow catechol-binding pocket bearing the SAM-binding site [653, 714, 715]. Although similar in structure, the additional 50 amino acids present on the N-terminal allow the MB-*COMT* isoform to remain embedded through a linker sequence, resulting in the exposed C-terminal catalytic domain in the extracellular space [711, 716]. The enzymatic mechanism described for *COMT* involves the methylation of catecholamine substrates through a series of steps; substrates bind to the enzyme's active site along with the cofactor SAM, followed by the transfer of a methyl group (SAM -> substrate), resulting in a methylated product [649, 717]. Subsequently, the product is released, SAM is regenerated, and the enzyme is reset for subsequent reactions [649, 717].

The enzyme regulates the bioavailability of the catechols, such as dopamine (DA), epinephrine (EP), norepinephrine (NEP), as well as catechol oestrogens (ER); [718]. These catecholamines are known to act as both neurotransmitters and hormones to maintain the balance within the autonomic nervous system (ANS), and physiologically, are responsible for the ”fight or flight” response [719]. Studies describe varying levels of catecholamines (excess/scarcity), such as in the

case of altered *COMT* activity, which may lead to the over/under-activation of the sympathetic nervous system (SNS); [719, 720]. Concerning pain, the SNS and pain are understood to interact within the neuro-axis and therefore implicate *COMT* enzymatic activity, particularly since altered levels of catecholamines are shown to result in persistent pain conditions [21, 657, 721-723]. Regarding this thesis, the functioning of *COMT* in pain modulation is complex but important to understanding pain mechanisms and chronicity.

This thesis acknowledges that the three specific genes, *ABCB1*, *OPRM1*, and *COMT*, play crucial roles in the opioid signalling pathway and have been individually studied in the context of various conditions including pain. However, the literature also emphasizes that complex conditions often involve multiple interacting factors. It is therefore important to explore how these three genes potentially interact with each other within the framework of opioid signalling and their influence on the development of chronic shoulder pain and disability.

1.5 GENE-GENE INTERACTION BETWEEN OPIOID SIGNALLING PATHWAY GENES

Considering the complex relationship between genes and pain; the role of multiple gene interactions has been advocated for, in the aetiology of chronic pain development [554, 680, 724]. Many genetic association studies determine and report on independent associations by allele, genotype, and haplotype for specific loci within candidate genes. Another evolving research approach is the stepwise incorporation of genetic loci within functionally associated genes located on different chromosomes and evaluating the effects of the specific loci while considering one another [554, 725]. With the primary aim of understanding how significant genetic markers/SNPs at various loci that function within the same biological pathway, might influence a complex phenotype or disease status [680, 726]. This research approach has been well used and described in various conditions including cardiovascular disease, cancer, neurodegenerative disease, as well as pain-related conditions [554, 557, 680, 727-733]. Moreover, these studies indicate that gene-gene interactions may exercise an increased, decreased or zero effect on the specific phenotype of interest.

To the best of the author's knowledge, no studies have yet explored the role of SNPs and the gene-gene interactions between *ABCB1*, *OPRM1* and *COMT*, in the development of chronic shoulder pain following surgery for breast cancer with a mixed ancestry background. In a 2012 study, the authors investigated the relationship between four *ABCB1* markers (rs9282564, rs1128503, rs2032582 and rs1045642), the *OPRM1* rs1799971 variant, and methadone concentration. The study found that among wild-type rs1799971 carriers, those with heterozygous and homozygous AGCTT (REV; TCGAA) haplotypes observed lower methadone dosage and plasma concentrations than those who had *ABCB1* wild-type haplotypes. The authors concluded that *ABCB1* and *OPRM1* variants were associated with

lower and higher dose requirements, respectively [734]. Whilst in independent genetic associations studies, it has been observed that both *ABCB1* and *OPRM1* have either been implicated or not, or in some cases, the results may implicate one of the two. An example of this can be seen in the study of opioid requirement in a Chinese population, reporting that individuals carrying the *OPRM1* rs1799971 G compared to A and *ABCB1* rs1045642 C compared to T carriers, consumed the most and least number of opioids, respectively [512]. Whereas another study reported no association was observed between *ABCB1* (rs1045642), *OPRM1* (rs1799971) and *COMT* (rs4680) variants and postoperative opioid requirements for a Caucasian population [518]. In addition, in a review conducted by, Choi *et al.* (2017)[735] it was reported that from fifty-one studies, the *OPRM1* rs1799971 A/A genotype was associated with fewer opioid requirements postoperatively, while no association was observed for the *ABCB1* marker.

Gene-gene interactions between *COMT* and *OPRM1* polymorphisms have been explored concerning pain sensitivity in preoperative cancer, gynaecological, postoperative orthopaedic, and general surgery settings [463, 680, 726, 736]. The basis for evaluating these interactions stems from studies reporting that *COMT* rs4680 G>A modulates *OPRM1* expression and receptor binding site availability in different brain structures [463, 736]. The *OPRM1* gene encodes the μ-opioid receptor 1 (MOR1), the primary action site for opioids and consequently influences both endogenous and exogenous analgesic responses [737-739]. Several studies have implicated the *OPRM1* rs1799971 A>G (A118G) polymorphism, the most prevalent polymorphism, in pain variability and response to opioids as it is shown to reduce signal transduction and *OPRM1* expression [468, 680, 740-742].

Studies exploring the interaction between *ABCB1* and *COMT*, or the *ABCB1-OPRM1-COMT* combination, have not been identified. Given the critical roles these three genes play in this pathway independently, and the interactive networking these three have across the pathway; It is important to understand the combined or additive effects the genes may have on the susceptibility of developing pain phenotypes. As described earlier, the literature reports for *ABCB1*, *OPRM1* and *COMT* are predominantly observed in Caucasian populations, but also in highly homogenous cohorts. The cohorts that have been described in this review are also noted to be diverse in their surgical settings with few focusing on postoperative pain conditions, and BCS data being very limited.

1.6 AIMS OF THE STUDY

Reviewing the literature shows that there has been an increasing focus on exploring chronic post-operative pain. Moreover, the emerging research is specifically aimed towards understanding how genes at distinct points along the pain-modulating pathway contribute to the onset of chronic shoulder pain and disability. Currently, there is a paucity of genetic research describing these genes in BCS. Moreover, the mixed ancestry population of the Western Cape Region of South Africa remains an understudied population in the context of BC pain management. The main aims of this thesis were to evaluate the role of single nucleotide polymorphisms (SNPs) in candidate genes forming part of the pain-modulating pathway in a BCS cohort with symptoms of chronic pain and disability using the Shoulder Pain and Disability Index (SPADI), within a SA population of mixed ancestry. Furthermore, the secondary aims included to:

i. Explore an *in-silico* approach to evaluate the potential functional effects of the polymorphisms within the candidate genes on their encoding protein.
ii. Highlight potential biologically relevant pain-related pathways specific to this BCS cohort.

1.6.1 The Objectives Included

i. Describing the prevalence of BCS and frequencies of those reporting symptoms of chronic pain and disability using the Shoulder Pain and Disability Index (SPADI) in the SA cohort of mixed ancestry.

ii. To conduct an exploratory analysis, evaluating the clinical profile of a subset of BCS during a 1-year treatment period relating to opioid administration and patient-reported outcome measures [SPADI, Hospital Anxiety and Depression Scale (HADS) and Positive and Negative Affect (PANAS)]

iii. To conduct a genetic association study evaluating the genotype and allele frequencies of eight prioritised SNPs in three candidate genes: (i) *ABCB1* (rs1045642 G>A; rs1128503 G>A), (ii) *OPRM1* (rs1799971 A>G; rs540825 T>A) and (iii) *COMT* (rs6269 A>G; rs4633 C>T; rs4818 C>G; rs4680 G>A).

iv. Construct haplotypes and allele-allele combinations as a proxy for (i) gene and (ii) gene-gene associations respectively, with chronic pain and disability.

v. Conduct bioinformatic analyses to (i) characterise the potential functional effects of the SNPs investigated, (ii) identify the functional associated networks related to the gene sets explored and (iii) identify the potential protein network partners underlying the pain-related associations identified in this study.

2 CHAPTER TWO: MATERIALS AND METHODS

2.1 INTRODUCTION

This study used clinical data and biological samples collected from a South African (SA) breast cancer survivors (BCS) cohort, previously recruited for a parent study (HREC: 312/2012) that aimed to examine the latent effects of breast cancer treatment.

2.2 STUDY DESIGN

A cross-sectional study (**Figure 2.1**) was conducted in accordance with the 'Strengthening the Reporting of Genetic Association studies' (STREGA) initiative, [743-745]. This initiative is an extension of the 'STrengthening the Reporting of OBservational Studies in Epidemiology' (STROBE) statement seeking to improve the precision of research reporting [746]. For the exploratory analyses, the focus group were new patients starting treatment or patients before commencing treatment for breast cancer. Ethical clearance was provided by the Human Research Ethics Committee of the Faculty of Health Sciences within the University of Cape Town (HREC REF: 312/2012, 125/2017); (Appendix A: Research Documentation). All participants were de-identified and assigned a study number.

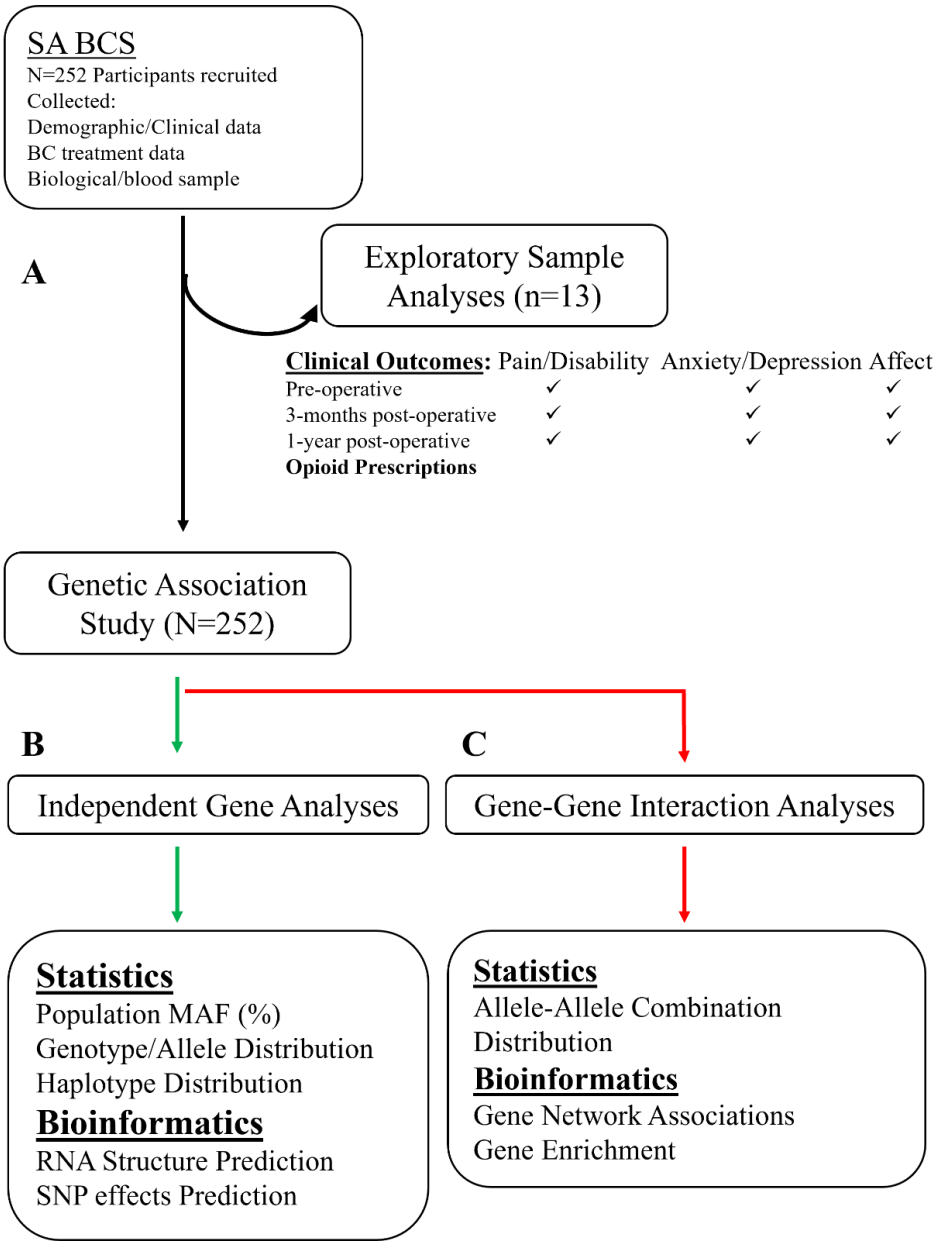


Figure 2.1: Study design. A cohort of N=252 SA BCS was recruited from the tertiary hospital GSH along with all relevant data as well as a blood sample. A) A subset of the study underwent an exploratory sample analysis, used to profile the clinical outcomes of pain, disability, anxiety, depression and affect during the 1-year course of breast cancer treatment. The genetic association study was conducted in two parts utilizing statistical and bioinformatic approaches. These were B) An independent study focusing on the implications of polymorphisms within the candidate genes and C) A gene-gene interaction study focussing on the collective implications of polymorphisms across the candidate genes, relating to the prevalence of chronic pain and disability in SA BCS.

2.3 PARTICIPANTS

Two hundred and fifty-two (N=252) women previously diagnosed with breast cancer were recruited from the Oncology clinic of a tertiary hospital [Groote Schuur Hospital (GSH)] in the Western Cape, South Africa, from August 2013 to June 2019. For the exploratory analysis, a convenience sample of thirteen (n=13) women diagnosed with breast cancer and a full data set, were recruited from GSH prospectively from April 2017 to June 2019. Volunteers were informed of all the procedures within the study and gave written informed consent after an inclusion and exclusion reviewing process. Eligible participants were SA women who self-identified as “coloured” or “mixed ancestry" (**Chapter 1**, **Section 1.2.1**) and were older than 18 years of age at the time of recruitment. The individuals had to be diagnosed with unilateral BC and would have had BC surgery no less than one year before the recruitment process. Non-eligible individuals were excluded if there was any history of shoulder and neck pathology before the BC diagnosis. Individuals with a history of any comorbidities, connective tissue disorders or contralateral shoulder conditions were excluded.

2.4 STUDY PROCEDURES

2.4.1 Demographical and Clinical Data Collection

Through the reviewing of hospital records, a case report form (CRF) was completed containing all relevant demographic and clinical data. The following variables were obtained: participants’ age at the time of surgery, side of primary (left vs right), breast cancer pathology (invasive ductal carcinoma, lymphovascular invasion, tumour grades), the total number of nodes examined and involved, surgery type [mastectomy (MTX) and wide local excision (WLE)], lymph node surgery type [axillary lymph node dissection (ALND) and sentinel lymph node biopsy (SLNB)], and adjuvant breast cancer therapies, for example, chemotherapy (CT). In

addition, the participants also completed a questionnaire that would serve as an outcome measure for pain and disability.

For the exploratory analyses, participants were assessed pre-operatively (T1), at three months (T2) and one year (T3) after surgery for primary outcome measures, a) pain and disability, b) anxiety and depression, and c) positive and negative affect. Participants received self-reported questionnaires and were regularly informed about all subsequent follow-up dates. Furthermore, medical records were screened for all and any prescriptions of opioid drugs during the 1 year.

2.4.2 Blood Sampling

Blood samples were drawn by a registered nurse. Using appropriately labelled ethylene diamine tetra acetic acid (EDTA); (The Scientific Group Pty LTD, Northriding, Randburg, SA) vacutainer tubes, 10ml of venous blood were drawn by venepuncture on the unaffected side. The blood was collected at the hospital site and transported in an icebox for storage at -20^{o}C before Deoxyribonucleic (DNA) extraction, to the Centre for Health, through Physical Activity, Lifestyle and Sports Research (HPALS), Department of Human Biology (HUB), at the University of Cape Town (UCT).

2.4.3 Deoxyribonucleic Acid (DNA) Extraction from Whole Bloods

A modified version of the standard DNA extraction protocol described by Lahiri and Nurnberger (1991)[747] was employed in this study using whole blood samples [748]. Formulations for all buffers used in this study are described in detail including the DNA Extraction Reagents (Appendix B: Outcome Measures)**.** In brief, the EDTA stored blood was transferred to 15ml

polypropylene tubes to which 10ml of TKM1 (10mM Tris-HCl pH 7.6, 10mM KCl, 10mM MgCl2 and 2mM EDTA) buffer was added containing 2.5% of Nonidet P-40. After mixing thoroughly and incubating for 10 minutes at room temperature, the samples were centrifuged at 3000rpm for 10 minutes at room temperature. 5ml of TKM1 (without NP40) buffer was added to the recovered pellet, and shaken until the pellet was dissolved, followed by centrifugation at 3000rpm for 10 minutes at room temperature. This was done repeatedly until the pellet appeared off-white to clear in colour to which 800μl of TKM2 (10mM Tris-HCl pH 7.6, 10mM KCl, 10mM MgCl2, 0.4M NaCl2 and 2mM EDTA) buffer and 50μl of 10% SDS solution was added.

The samples were subjected to a 60-minute incubation period at 55℃ to facilitate the lyses of white blood cells. 150μl of 5M $NaCLO_4$ and 500μl molecular grade chloroform (Sigma Aldrich, Missouri, United States) were then added to the dissolved pellet and vortexed. Samples were transferred to a sterile 1.5ml microfuge tube and centrifuged at 1300rpm at room temperature for 5 minutes. Carefully ensuring to prevent mixing of the top and bottom phases, the aqueous solution was transferred to a new sterile microfuge tube, to which 1ml of absolute ethanol was added. The tubes were inverted and centrifuged at 1300 rpms for 5 to 10 minutes at room temperature to pellet the precipitated DNA. The supernatant was decanted, and the DNA pellet was left to air dry for no less than 60 minutes and resuspended in 200μl of 1X TE (10mM Tris-HCl, 1mM EDTA, pH 8.0) buffer. Following the resuspension, the sample was then incubated at 65℃ for 15 minutes in a heating block and once cooled, was stored at 4℃.

2.4.4 DNA Quality and Quantity

The DNA samples were quantified using the Take3 microvolume plates and the BioTek® *Synergy™ HT* multi-mode reader (Agilent Technologies, CA, USA) Using n=50 of the total number of samples, 1μ1 of DNA was aliquoted to a sterile Take3 plate and measured for sample purity and concentration. DNA purity and concentrations were recorded, reporting a range of 1.2 to 2.165 (A260/A280 ratio) and 24ng/µl to 389ng/µl, respectively. All de-identified DNA samples were stored in polypropylene tubes labelled with the assigned study number for genotyping analysis at -20^{o}C.

2.5 OUTCOME MEASURES

2.5.1 Shoulder Pain and Disability Index (SPADI)

The SPADI index, a self-reported outcome measure, was used to assess pain and disability associated with musculoskeletal pathologies of the shoulder. The index has two domains – namely, pain (5 items) and disability (8 items) that describe daily activities (Appendix B: Outcome Measures). For each domain, the items are scored on a scale of 0 (no pain/difficulty) to 10 (worst pain/difficulty). The total scores for each domain were added and converted to a percentage as a representation of patient-reported pain or disability. Furthermore, for each domain, the end score (in %) was used to categorize participants into no-low (scores <30%) and moderate-high (scores ≥30%) groups. Previous literature has shown that SPADI scores of >30% can affect routine activities of day-to-day living in affected individuals [749, 750]. Consistent with cases presenting with moderate-high pain scores on the visual analogue scale, those scoring >30-50% on SPADI reported major setbacks in their ability to conduct daily life activities due to impaired mobility and pain [749]. This study, therefore, used these parameters with a margin of 30%, to categorize participants into groups of no-low and moderate-high

scores [751]. Reliability and validity studies show that the SPADI index exhibits excellent reliability (test–retest ICC: ~0.89) with factor analysis indicating the items to correspond to pain and disability characteristics observed in several shoulder pathologies [750, 752, 753]. Utilizing SPADI, this study evaluated patient-reported symptoms of pain, disability and combined (symptoms of pain and disability) related to shoulder movement following treatment for breast cancer.

2.5.2 Hospital anxiety and Depression scale (HADS)

The HADS tool is a 14-item questionnaire that was used to assess anxiety (7 items) and depression (7 items) symptoms [754] in the participants (Appendix B: Outcome Measures). The items for both mental states is each coded from 0 to 3, and therefore, scores may range from 0 to 21, representing the presence and severity of anxiety and depression [755]. The total scores for anxiety and depression once answered, were added, and used to sort participants as either exhibiting normal (0-7), borderline abnormal (8-10) or Abnormal/Case (11-21) symptoms. Validity and reliability studies revealed the tool is appropriate for the detection of anxiety and depression symptoms amongst individuals, and that a threshold of 8 is highly supportive of anxiety and depression symptoms [755-761].

2.5.3 Positive and Negative Affect Scale (PANAS)

The PANAS Scale is a self-reported questionnaire consisting of two scales with 10 items each used to measure the positive (PA) and negative (NA) affect in the breast cancer participants of this study [762] (Appendix B: Outcome Measures). Each item is graded on a scale ranging from 1 (“very slightly or not at all”) to 5 (extremely), with the PA scores calculated from items 1,

3, 5, 9, 10, 12, 14, 16, 17, and 19, and the NA scores calculated from the remaining items [763]. Scores once added, can range from 10 to 50, where mean scores >33.3±7.2 (Mean±SD) indicate high levels of PA, and <17.4±6.2 indicate lower levels of NA. Factory analysis of PANAS indicates the scale presents validity and internal consistency that is sufficiently sensitive [763].

2.5.4 Pain Medication Data Collection

Data was collected for the total amounts of opioids prescribed to participants during the 1 year following surgery for breast cancer. All scripts were systematically screened for the terms "tramadol" and "paracetamol", documenting the corresponding dose, frequency per day, and duration of the script (total number of days). Participants were prescribed tramadol, an opioid derivative, in either 50mg or 100mg dosage. The total dosage of tramadol was converted to milligram morphine equivalents (MME) to standardize opioid intake when describing opioid trends[764]. The total dosage of paracetamol was calculated in grams.

2.5.5 Predesigned TaqMan® SNP Genotyping Assays

SNP Selection

The genes *ABCB1*, *OPRM1*, and *COMT* were chosen using a candidate gene approach because their encoded proteins are fundamental to drug distribution, opioid signalling, and pain modulation [496, 547, 685]. Additionally, the selection of polymorphisms within these genes was driven by the hypothesis that the polymorphisms may have a notable impact on the biological processes and functions associated with the protein product [21, 490, 551, 584, 765, 766].

In the context of this study, focus was placed on the *ABCB1* SNPs, rs1128503 G>A and rs1045642 G>A. A schematic representation of the *ABCB1* gene organisation and the approximate genomic locations for rs1128503 G>A and rs1045642 G>A is shown in **Figure 2.2** and highlighted with red arrows.

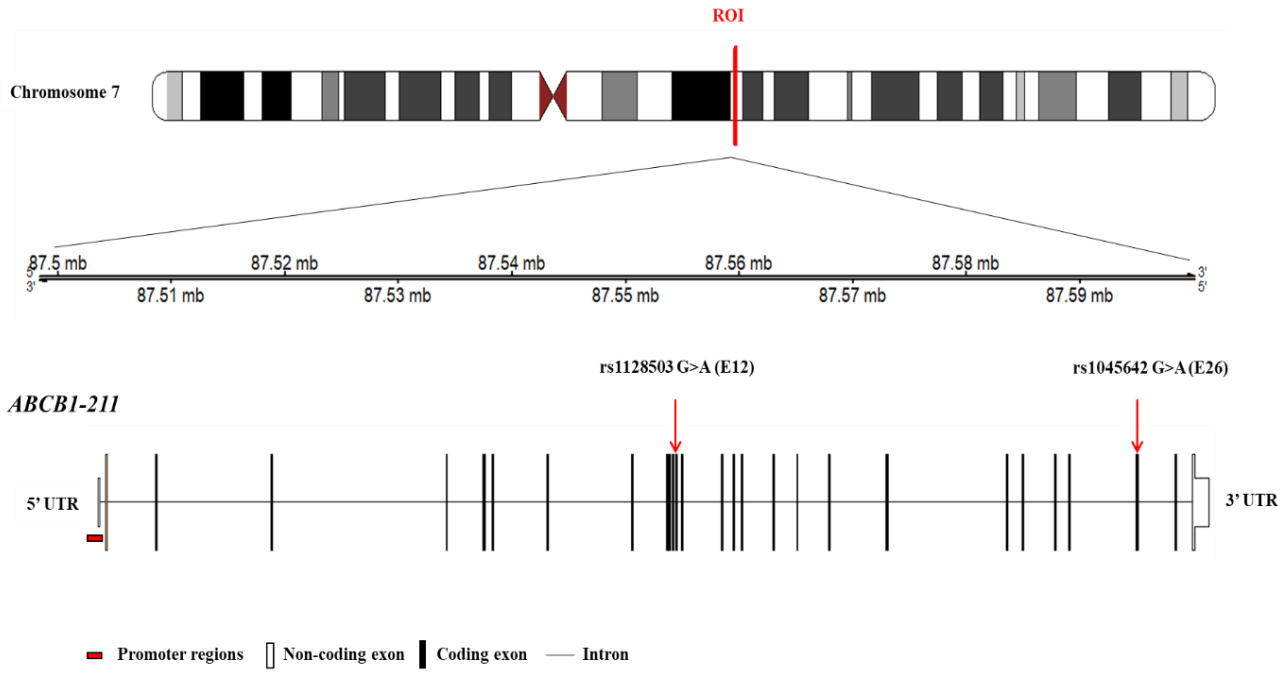


Figure 2.2:The genomic organization of the ATP-Binding Cassette subfamily B member 1 (*ABCB1*) gene. A) The graphic illustration of the genomic size and cytogenic location of the *ABCB1* gene on chromosome 7 (7q21.1: 87,503,017 to 87,600,887 [reverse strand]). The legend describes all coding (black blocks) and non-coding (white blocks), intronic (black line) and promoter (red blocks) regions for the gene. B) Red arrows indicate the location of the SNPs in the 5' to 3' direction, rs1128503 G>A (exon 12) and rs1045642 G>A (exon 26) on *ABCB1* containing 28 exons and introns. This figure was constructed using the R packages, Bioconductor package "Gviz" V1.40.1, "biomatrix" V2.52.0, and the Ensembl (https://www.ensemble.org) V108 assembly, presenting the primary transcript for the gene, *ABCB1-211*.

A schematic representation of *OPRM1* genomic organisation can be seen in **Figure 2.3**. Indicated with red arrows, the SNP rs1799971 A>G in the first exon (E1) of the main transcript for *OPRM1*, *OPRM1*-202, and rs540825 T>A, in exon X, located on an alternative splicing isoform for the gene, *OPRM1-201*.

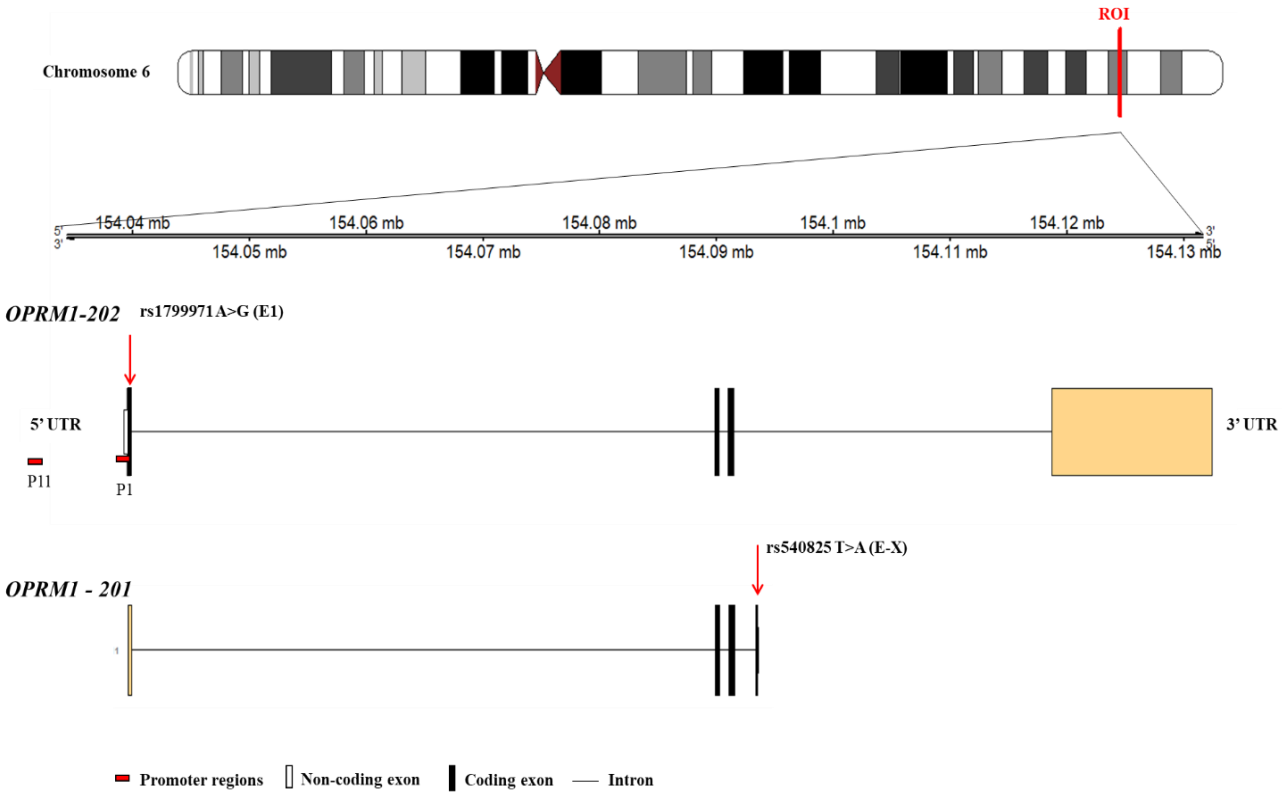


Figure 2.3: The genomic organization of the mu-Opioid receptor 1 (*OPRM1*) gene. A) The graphic illustration of the genomic size and cytogenic location of the *OPRM1* gene on chromosome 6 (6q21.1: 154,010,496 to 154,246,867 [forward strand]). The legend describes all coding (black blocks) and non-coding (white blocks), intronic (black line) and promoter (red blocks) regions for the gene. B) Red arrows indicate the location of the SNP rs1799971 A>G (exon 1) of the major transcript (*OPRM1*-202) and C) the SNP rs540825 T>A (exon X) located on a different exon of an alternative transcript (*OPRM1*-201). The Bioconductor package "Gviz" V1.40.1, "biomaRt" V2.52.0, and the Ensembl (https://www.ensemble.org) V108 assembly were used to generate the illustration via R studio.

The *COMT* SNPs, rs6269 A>G, rs4633 C>T, rs4818 C>G and rs4680 G>A were selected for examination in this study. A schematic representation of the *COMT* genomic organisation is shown in **Figure 2.4**. Red arrows depict the approximate locations of rs6269 A>G in intron 2 (E2), rs4633 C>T in exon 3 (E3), rs4818 C>G and rs4680 G>A in exon 4 (E4), on the main transcript *COMT*-202.

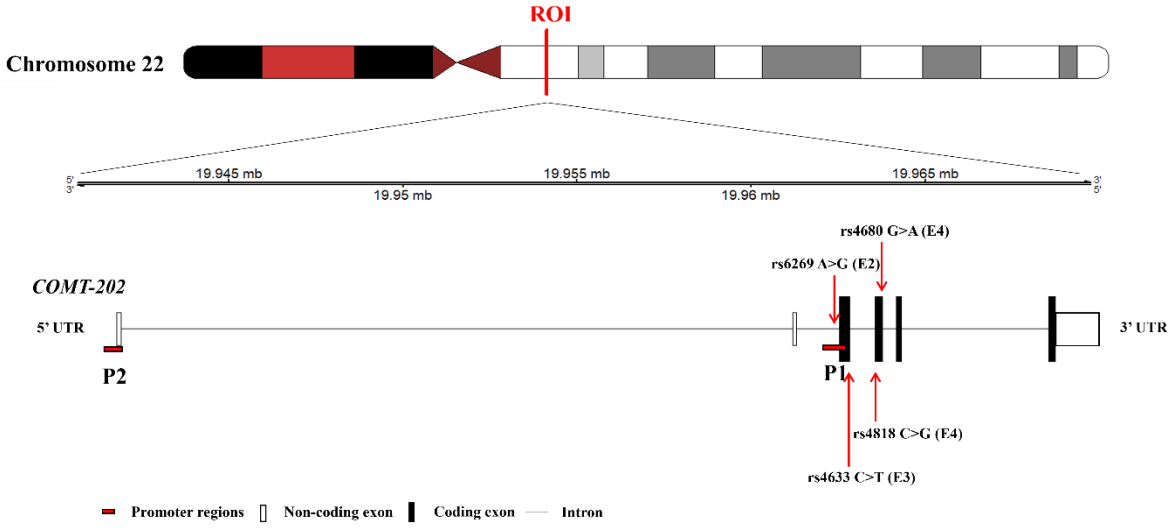


Figure 2.4 The genomic organization of the Catechol-O-methyl transferase (*COMT*) gene. A) The graphic illustration of the genomic size and cytogenic location of the *COMT* gene on chromosome 2 (22q11.21: 19,941,371 to 19,969,975 [forward strand]). The legend describes all coding (black blocks) and non-coding (white blocks), intronic (black line) and promoter (red blocks) regions for the gene. B) Red arrows indicate the location of the SNPs in the 5' to 3' direction, rs6269 A>G (intron2), rs4633 C>T (exon3), rs4818 C>G (exon 4) and rs4680 G>A (exon 4). The Bioconductor package "Gviz" V1.40.1, "biomaRt" V2.52.0, and the Ensembl (https://www.ensemble.org) V108 assembly were used to generate the illustration via R studio.

TaqMan® Technology

TaqMan® SNP genotyping assays were used to genotype the SA BCS for the *ABCB1* (rs1128503 G>A, rs1045642 G>A), *OPRM1* (rs1799971 A>G, rs540825 T>A) and *COMT* (rs6269 A>G, rs4633 C>T, rs4818 C>G, rs4680 G>A) SNPs. TaqMan® assays were obtained from Applied Biosystems™ (Applied Biosystems, Foster City, CA, USA). Genotyping was performed in MicroAmp™ Fast Optical 96-well reaction plates (Applied Biosystems™, Foster City, CA, USA) according to the manufacturer's instructions. Assays for the *ABCB1*, *OPRM1* and *COMT* SNPs were validated and functionally tested by Thermofisher Scientific. Accordingly, *ABCB1* and *COMT* TaqMan® SNPs are classified as drug-metabolizing enzyme (DME) assays, whereas *OPRM1* SNPs are classified as standard assays (non-DME). All assays were used according to the manufacturer's instructions.

TaqMan® is a robust and effective technology that utilised the use of TaqMan® 5'-nuclease chemistry to amplify and detect the specific SNPs of interest in a purified genomic DNA sample, known as 5'-nuclease allelic discrimination [767]. The assay employs the use of fluorescence-based technology, where the flanking regions are labelled with fluorescent probes allowing for detection during the amplification process [767]. In TaqMan® assays, the two primary fluorescent probes used are, 2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein and, 6-carboxyfluorescein also known as VIC and FAM [768]. In addition, the assays contained sequence-specific primers that are targeted to the region of interest with the two minor groove binder (MGB) probes (VIC or FAM), able to specifically detect Allele 1 and Allele 2, respectively. The robustness of TaqMan® assays is the end-point detection which results from the stringent primer design, making sequence binding highly specific, and the emitted

fluorescence measured as an indication of the total quantity of amplified and targeted DNA [743-745].

2.5.6 Polymerase Chain Reaction (PCR)

Sample Preparation

For non-DME assays, each sample well contained 0.2μl of TaqMan® allele-specific primer (final concentrations of 1X), 4μl of TaqMan® PCR master mix containing ampliTaq DNA polymerase Gold, 2.8μl of *d*H_2O and 1µl of DNA template (template [DNA] 1–10*ng*) to a final volume of 8μl. For the DME assays, each sample contained 0.4 μl of TaqMan® allele-specific primer (final concentrations of 1x), 4μl of TaqMan® PCR master mix containing ampliTaq DNA polymerase Gold, 2.6μl of *d*H_2O and 1μl of DNA template (template [DNA] 1–10*ng*). As a measure of PCR quality control, each reaction plate was loaded with dH_2O (n=5) which functioned as a no template control (NTC), that is the absence of DNA. In addition, each reaction plate was loaded with positive controls of DNA samples, with known genotypes (n=5).

PCR Reaction

Standard PCR conditions were applied for the *OPRM1* and *COMT* SNPS, with 1 cycle of activation for 10 minutes (95°C), followed up by 40 cycles of denaturation for 15 seconds (92°C) and annealing for 60-seconds (60°C), and holding at 10-minutes (60°C). Amended DME PCR conditions were applied for the *ABCB1* SNPS, with 1 cycle of activation for 10 minutes (95°C), 50 cycles of denaturation for 15 seconds (95°C) and annealing for 90 seconds (60°C) and holding at 10-minutes (60°C).

All PCR reactions were performed using the Quantstudio™ 3 real-time PCR (Thermo Fisher Scientific, Applied Biosystems™, CA, USA) system. The resulting genotypes were automatically called using endpoint fluorescence and analyzed using the Thermo Fisher Design and Analysis software V 2.6.0. In addition, a two-person inspection was performed to manually verify and check allele-probe intensity results as a measure of quality control for genotyping calls. This also included the consideration of the allelic discrimination plots, creating clusters for each of the homozygous (major and minor) and heterozygous groups. Depicted in **Figure 2.5** is an example of the allele discrimination plot (AD plot) generated using TaqMan® assays.

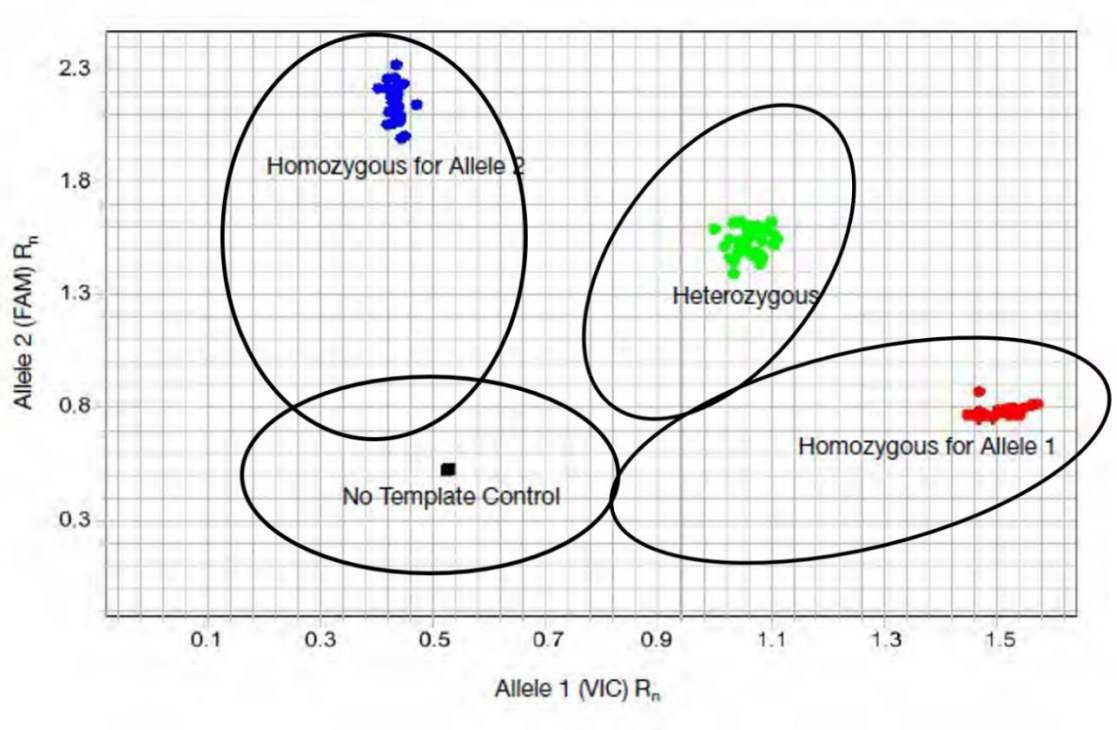


Figure 2.5: A typical allele discrimination plot generated from the TaqMan® SNP genotyping assays. Shown here is the x-axis denoting the VIC-labelled probe as allele 1, and the y-axis denoting the FAM-labelled probe as allele 2. The characteristic clustering of homozygous major (Allele1/Allele1), minor (Allele2/Allele2) and heterozygous (Allele1/Allele2) genotypes are highlighted relative to the negative template controls.

Successful genotyping was considered if the sample was amplified for all polymorphisms, except when failing to amplify after two PCR repeats. All laboratory work was conducted at the Genomics solution to MusculoSkeletal injuries (GenMS) research lab within the Centre for Health through Physical Activity, Lifestyle and Sports (HPALS), University of Cape Town (SA).

2.5.7 Data Management and Quality Control (QC)

A new database was generated using the web-based system, RedCap™. All relevant clinical and demographical data was collected from the participant's medical records and recorded into a source document (case report form). The data was independently captured from the source documents into the secure and access-controlled RedCap™ database. Participants' data was anonymised and quality-checked using a two-person call and check system. Subsequently, the captured data was exported from the RedCap™ database to the R' programme for analysis. All source documents were stored in a locked fireproof filing cabinet and will be archived for a maximum period of 5 years.

2.6 STATISTICAL ANALYSIS

Summarized in **Table 2.3** is a list of all statistical tests and analyses performed in this study. Statistical analyses were conducted on all parameters described in the **Sections 2.4** and **2.5**.

2.6.1 Sample Size Calculation

Defining SPADI Categories

To determine the sample size (N) sufficient to detect a 31% difference in SPADI scores between no-low and moderate-high groups [769]. Using the formula described by Kadam and Bhalerao (2010)[770], *N* was calculated with a 2-sided error of 5% (Z_{α}), 80% ($Z_{1-\beta}$) power, and an estimated standard deviation (σ) of 0.6 computed from the 31% (Δ) variability, as follows:

$$N = \frac{2\,(Z_{\alpha} + Z_{1-\beta})^2\,\sigma^2}{\Delta^2} \quad \rightarrow \quad N = \frac{2\,(1.96+0.8416)^2\,(0.6)^2}{(0.31)^2} \quad \rightarrow \quad N = 63{,}06.$$

A total sample size of N=126, n=63 within each category, is therefore required to detect a 31% difference between categories.

Minor Allele Frequencies

An estimated odds ratios of 1.5 to 2.5, with an 80% power to detect the minor allele frequencies were used to calculate the sample size of the study for each of the *ABCB1* (rs1128503; rs1045642), *OPRM1* (rs1799971; rs540825), and *COMT* (rs6269; rs4633; rs4818; rs4680) polymorphisms. However, for these specific SNPs, no allele frequency data was available for the SA mixed ancestry population in the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/snp/). Sample size calculation was therefore determined using minor allele frequencies of 0.1, 0.2, 0.3, 0.4, and 0.5.

This calculation was completed in two ways, i) using an assumed, literature-inferred, baseline risk of 45% [8, 10, 771-774] for chronic pain (~37%) [7, 10, 427, 771, 775] and disability (~34%)[10, 771, 773, 776, 777] in BCS, and ii) using the frequencies (22%, 28%, and 17%) as reported in Kramer *et al.* (2019)[769]. Sample size requirements were calculated using the QUANTO v1.2.4.49 software [778-780].

A total sample of n=63 South African (SA) breast cancer survivors (BCS) within each category, was required to allow for the detection of differences between (i) no-low and (ii) moderate-high, pain and disability scores. For the sample size calculation using the MAF of the individual SNPs, two different prevalence rates were used. The prevalence rate used was (i) determined by averaging data from various independent studies, and (ii) using the prevalence rate from a previous study within the SA BCS cohort [769]. The sample size (n) calculations were found to be similar and adequate to detect large effect sizes (OR>2.0). To identify odds ratios (OR) >2 with 80% power for MAF (0.1, 0.2, 0.3, 0.4 and 0.5) in the dominant and log additive models, n=159 and n=138, respectively, within each category were found to be sufficient when using the mean reported rates (**Supplemental Table 1**). In contrast, the recessive model, n=76 within each category was adequate to identify OR: 2.5, with 80% power for the MAF of 0.5 only. Using the SA BCS frequencies described by Kramer *et al.* (2019)[769], a sample size of n=144 and n=121 in the dominant and log additive models, respectively, were sufficient to detect OR >2 with 80% power for MAF (0.1-0.5) (Supplemental Table 2). For the recessive model, a sample size of n= 69 was sufficient to detect OR=2.5 with 80% power for MAF of 0.5 only (**Supplemental Table 2**).

This study included N=252 participants, among whom n=68, n=48, and n=55 reported moderate-high pain, disability, and total SPADI (pain and disability) scores, respectively. All participants were genotyped for the *ABCB1*, *OPRM1* and *COMT* SNPs. Subsequently, post-hoc power analyses were performed to determine the adequacy of the sample sizes noted concerning the MAF for the *ABCB1* (rs1128503 G>A, rs1045642 G>A), *OPRM1* (rs1799971 A>G, rs540825 T>A) and *COMT* (rs6269 A>G, rs4633 C>T, rs4818 C>G, rs4680 G>A) SNPs. Using the independent populations' frequencies, posthoc power analyses showed >80% power for OR 2-2.5 in the dominant (pain) and log additive (pain, disability and combined) models only (**Supplemental Table 3**). Similarly, when using previous SA BCS frequencies, >80% power was highlighted for OR 2-2.5 in the dominant (pain) and log additive (pain, disability and combined) models only (**Supplemental Table 4**). For the MAF of *ABCB1*, *OPRM1* and *COMT* SNPs, the sample size was underpowered (<80%) in the recessive model for each category (pain, disability and combined).

2.6.2 Exploratory Sample Analyses

No formal sample size calculation was performed. Basic descriptive statistics were conducted to describe the cohort using GraphPad Prism software [781]. A normality test was performed for quantitative variables using the Shapiro-Wilk test (**Supplemental Table 6**). An abnormal distribution was observed for the variable's tramadol and paracetamol total dose. Correlations between quantitative and qualitative variables, and total drug dosages were evaluated using the Spearman's correlation and Mann-Whitney U tests, respectively (**Table 2.2**). Examination of differences in outcome measure scores between the three-time points, T1 (pre-operative), T2 (3-month post-operative), and T3 (1-year post-operative) were conducted using the Wilcoxon signed-rank test. Additionally, Spearman's statistics R was used to test for pairing effectiveness

between the means of different time points with 95% estimated confidence intervals and significance accepted at $p<0.05$ (**Table 2.2**).

2.6.3 Descriptive Variables

Comparative analysis of demographic and clinical data was assessed using Statistica V13.5.0.17 [782]. All quantitative variables were tested for normality using the Shapiro-Wilk test. Consequently, quantitative variables presenting with non-normal distributions were analysed for differences between groups using the nonparametric Mann-Whitney U test. However, to ensure a comprehensive statical analysis was performed, parametric tests were applied and differences between groups were observed (**Supplemental Table 8**). To test for differences between groups of categorical variables, Pearson's Chi-square ($\chi 2$) and Fisher's exact tests (if $n<10$) were used (**Table 2.3**).

2.6.4 Genotype Frequency Distribution Across Descriptive Variables

Statistical analysis was applied to identify any potential confounders between the descriptive and the genetic (*ABCB1, OPRM1* and *COMT*) variables. To examine the genotype effects of each SNP on the participants' quantitative variables, the nonparametric Kruskal Wallis test was applied. To evaluate differences in genotype frequency distribution across categorical parameters, Pearson's Chi-square ($\chi 2$) and Fisher's exact tests (if $n<10$) were employed (**Table 2.3**).

2.6.5 Genotype and Allele Frequency Distribution

The R studio V1.3.1056 running R V4.0.4 language and programming environment (http://www.r-project.org) was used to analyse all genotype data [783], summarised in **Table 2.3**. Pearson's Chi-square ($\chi 2$) and Fisher's exact tests (if $n<10$) tests were applied to evaluate differences in genotype and allele frequency distribution between no-low and moderate-high groups of pain, disability and combined (pain and disability). The "genetics" v1.3.8.1.3 package was used to determine the Hardy-Weinberg equilibrium (HWE) probabilities and linkage disequilibrium (LD) for *ABCB1* (rs1128503 G>A; rs1045642 G>A), *OPRM1* (rs1799971 A>G; rs540825 T>A) and *COMT* (rs6269 A>G; rs4633 C>T; rs4818 C>G; rs4680 G>A) SNPs [784]. Furthermore, logistic regression analysis was applied to evaluate the association between genotypes and pain/disability categories using the "SNPassoc" v2.0.2 packages (**Table 2.3**); [785].

2.6.6 Inferred Haplotype Frequency Distribution

Inferred haplotypes were constructed using the individual genotype data for *ABCB1* (rs1128503 G>A and rs1045642 G>A) and *OPRM1* (rs1799971 A>G and rs540825 T>A). For *COMT*, the genotype data for rs6269 A>G and rs4680 G>A were used to construct the inferred haplotype which represents the genomic region spanning the central (second) haploblock described in the literature [21, 786-789].

The evaluation of statistically inferred haplotypes for the *COMT* SNPs was performed in the following manner. Applying the individual genotype data of the *COMT* rs6269 A>G, rs4633 C>T, rs4818 C>G and rs4680 G>A SNPs, inferred haplotypes were constructed using a stepwise design (**Table 2.1**). Differences in inferred haplotype frequency distribution patterns

between no-low and moderate-high groups of pain, disability and combined, were compared using Pearson's Chi-square ($\chi 2$) and Fisher's exact tests (**Table 2.3**). The inferred haplotype frequency distribution patterns between no-low and moderate-high groups were analysed using the "haplo.stats" v1.8.6. package [790, 791].

Table 2.1: Inferred haplotypes constructed for *COMT* SNPs, rs6269 A>G, rs4633 C>T, rs4818 C>G and rs4680 G>A.

	COMT SNPs			
Inferred Haplotypes	rs6269 A>G	rs4633 C>T	rs4818 C>G	rs4680 G>A
H1	✓	✓	✓	✓
H2		✓	✓	✓
H3			✓	✓
H4		✓		✓
H5	✓			✓

2.6.7 Inferred Allele-Allele Combination Frequency Distribution

As a proxy for gene-gene interactions, stepwise inferred allele-allele combination constructs were generated using the individual genotype data for the *ABCB1*, *OPRM1* and *COMT* SNPs. The allele-allele combination constructs were used to evaluate the gene-gene interaction association between the *ABCB1, OPRM1 and COMT* genes and pain/disability and combined (pain and disability) categories. The "haplo.stats" (v1.8.6.) package was used to analyse inferred allele-allele combination frequency distribution between the no-low and moderate-high groups (**Table 2.3**); [790, 791].

The analysis data for quantitative variables were expressed as medians (interquartile range; quartile 1, quartile 3), while qualitative variables were expressed as percentages (%). Odds ratios [OR], confidence intervals at 95% [95% CI], and statistical significance accepted at $p < 0.05$ were reported as part of the regression analysis. All logistic regression analysis was adjusted for the confounder participants' age at the time of surgery. Subsequently, all unadjusted data are provided in the supplementary **Section 7.4.1**. and adjusted data presented in the chapter to follow. Furthermore, no corrections were made for multiple tests, given the limited sample size and that more than two SNPs were evaluated. Finally, all graphical illustrations for the genetic association analyses in this thesis were generated utilizing the GraphPad Prism software V8.4.3 (686) [781].

Table 2.2: A summary of the statistical methods used to analyse the clinical profile of a subset of participants during a 1-year period of breast cancer treatment.

					Testing Between Groups/levels	
Variable	**Type**	**Level**	**Normality**	**Tests**	**Pre-op/3-months/1-year**	**Drug dosage**
Outcome Measures						
Pain	Quantitative	Continuous	Non-normal distribution	Non-Parametric	Wilcoxon-signed rank test	Spearman's correlation
Disability	Quantitative	Continuous	Non-normal distribution	Non-Parametric	Wilcoxon-signed rank test	Spearman's correlation
Combined	Quantitative	Continuous	Non-normal distribution	Non-Parametric	Wilcoxon-signed rank test	Spearman's correlation
Anxiety	Quantitative	Continuous	Non-normal distribution	Non-Parametric	Wilcoxon-signed rank test	Spearman's correlation
Depression	Quantitative	Continuous	Non-normal distribution	Non-Parametric	Wilcoxon-signed rank test	Spearman's correlation
Positive Affect	Quantitative	Continuous	Non-normal distribution	Non-Parametric	Wilcoxon-signed rank test	Spearman's correlation
Negative Affect	Quantitative	Continuous	Non-normal distribution	Non-Parametric	Wilcoxon-signed rank test	Spearman's correlation
Demographical, Clinical And BC Treatment Data						
Age At Surgery	Quantitative	Continuous	Non-normal distribution	Non-Parametric	Mann-Whitney	Spearman's correlation
BMI	Quantitative	Continuous	Non-normal distribution	Non-Parametric	Mann-Whitney	Spearman's correlation
Surgery Type	Categorical	Binary		Non-Parametric		Mann-Whitney
Lymph Node Surgery	Categorical	Binary		Non-Parametric		Mann-Whitney
Neo CT	Categorical	Binary		Non-Parametric		Mann-Whitney
Adjuvant CT	Categorical	Binary		Non-Parametric		Mann-Whitney
Adjuvant RT	Categorical	Binary		Non-Parametric		Mann-Whitney
Adjuvant HT	Categorical	Binary		Non-Parametric		Mann-Whitney
HT Given	Categorical	Nominal		Non-Parametric		Mann-Whitney
Total Drug Dosage						
Tramadol Dosage	Quantitative	Continuous	Non-normal Distribution			
Paracetamol Dosage	Quantitative	Continuous	Non-normal Distribution			

Table 2.3: A summary of the statistical methods used to analyse the relationship between all the study variables featured in the complete cohort.

Variable	Type	Level	Normality	Tests	Testing Between Groups/levels		Tool
					Pain/Disability/Combined	Genotypes	
SPADI Data							
Pain	Quantitative	Continuous	Non-normal distribution				Statistica
Disability	Quantitative	Continuous	Non-normal distribution				Statistica
Combined	Quantitative	Continuous	Non-normal distribution				Statistica
Demographic and Clinical Data							
Age At Surgery	Quantitative	Continuous	Non-normal distribution	Non-Parametric	Mann-Whitney	Kruskal Wallis	Statistica
Time Since Surgery (Yr.)	Quantitative	Continuous	Non-normal distribution	Non-Parametric	Mann-Whitney	Kruskal Wallis	Statistica
Total Nodes Examined	Quantitative	Discrete	Non-normal distribution	Non-Parametric	Mann-Whitney	Kruskal Wallis	Statistica
Total Nodes Involved	Quantitative	Discrete	Non-normal distribution	Non-Parametric	Mann-Whitney	Kruskal Wallis	Statistica
Side Of Primary	Categorical	Binary		Non-Parametric	Fishers Exact/Pearson's X^2	Pearson's X^2	Statistica
Invasive Ductal Carcinoma	Categorical	Binary		Non-Parametric	Fishers Exact/Pearson's X^2	Pearson's X^2	Statistica
Lymphovascular Invasion	Categorical	Binary		Non-Parametric	Fishers Exact/Pearson's X^2	Pearson's X^2	Statistica
Tumour Grade	Categorical	Ordinal		Non-Parametric	Pearson's X^2	Pearson's X^2	Statistica
Breast Cancer Treatment Data							
Surgery Type	Categorical	Binary		Non-Parametric	Fishers Exact/Pearson's X^2	Pearson's X^2	Statistica
Lymph Node Surgery	Categorical	Binary		Non-Parametric	Fishers Exact/Pearson's X^2	Pearson's X^2	Statistica
Neo CT	Categorical	Binary		Non-Parametric	Fishers Exact/Pearson's X^2	Pearson's X^2	Statistica
Adjuvant CT	Categorical	Binary		Non-Parametric	Fishers Exact/Pearson's X^2	Pearson's X^2	Statistica
Adjuvant RT	Categorical	Binary		Non-Parametric	Fishers Exact/Pearson's X^2	Pearson's X^2	Statistica
Adjuvant HT	Categorical	Binary		Non-Parametric	Fishers Exact/Pearson's X^2	Pearson's X^2	Statistica
HT Given	Categorical	Nominal		Non-Parametric	Pearson's X^2	Pearson's X^2	Statistica
Genetic data							
Genotypes	Categorical			Non-Parametric	Pearson's X^2		R Studio
Alleles	Categorical			Non-Parametric	Fishers Exact/Pearson's X^2		R Studio
Inferred Haplotypes	Categorical				Logistic Regression		R Studio
Inferred Allele-Allele Combinations	Categorical				Logistic Regression		R Studio

2.7 BIOINFORMATIC ANALYSES

Bioinformatic analyses were used for the *in-silico* approach to (i) explore the functional effects of each of the SNPs using various online programs (SIFT, PolyPhen-2, FATHMM-MKL, and *S*FOLD), (ii) identify and visualize gene-gene interactions and functional gene-associated network, (iii) explore biological pathways associated specifically to the study gene set *ABCB1*, *OPRM1* and *COMT*.

2.7.1 Computational Prediction of Effects of SNPs for *ABCB1*, *OPRM1* and *COMT*

SIFT, PolyPhen-2, and FATHMM-MKL

The consequences of *ABCB1* (rs1045642 G>A; rs1128503 G>A), *OPRM1* (rs1799971 A>G; rs540825 T>A) and *COMT* (rs6269 A>G; rs4633 C>T; rs4818 C>G; rs4680 G>A) SNPs on the translated protein structure and function were investigated using the, Sorting Intolerant from Tolerant (SIFT), Polymorphism Phenotyping 2 (PolyPhen-2), and Functional Analysis through Hidden Markov Models (FATHMM) algorithms.

The SIFT V 5.1.1 algorithm [SIFT dbSNP (a-star.edu.sg), updated version of 31 Mar 2022, accessed on 27 October 2022] predicts the functional effects of non-synonymous SNPs on the translated protein product through sequence homology that may modify the gene-associated phenotype [792-794]. A SIFT search will, based on multiple alignments, compute a tolerance index score that ranges between 0 (deleterious) to 1 (neutral); [794, 795]. SNPs are predicted to be deleterious with scores <0.049, and tolerant with scores >0.049. Predictions are most confident the closest the scores approach 0 and 1.

PolyPhen-2 V 2 [PolyPhen-2: prediction of functional effects of human nsSNPs (harvard.edu), updated version of 21 June 2021, accessed on 27 October 2022] is an online tool that predicts the functional effects of SNPs through amino acid substitution on the translated protein product through structural, and comparative evolutionary factors [796, 797]. PolyPhen-2 predictions score amino acid substitutions between 0 (neutral) and 1 (deleterious). Furthermore, predicted substitutions may have a benign (0.00-0.014), possibly damaging (0.15-0.84) or damaging (0.85-1) effect on the resulting protein.

FATHMM-MKL V 2.3 [fathmmMKL – Predict the Functional Consequences of Single Nucleotide Variants (SNVs) (biocompute.org.uk), accessed on 27 October 2022] is a software program that predicts the effects of non-synonymous SNPs, both coding and non-coding, on the resulting protein product, through weighted models for human mutations [798, 799]. FATHMM predictions are presented in the form of p-values in the range of (0 to 1), where $p > 0.5$ is predicted as deleterious, and $p < 0.5$ is predicted as neutral or benign.

Two-Dimensional (2D) RNA Structures

Two-dimensional (2D) RNA structures were computationally predicted for the individual *ABCB1*, *OPRM1* and *COMT* SNPs, using the online Software for Statistical Folding of Nucleic Acids and Studies of Regulatory RNAs [*S*FOLD, Sfold – Software for Statistical Folding and Studies of Regulatory RNAs (wadsworth.org), accessed on 19 April 2022]. Using the nucleotide sequence obtained from the NCBI variation viewer (assembly GRCh38.13) for each SNP, (limited to 250 bases), this interactive platform predicted the highest-scoring potential RNA conformations. This was evaluated for each SNP sequence containing both minor and major alleles. Furthermore, all RNA structures and conformations were predicted to the folding temperature of 37^{o}C.

2.7.2 Gene-Associated Network Analyses

The GeneMANIA platform (available at https://genemania.org/, accessed on 21 April 2022), was used to evaluate and predict the functional and gene-associated networks between *ABCB1*, *OPRM1* and *COMT*. The association data within this platform describes various networks across the gene set such as protein and genetic interactions, pathways, co-expression and -localization, shared protein domains and analogues. The platform functions on weighted data of interaction networks generated from various sources, processed, and categorized into the respective networks [800].

2.7.3 Gene Set Enrichment Analyses

Gene set enrichment analysis was performed for *ABCB1*, *OPRM1* and *COMT* using the online EnrichR platform at (Enrichr (Malayalam.cloud), accessed on 21 April 2022). The platform allows for the screening of the prioritised gene set against 199 libraries, 403 883 functional terms and more than 50 million sets of annotated data. Several selective libraries associated with the study gene set were identified and reported here [801].

3 CHAPTER THREE: RESULTS

3.1 EXPLORING THE CLINICAL PROFILE OF SA BCS

The primary objective of this analysis was to provide an insight into the clinical spectrum of BCS within the first year period, post-treatment. Thirteen BC participants were observed at three distinct time intervals: preoperatively (before surgery), at 3 months, and 1 year post-surgery. The assessment of patients' pain, disability, anxiety, depression, and affect outcomes, was performed at each designated time point, using the SPADI, HADS, and PANAS questionnaires. Furthermore, the total tramadol and paracetamol medication prescribed was documented throughout the entire study duration.

This study included N=13 participants, with the variables, age, body mass index (BMI), and treatments received summarised in **Supplemental Table 5**. Initial analyses revealed a normal distribution for the variable age [(means±SD) 53,3±13,2; p=0.704). Analyses of BMI (kg/m^2), however, did not show a normal distribution [(median (IRQ)) 33.1 (27.6-38.3); p=0.022]. Five and eight participants received an MTX and WLE, and four and eight received an ALND and SLNB. Three received neo-adjuvant chemotherapy, eight received radiation therapy and more than 90% (n=12) of participants received tamoxifen as hormonal treatment (**Supplemental Table 5**).

3.1.1 Participant Variables and Drug Dosage

Clinical effects were evaluated to assess if any relationship exists between opioid dosage and participant variables. No significant correlations were evident between the total tramadol dosage and the participant's age, and/or BMI, p>0.05 (**Figure 3.1** A and B).

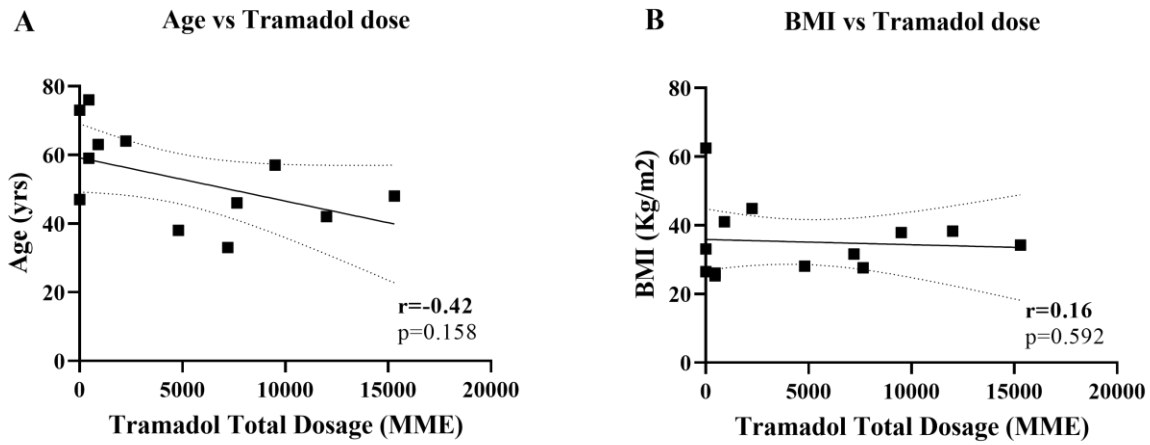


Figure 3.1: Spearman correlation plot for total tramadol (MME) dose for A) age, and B) BMI. Depicted is the linear regression (solid line) with confidence intervals (curved dotted lines). Lower right corner with r, Spearman correlation coefficient, and p, associated p-value, with significant p-values in bold.

Similarly, the results noted no significant correlation between the total paracetamol dosage and age, and BMI, p>0.05 (**Figure 3.2**A and B). Furthermore, no other significant correlations were observed for the total tramadol and paracetamol dose, and the different BC treatments received (surgery type, lymph node surgery type, and adjuvant treatments), p>0.05 (**Figure 3.3**).

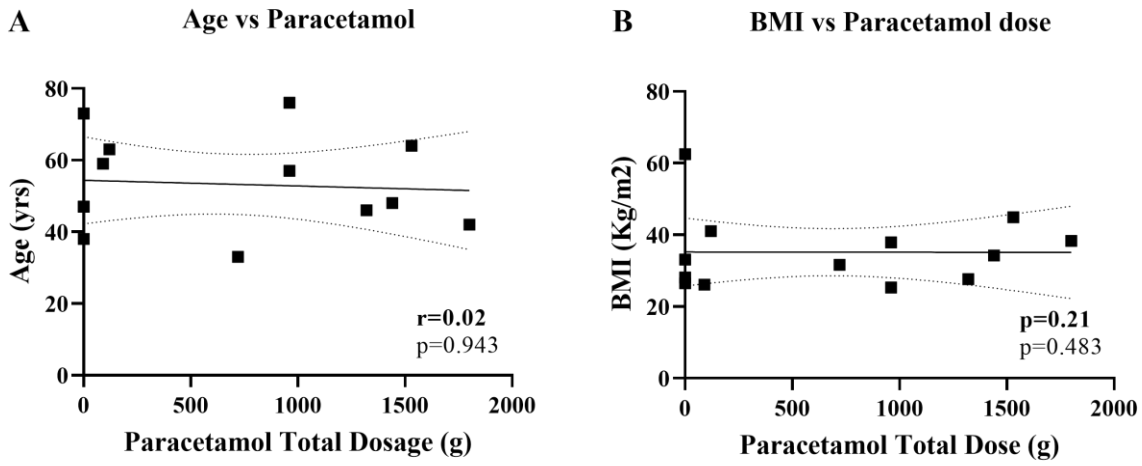


Figure 3.2: Spearman correlation plot for the total paracetamol (g) dose for A) age, and B) BMI. Depicted is the linear regression (solid line) with confidence intervals (curved dotted lines). Lower right corner with r, Spearman correlation coefficient, and p, associated p-value, with significant p-values in bold.

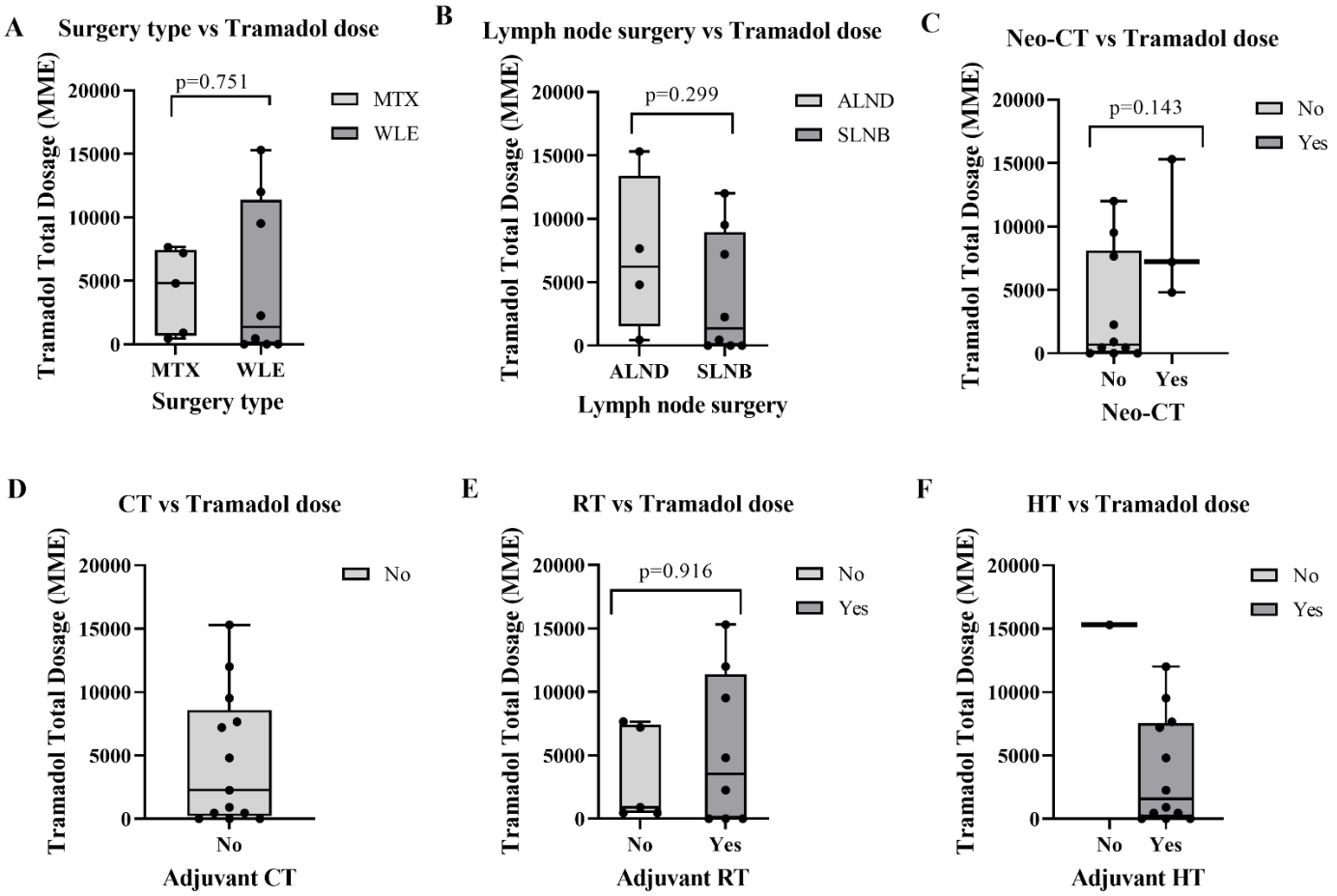


Figure 3.3: The total tramadol (MME) dose for the different breast cancer treatments received. Depicted is A) Surgery type, B) Lymph node surgery, C) Neo chemotherapy, D) Chemotherapy, E) radiation therapy and F) Hormone therapy. Significant p-values between the categories of each treatment in Mann-Whitney's U tests are shown in bold.

3.1.2 Patient-Reported Outcome Measures (PROMS)

Mean±SD scores for each outcome during the three designated intervals of BC treatment are summarised in **Table 3.1.**

Shoulder Pain and disability (SPADI)

Pain, disability and combined (pain and disability) scores of <30% and ≥30% were categorised as no-low and moderate-high, respectively. Means scores indicate pain, at all three time points were low, the lowest noted for pain (5,69±14,1) pre-operatively (**Table 3.1**). Paired t-test analysis noted a difference in mean scores of 43 (p=0.005, 95% CI: 6.0-52.0), -22 (p=0.012, 95% CI: -36.0-0.0), and 8 (p=0.020, 95% CI: 0.0-30.0) for T1-T2, T2-T3, and T1-T3, respectively (**Table 3.2**). The disability mean scores were low at all three time points, with the lowest noted pre-operatively (12,5±24,9); (**Table 3.1**). No significant differences were evident between the three time points, p>0.05 (**Table 3.2**).

For combined (pain and disability) symptoms, the mean scores indicated participants experienced low scores pre-operatively (18,2±37,9), and thereafter moderate-high scores at 3-months (52,3±38,1) and 1-year (34,4±39,7) post-operatively (**Table 3.1**). Paired t-test analysis noted significant differences in the means of 23 (p=0.007, 95% CI: 6.9-51.5), -10 (p=0.023, 95% CI:-30.8- -3.1), and 4 (0.0-44.3) for T1-T2, T2-T3, and T1-T3, respectively (**Table 3.2**).

Table 3.1: The outcome measures, SPADI, HADS and PANAS mean scores at three time points during the 1-year of breast cancer treatment.

Outcome	Pre-operative (T1)	3-month (T2)	1-year (T3)
SPADI			
Pain	5,69 ± 14,1	23,6 ± 15,7	13,3 ± 14,5
Disability	12,5 ± 24,9	28,7 ± 23	21,1 ± 25,6
Pain and Disability	18,2 ± 37,9	52,3 ± 38,1	34,4 ± 39,7
HADS			
Anxiety	7,0 ± 4,56	7,67 ± 6,93	8,69 ± 7,02
Depression	8,54 ± 5,47	9,33 ± 3,31	11 ± 3,89
PANAS			
Positive affect	43,5 ± 5,25	41,9 ± 4,54	38,1 ± 9,6
Negative affect	19,7,± 8,54	22,9 ± 12,5	21,8 ± 9,58

Abbreviations: HADS, Hospital Anxiety and Depression Scale; PANAS, Positive and Negative Affect Scale; SPADI, Shoulder Pain and Disability Index

Anxiety and Depression-HADS

Based on the cohort size and scoring of the HADS tool, participants reported either normal (≤7) or borderline-to-case (>7) levels of anxiety and depression. Mean scores for anxiety among participants were noted to be normal preoperatively and borderline-case at 3 months and 1 year' post-operatively (**Table 3.1**). However, no significant differences were noted across the three time points, p>0.05 (**Table 3.2**). In contrast, depression mean scores were borderline-case high (>7) at all three time points, with the highest level of depression noted at 1 year (11±3,89) after surgery (**Table 3.1**). The paired t-test analysis, however, noted no significant differences, p>0.05 (**Table 3.2**).

Positive and Negative Affect-PANAS

Positive affect was highest pre-operatively (43,5,0±5,25) and thereafter noted a decline at 3 months (41,9±4,54) and 1-year (38,1±9,6) post-operatively, with a significant difference in means [-2 (p=0.039, 95% CI: -12.20)] noted between time points T1 and T3 (**Table 3.1, Table 3.2**). No significant differences were further noted. Negative affect mean scores were at their lowest pre-operatively (19,7,0±8,54), highest at 3 months (22,9±12,5), and noting a decline at 1-year (21,8±9,58) post-operatively (**Table 3.1**). No significant differences were, however, evident in the paired t-test analysis, p>0.05 (**Table 3.2**).

A one-tailed p-value using Spearmen's statistic R was used to measure the pairing effectiveness for all three time intervals within each outcome measured with significantly paired t-tests at p<0.05 (**Table 3.2**). Apart from the pairing of scores for the outcome depression (T1-T2, and T1-T3), positive affect (T1-T2), and negative affect (T1-T3).

Table 3.2: Pairwise t-test for differences between pre-operative, 3-months, and 1-year post-operative means for all PROMS.

			Wilcoxon signed-rank test			Median of differences		Pairing effectiveness	
Outcome	**Timepoint pairing**		**Sum (W)**	**P value[a]**	**Pairs**	**Median**	**> 95% CI**	**Spearman's statistic**	**P value[b]**
Pain	Pre-OP	3-months	60	**0,005**	12	43	(6,0 - 52,0)	0,67	**0,009**
	3-months	1-year	-41	**0,012**	12	-22	(-36,0 - 0,0)	0,82	**0,001**
	Pre-OP	1-year	39	**0,02**	13	8	(0,0 - 30,0)	0,54	**0,032**
Disability	Pre-OP	3-months	38	0,102	12	15	(0,0 - 47,5)	0,55	**0,032**
	3-months	1-year	-31	0,131	12	-5	(-21,3 - 0,0)	0,79	**0,002**
	Pre-OP	1-year	18	0,25	13	0	(0,0 - 46,3)	0,58	**0,022**
Pain and Disability	Pre-Op	3-months	58	**0,007**	12	23,1	(6,9 - 51,5)	0,56	**0,031**
	3-months	1-year	-50	**0,023**	12	-9,62	(-30,8 - -3,1)	0,8	**0,001**
	Pre-Op	1-year	43	**0,027**	13	3,85	(0,0 - 44,3)	0,55	**0,027**
Anxiety	Pre-Op	3-months	8	0,68	12	0	(-5,0 - 5,0)	0,63	**0,016**
	3-months	1-year	13	0,477	12	0,5	(-2,0 - 4,0)	0,82	**0,001**
	Pre-Op	1-year	21	0,376	13	0	(-3,0 - 9,0)	0,61	**0,015**
Depression	Pre-Op	3-months	23	0,391	12	3	(-3,0 - 4,0)	0,21	0,254
	3-months	1-year	20	0,392	12	1	(-3,0 - 5,0)	0,25	**0,216**
	Pre-Op	1-year	40	0,127	13	4	(-2,0 - 7,0)	0,45	0,064
Positive affect	Pre-Op	3-months	-23	0,338	12	-1	(-6,0 - 2,0)	0,47	0,063
	3-months	1-year	-34	0,141	12	-4	(-13,0 - 4,0)	0,3	**0,168**
	Pre-Op	1-year	-46	**0,039**	13	-2	(-12,0 - 2,0)	0,61	**0,015**
Negative Affect	Pre-Op	3-months	31	0,249	12	2,5	(-3,0 - 10,0)	0,5	**0,049**
	3-months	1-year	-15	0,53	12	-1,5	(-7,0 - 6,0)	0,67	**0,01**
	Pre-Op	1-year	10	0,748	13	-2	(-6,0 - 15,0)	0,26	0,195

[a] two-tailed P value, [b] one-tailed P value

3.1.3 Tramadol Prescription Patterns

The total tramadol (MME) dose prescribed was evaluated concerning perceived pain, disability, anxiety, depression and affect.

Pain, Disability, and Combined (pain and disability)

Scores of pain (p=0.002, r=0.81, 95% CI: 0.42-0.95), disability (p=0.001, r=87, 95% CI: 0.58-0.96) and combined (pain and disability); (p<0.001, r=0.88, 95% CI: 0.60-0.97) at 3-months post-operatively, correlated with increasing tramadol dosage (**Figure 3.4**A-C). Similarly, scores for pain (p=0.004, r=76, 95% CI: 0.34-0.93), disability (p=0.015, r=0.67, 95% CI: 0.18-0.90) and combined (pain and disability) (p=0.012, r=0.69, 95% CI: 0.20-0.90) at 1-year post-operatively, correlated with increasing tramadol dosage (**Figure 3.4**D-F).

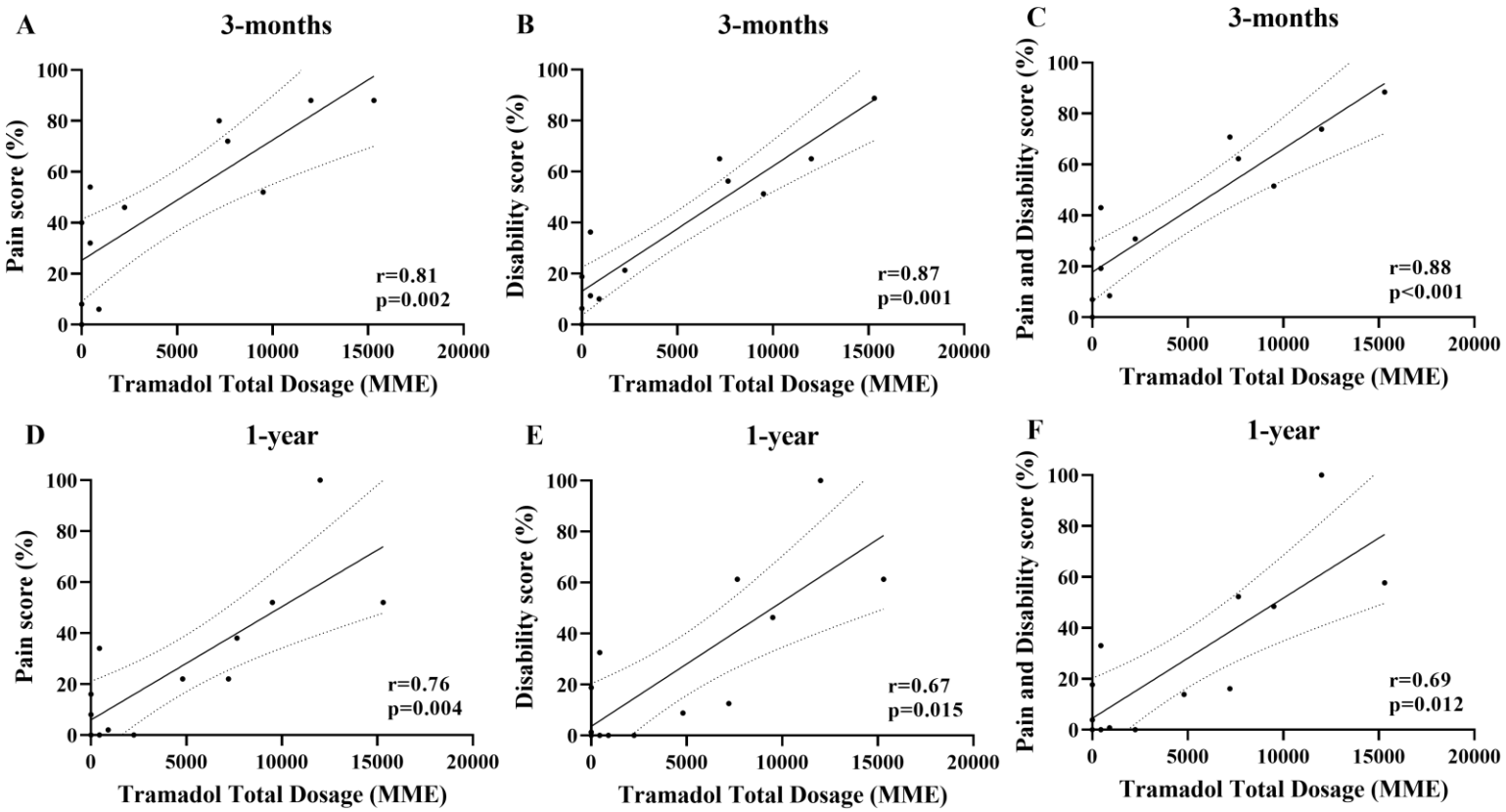


Figure 3.4: Spearman correlation plot for total tramadol (MME) dose prescribed for A/D) pain, and B/E) disability and C/F) combined (pain and disability) at 3 months and 1 year post-treatment. Depicted is the linear regression (solid line) with confidence intervals (curved dotted lines). Lower right corner with r, Spearman correlation coefficient, and p, associated p-value, with significant p-values in bold.

Anxiety and Depression

Higher levels of anxiety at 3 months (p=0.013, r=0.70, 95% CI: 0.20-0.91) and 1-year (p=0.014, r=0.68, 95% CI: 0.18-0.90) post-operatively, correlated with increasing tramadol dosage (**Figure 3.5A** and C). In contrast, no correlation was observed between depression levels and tramadol dosage (**Figure 3.5**D-F).

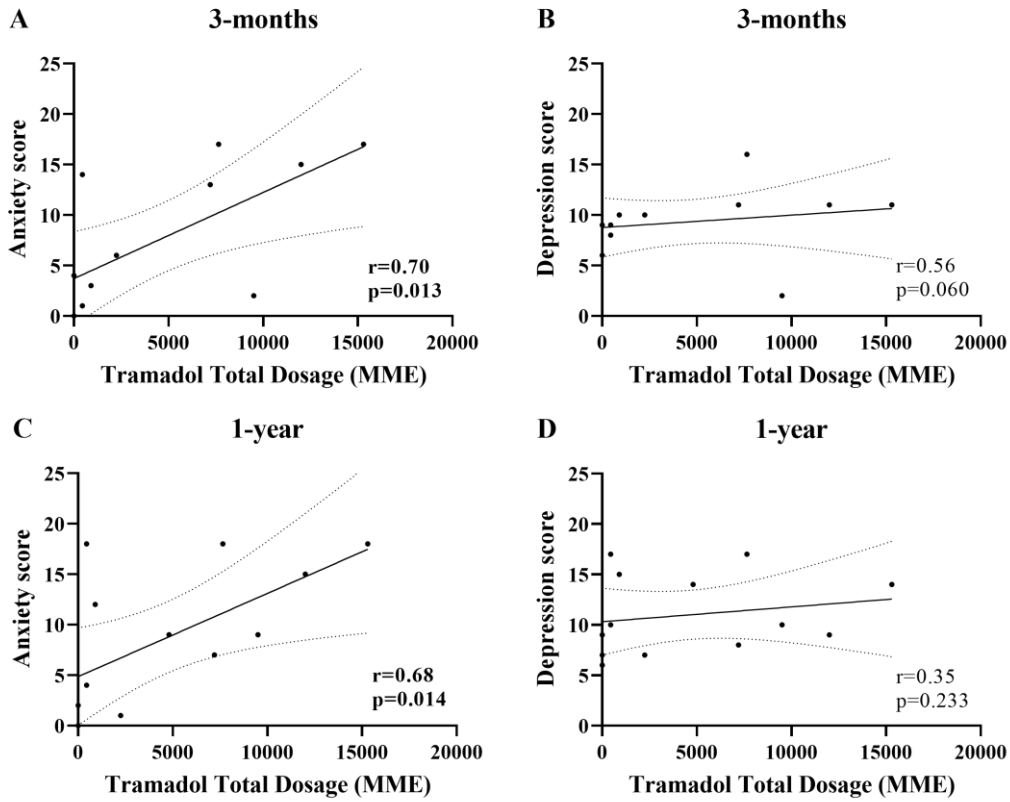


Figure 3.5: Spearman correlation plot for total paracetamol dose prescribed for A/C) anxiety, and B/D) depression at 3 months and 1 year post-treatment. Depicted is the linear regression (solid line) with confidence intervals (curved dotted lines). Lower right corner with r, Spearman correlation coefficient, and p, associated p-value, with significant p-values in bold.

Positive and Negative Affect

No significant correlations were found between positive affect and tramadol dosage, as depicted in **Figure 3.6**A and C. In contrast, a correlation was observed between negative affect and tramadol dosage at 3 months post-operatively (p=0.038, r=0.61, 95% CI: 0.01-0.88). However, there was no significant correlation between negative affect and tramadol dosage at 1 year post-operatively (**Figure 3.6**B and D).

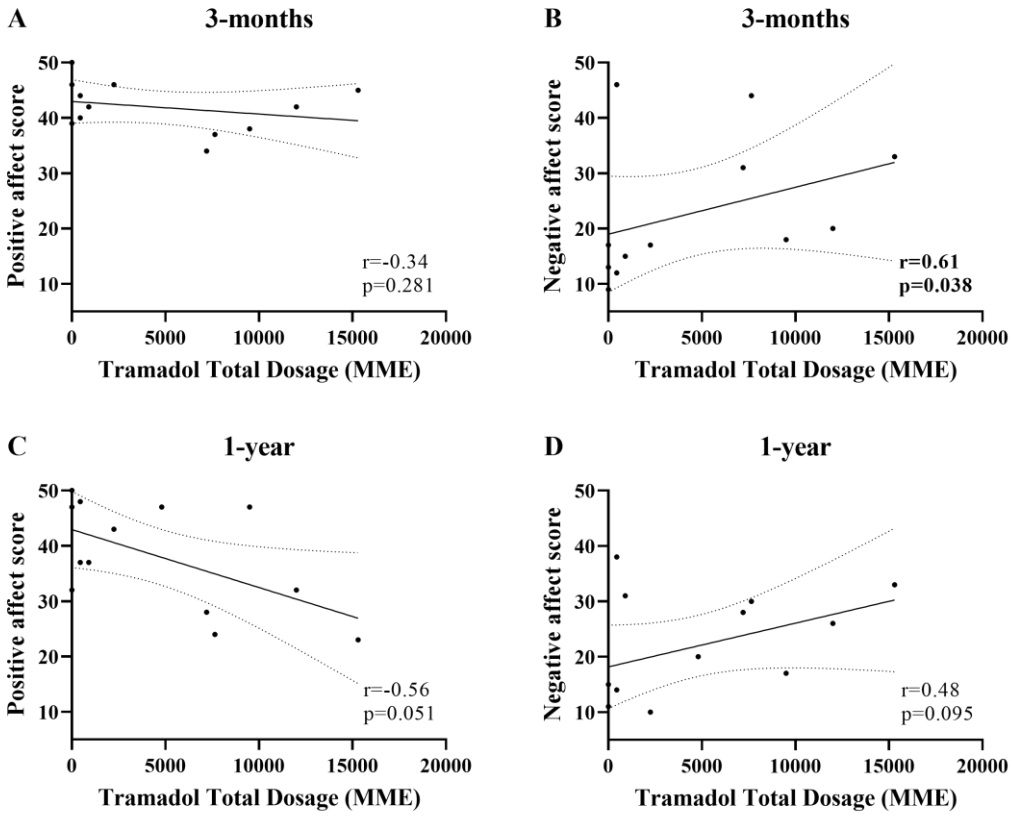


Figure 3.6: Spearman correlation plot for total paracetamol dose prescribed for A/C) positive, and B/D) negative affect at 3 months and 1 year post-treatment (n=13). Depicted is the linear regression (solid line) with confidence intervals (curved dotted lines). Lower right corner with r, Spearman correlation coefficient, and p, associated p-value, with significant p-values in bold.

3.1.4 Paracetamol Prescription Patterns

The total paracetamol (g) dose prescribed was evaluated concerning perceived pain, disability, anxiety, depression and affect.

Pain, Disability, and Combined (pain and disability)

Scores of pain (p=0.005, r=0.77, 95% CI: 0.34-0.94), disability (p=0.005, r=0.77, 95% CI: 0.33-0.93) and combined (pain and disability); (p=0.004, r=0.79, 95% CI: 0.37-0.94) symptoms at 3-months post-operatively correlated with increasing paracetamol dosage (**Figure 3.7** A-C). In contrast, no correlations were observed at 1 year post-operatively as seen in **Figure 3.7** (D-F).

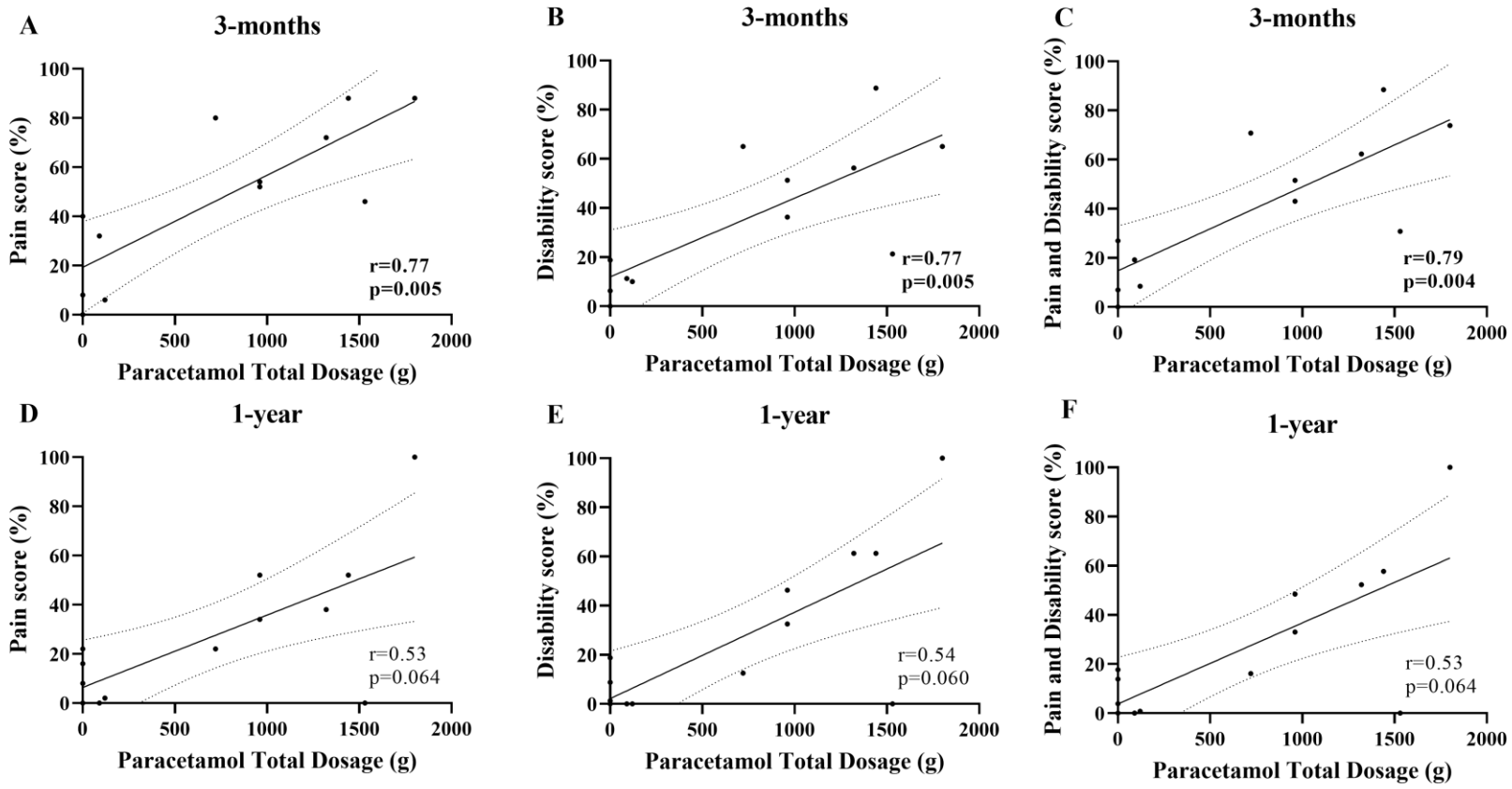


Figure 3.7: Spearman correlation plot for total paracetamol (g) dose prescribed for A/D) pain, B/E) disability, and C/F) combined (pain and disability) at 3 months and 1 year post-treatment. Depicted is the linear regression (solid line) with confidence intervals (curved dotted lines). Lower right corner with r, Spearman correlation coefficient, and p, associated p-value, with significant p-values in bold.

Anxiety and Depression

Higher levels of anxiety at 3 months (p=0.005, r=0.77, 95% CI: 0.34-0.94) post-operatively, correlated with increasing dosage prescribed for paracetamol dosage Figure 3.8A). Similarly, the levels of anxiety at 1-year (p=0.039, r=0.59, 95% CI: 0.03-0.86) post-operatively, correlated with the increasing paracetamol dosage (**Figure 3.8**C). At 3 months (p=0.048, r=0.59, 95% CI: 0.00-0.87) post-operatively, levels of depression noted a weak correlation with the increasing dosage of paracetamol (**Figure 3.8**B). Furthermore, no correlation was observed for depression at 1-year postoperatively (**Figure 3.8**D).

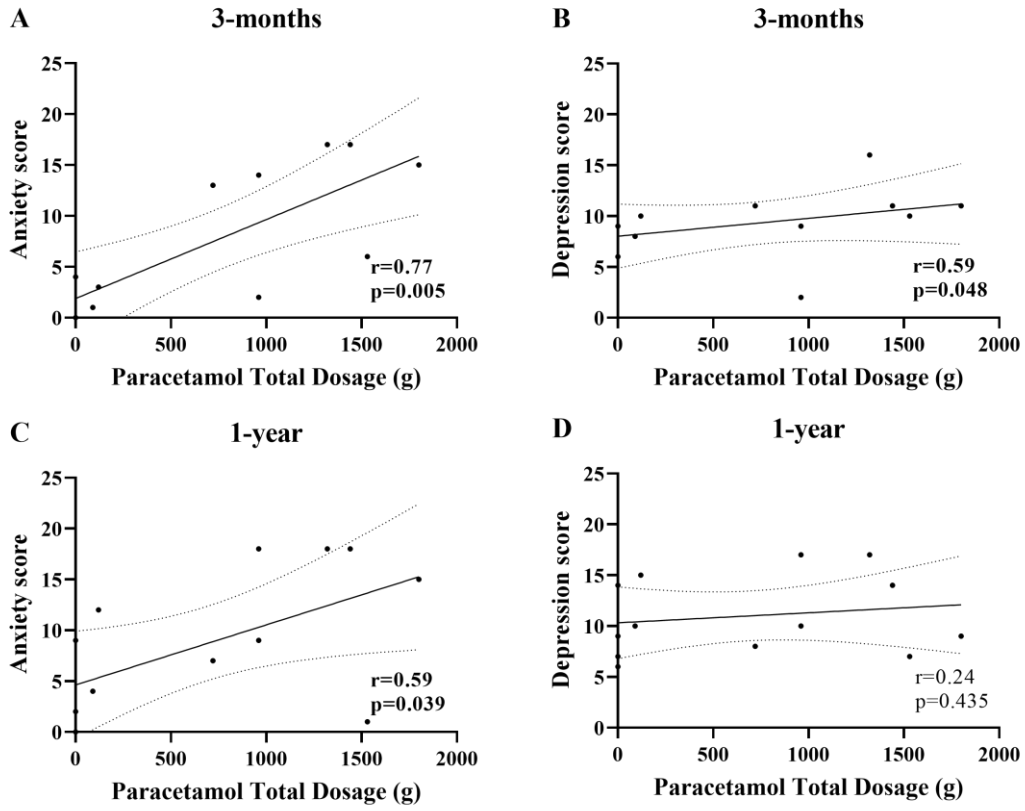


Figure 3.8: Spearman correlation for total paracetamol dose prescribed for A/C) anxiety, and B/D) depression at 3 months and 1-year post-treatment. Depicted is the linear regression (solid line) with confidence intervals (curved dotted lines). Lower right corner with r, Spearman correlation coefficient, and p, associated p-value, with significant p-values in bold.

Positive and Negative Affect

Analysis of the relationship between positive affect and paracetamol dosage noted no significant correlations at 3 months and 1 year postoperatively, p>0.05 (**Figure 3.9**A and C). A fair correlation was observed between the negative affect at 3 months post-operative and the total dosage of paracetamol prescribed (p=0.025, r=0.065, 95% CI: 0.10-0.90) (**Figure 3.9**B). Furthermore, no significant correlation was noted for total paracetamol dosage prescribed and negative affect at 1 year after surgery for BC, p<0.05 (**Figure 3.9**D).

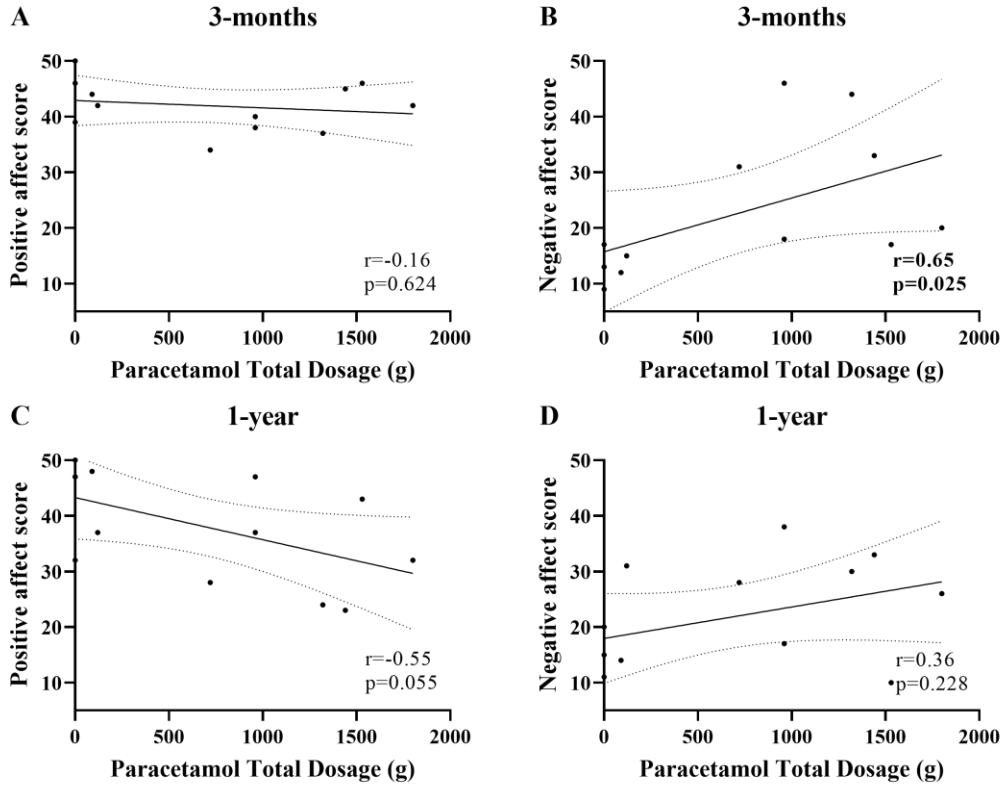


Figure 3.9: Spearman correlation plot for total paracetamol dose prescribed for A/C) positive, and B/D) negative affect at 3 months and 1-year post-treatment. Depicted is the linear regression (solid line) with confidence intervals (curved dotted lines). Lower right corner with r, Spearman correlation coefficient, and p, associated p-value, with significant p-values in bold.

In summary, in a sample of n=13 BCS, the data showed increasing patterns of pain, disability and combined symptoms, anxiety, depression, and negative affect over the 1 year, while positive affect noted a decrease. Assessment of differences in levels highlights significant changes over time for pain and combined symptoms, as well as positive affect. Over the 1 year, increasing the tramadol dosage prescribed correlated with increasing pain, disability, combined symptoms, anxiety, and negative affect (3 months only). Furthermore, increasing paracetamol dosage correlated with pain, disability and combined (pain and disability) symptoms (3 months), anxiety (3 months and 1 year), depression (3 months only) and negative affect (3 months only).

3.2 EXPLORING THE DESCRIPTIVE PROFILE OF SA BCS

Differences in participant variables between the no-low and moderate-high groups of pain, disability and combined (pain and disability) scores were evaluated. Comparative analysis of basic clinical data in the pain category noted that 73.0% and 27.0% (n=68) of individuals reported no-low and moderate-high pain respectively, on the SPADI index (**Table 3.3**). Initial analysis to test for normal distribution of the qualitative variables (age at surgery, time since surgery, total number of nodes examined and involved), showed the variables were non-normally distributed, $p<0.05$ (**Supplementary Section 7.4.1**). Significant differences were noted for age at the time of surgery ($p=0.002$), where the median [interquartile range (IQR; Q1, Q3)] age was older in the no-low [56 (49,62)] group compared to the moderate-high [50 (43,58)] group. No significant differences were evident for the remaining variables in this category (Table 3.3). Frequency distribution patterns were similar between the groups when nodal involvement, primary affected side, invasive ductal carcinoma, lymphovascular invasion and tumour grades were evaluated ($p> 0.05$).

In the disability category, 81.0% and 19% (n=48) of individuals reported no-low and moderate-high disability, respectively on the SPADI index (**Table 3.3**). Age at the time of surgery differed significantly ($p=0.011$) in this group. The median (IQR) in the no-low and moderate-high groups were 56 (49,62) and 49.5 (42,58), respectively. The total number of nodes involved ($p=0.025$) was also significantly different in this category. The median (IQR) was greater in the no-low [2 (1,5)] group, compared to the moderate-high [1 (1,2)] group. No other differences were noted in this category, $p>0.05$ (**Table 3.3**).

The combined (pain and disability) category noted 78.0% and 22.0%(n=55) of individuals reporting no-low and moderate-high pain and disability seen in Table 3.3. Once more, age at the time of surgery was significantly different between the groups, p=0.003. Consistent with the pain and disability categories, the median (IQR) age was greater in the no-low [56 (49,62)] group compared to the moderate-high [50 (43,57)] group (**Table 3.3**). Compared to the no-low [2 (1,5)] group, younger participants also reported having fewer nodes involved in the moderate-high [1 (1,3)] group, p=0.034. No other significant differences were observed between the descriptive variables and the combined (pain and disability) category (**Table 3.3**).

Table 3.3: Breast Cancer Survivors' Clinical Variables Between No-Low And Moderate-High Groups Of Pain, Disability, And The Combined (Pain And Disability) Categories.

	Pain			Disability			Pain and Disability		
Variables	**No-Low**	**Mod-High**	***P***	**No-Low**	**Mod-High**	***P***	**No-Low**	**Mod-High**	***P***
N=252	73 (184)	27 (68)		81 (204)	19 (48)		78 (197)	22 (55)	
Age at surgery	56 (49,62)	50 (43,58)	**0,002**	56 (49,62)	49,5 (42,58)	**0,011**	56 (49,62)	50 (43,57)	**0,003**
Time since surgery (yr.)	3 (2,4)	2 (2,4)	0,116	3 (2,4)	2 (2,4)	0,482	3 (2,4)	2 (2,4)	0,133
Total nodes examined	11 (5,15)	9 (5,12)	0,155	11 (5,14)	9 (5,13)	0,177	11 (5,5,14)	8 (4,12)	0,066
Total nodes involved	2 (1,4)	1,5 (1.4,5)	0,145	2 (1,5)	1 (1,2)	**0,025**	2 (1,5)	1 (1,3)	**0,034**
Side of primary									
Left	49,2 (90)	57,4 (39)	0,250	49,8 (101)	58,3 (28)	0,285	49,5 (97)	58,2 (32)	0,254
Right	50,8 (93)	42,6 (29)		50,3 (102)	41,7 (20)		50,5 (99)	41,8 (23)	
Invasive ductal carcinoma									
Yes	78,7 (144)	80,9 (55)	0,768	79,8 (162)	77,1 (37)	0,913	79,1 (155)	80,0 (44)	0,985
No	3,3 (6)	4,4 (3)		3,5 (7)	4,2 (2)		3,6 (7)	3,6 (2)	
Not done	18,0 (33)	14,7 (10)		16,8 (34)	18,8 (9)		17,4 (34)	16,4 (9)	
Lymphovascular invasion									
Yes	35,7 (55)	29,8 (17)	0,423	35,6 (62)	27,0 (10)	0,316	35,7 (60)	27,9 (12)	0,335
No	64,3 (99)	70,2 (40)		64,4 (112)	73,0 (27)		64,3 (108)	72,1 (31)	
Tumour grade									
I	26,7 (43)	27,6 (16)	0,914	25,6 (46)	33,3 (13)	0,379	26,0 (45)	30,4 (14)	0,132
II	49,7 (80)	48,3 (28)		51,1 (92)	41,0 (16)		51,5 (89)	41,3 (19)	
III	21,7 (35)	20,7 (12)		21,7 (39)	20,5 (8)		21,4 (37)	21,7 (10)	
Not known	1,9 (3)	3,5 (2)		1,7 (3)	5,1 (2)		1,2 (2)	6,5 (3)	

Notes: Data presented as median (interquartile range, Quartile 1, Quartile 3) or % (n). P-values (*P*) in **bold** typeset indicate significance ($P<0.05$). Tests used for comparative analysis include the Mann-Whitney U test (Independent sample T-test); Fisher's exact test (*when n <10); and Chi-squared test Abbreviations: Mod-High: Moderate-High

3.3 BREAST CANCER TREATMENT MODALITIES OF SA BCS

The different treatment modalities received by the SA BCS cohort are summarised in **Table 3.4**. Participants received two specific surgery types, the surgical removal of, i) breast cancer (MTX or WLE), and ii) affected lymph nodes (ALND or SLNB). The two surgical treatments were carried out concurrently, and in any combination for example an MTX+ALND, MTX+SLNB, WLE+ALND, WLE+SLNB or MTX/WLE only. In addition, adjuvant therapies suitable to the diagnoses were also administered, including neo- and adjuvant chemotherapy, radiation, and hormonal therapy.

The frequencies of BC (MTX vs WLE: p=0.315) and lymph node (ALND vs SLND: p=0.490) surgeries were comparable between no-low (78.1% and 21.9%) and moderate-high (71.9% and 28.1%) groups of pain (**Table 3.4**). For the adjuvants, Neo-CT (p=0.708), CT (p=0.943), RT (p=0.483), and HT (p=0.765), there were also no distinct differences in frequency distribution between no-low and moderate-high groups of pain. Similarly, for the choice of HR administered (p=0.892), no significant differences were noted between the no-low and moderate-high groups of pain. The results, however, highlighted that the frequency of participants receiving an MTX, an ALND and all adjuvants, were greater than those receiving the alternative in that category, **Table 3.4**. Equally, no significant frequency distribution differences were noted for BC and lymph node surgeries, and adjuvant treatments between the no-low and moderate-high groups of disability and combined (pain and disability) categories, p>0.05 (**Table 3.4**).

Table 3.4.: Breast Cancer Treatment Modalities Observed In Breast Cancer Survivors, Compared Between No-Low And Moderate-High Groups Of Pain, Disability, And Combined (Pain And Disability) Categories.

	Pain			Disability			Pain and Disability		
Treatment	**No-Low**	**Mod -High**	***P***	**No-Low**	**Mod-High**	***P***	**No-Low**	**Mod-High**	***P***
N=252	**(n=184)**	**(n=68)**		**(n= 204)**	**(n=48)**		**(n=197)**	**(n=55)**	
Surgery type									
MTX	78,1 (139)	71,9 (46)	0,315	77,6 (152)	71,7 (33)	0,403	77,8 (147)	71,7 (38)	0,357
WLE	21,9 (39)	28,1 (18)		22,5 (44)	28,3 (13)		22,2 (42)	28,3 (15)	
Lymph node surgery									
ALND	85,3 (139)	81,5 (44)	0,490	84,4 (151)	84,2 (32)	0,795	85,6 (149)	79,1 (34)	0,357
SLNB	1,2 (2)	0,0 (0)		1,1 (2)	0,0 (0)		1,2 (2)	0,0 (0)	
None	13,5 (22)	18,5 (10)		14,5 (26)	15,8 (6)		13,2 (23)	20,9 (9)	
Neo CT									
Yes	65,4 (17)	60,0 (12)	0,708	68,8 (22)	50,0 (7)	0,225	66,7 (20)	56,3 (9)	0,486
No	34,6 (9)	40,0 (8)		31,3 (10)	50,0 (7)		33,3 (10)	43,8 (7)	
Adjuvant CT									
Yes	63,7 (114)	64,2 (43)	0,943	63,6 (126)	64,6 (31)	0,903	63,9 (122)	63,6 (35)	0,974
No	36,3 (65)	35,8 (24)		36,4 (72)	35,4 (17)		36,1 (69)	36,4 (20)	
Adjuvant RT									
Yes	90,8 (108)	87,3 (48)	0,483	90,1 (127)	87,9 (29)	0,710	91,0 (122)	85,0 (34)	0,271
No	9,2 (11)	12,7 (7)		9,9 (14)	12,1 (4)		9,0 (12)	15,0 (6)	
Adjuvant HT									
Yes	82,9 (136)	81,3 (52)	0,765	82,5 (151)	82,2 (37)	0,963	83,0 (146)	80,8 (42)	0,716
No	17,1 (28)	18,8 (12)		17,5 (32)	17,8 (8)		17,1 (30)	19,2 (10)	
HT given									
Tamoxifen	2,3 (3)	1,9 (1)	0,892	2,0 (3)	2,7 (1)	0,831	2,1 (3)	2,4 (1)	0,979
AI	12,8 (17)	15,4 (8)		12,8 (19)	16,2 (6)		13,3 (19)	14,3 (6)	
Other	85,0 (113)	82,7 (43)		85,1 (126)	81,1 (30)		84,6 (121)	83,3 (35)	

Data presented as percentage (n). P-values (*P*) in **bold** typeset indicate significance ($P<0.05$). Abbreviations: MTX, Mastectomy; WLE, Wide local excision; ALND, Axillary lymph node dissection; SLNB, Sentinel lymph node biopsy; Tests used for comparative analysis include Mann-Whitney U test (Independent sample T-test); Fisher's exact test (*when a sample was <10); Chi-squared test.

3.4 GENETIC PROFILING OF SA BCS

The candidate genes *ABCB1*, *OPRM1* and *COMT* were explored to establish and characterize the SNP frequencies within the SA BCS cohort. The following results describe the genotyping success rates and SNP frequencies within the SA BCS cohort.

3.4.1 *ABCB1*, *OPRM1* and *COMT* Genotyping Amplification Rates

The *ABCB1* rs1128503 G>A and rs1045642 G>A SNPs were successfully amplified in the SA BCS cohort, with rates of 98% (**Supplemental Table 9**). The allelic discrimination plots revealed significant clustering of the three different genotypes for *ABCB1* SNPs A) rs1128503 G>A and B) rs1045642 G>A **(Figure 3.10**). The *OPRM1* rs1799971 A>G and rs540825 T>A SNPs were also successfully amplified in the SA BCS cohort, with rates of 98% and 93%, respectively (**Supplemental Table 9**). Cluster formations are noted for each *OPRM1* SNP (**Figure 3.10**C-D). Similarly, the amplification rates for the *COMT* SNPs were between 96% and 98% in the SA BCS cohort (**Supplemental Table 9**). Allelic discrimination of the major, minor, and heterozygous genotypes was successful for each of the four *COMT* SNPs and represented in **Figure 3.10** E-H.

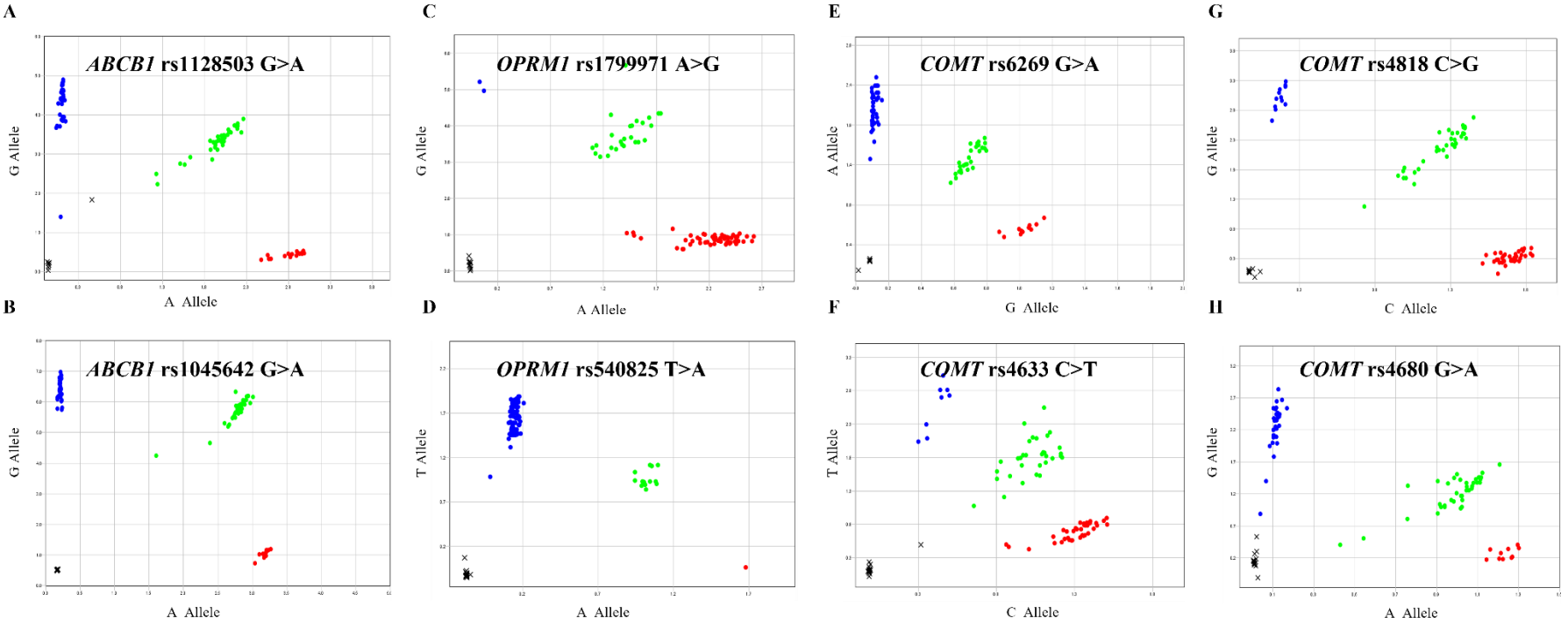


Figure 3.10: Allele discrimination plots (AD plots). Depicted are the AD plots generated from the TaqMan® SNP genotyping assays, used to genotype the South African breast cancer survivors cohort for the A) *ABCB1* rs1128503 G>A, B) *ABCB1* rs1045642 G>A, C) *OPRM1* rs1799971 A>G, D) *OPRM1* rs540825 T>A, E) rs6269 A>G, F) rs4633 C>T, G) rs4818 C>G and H) rs4680 G>A SNPs. Plots show clusters of the major and minor homozygous (blue and/or red), and heterozygous genotypes (green), as well as no template controls and undetermined (X mark) genotypes.

3.4.2 *ABCB1* rs1128503 G>A and rs1045642 G>A Minor Allele Frequencies

The minor allele frequency (MAF) distributions of *ABCB1* rs1128503 G>A within the SA BCS cohort revealed comparable patterns to the global population's frequencies reported in the 1000 Genomes Project, phase3 release version 3+ [Homo sapiens (ID 257713) - BioProject - NCBI (nih.gov)], p>0.05. The rs1128503 A MAF was underrepresented in the SA BCS cohort compared to the G allele (**Figure 3.11**A). A frequency of 40.1% in SA BCS and 41.6% in the global population was noted for the A allele, respectively. The highest reported frequency for the minor allele A was noted in the East Asian population (62.7%), and the lowest in the African population (13.6%); (**Figure 3.11**A).

The MAF distribution of *ABCB1* rs1045642 G>A within the SA BCS cohort revealed significant frequency differences compared to the global population's frequencies reported in the 1000 Genomes project, p<0.05. The rs1045642 A MAF was underrepresented in the SA BCS cohort, compared to the major G allele (**Figure 3.11**B). The A allele noted a frequency of 34.7% in the SA BCS, compared to 39.5% in the global populations. The European population had the highest reported MAF (51.8%) while the lowest frequency was reported in the African population (15.0%); (**Figure 3.11**B). The LD between rs1128503 G>A and rs1045642 G>A was found to be moderate with a D' = 0.73 (p<0.001) within the SA BCS cohort (**Figure 3.12**) similar to that of the global LD values reported for the global superpopulations. Global reported LD (1000 Genomes Project) scores were obtained using the LDpop tool, [LDlink | An Interactive Web Tool for Exploring Linkage Disequilibrium in Population Groups (nih.gov), updated version LDlink 5.6.4 (7/6/2023), accessed on 7 July 2023], using the GRCh38 genome build (**Figure 3.12**).

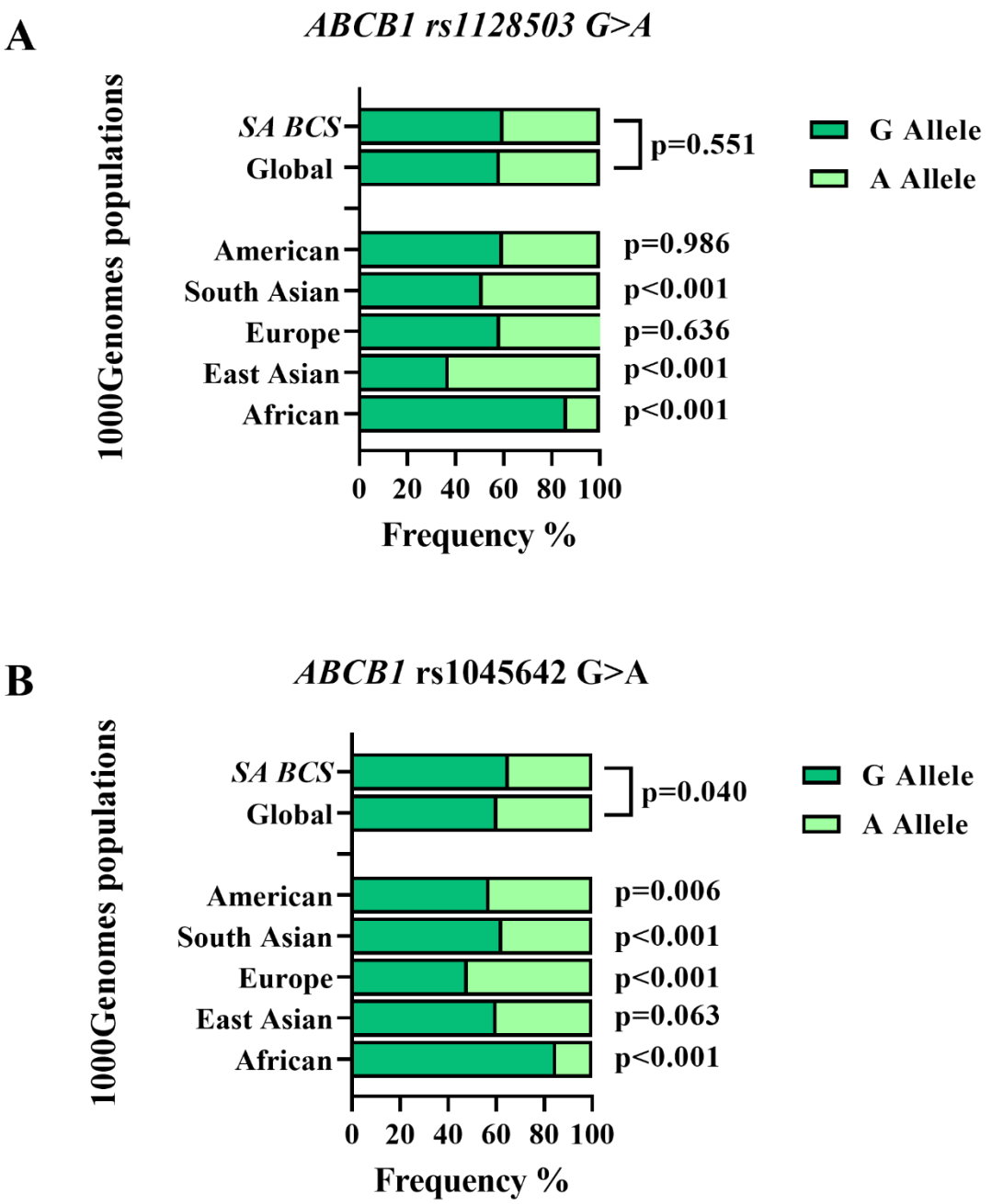


Figure 3.11: The global frequency distribution patterns for the *ABCB1* SNPs evaluated in this study. Global population frequencies were obtained from the public database NCBI-1000Genomes project- (https://www.ncbi.nlm.nih.gov/snp/). Major (green) and minor (white) allele frequencies for A) rs1128503 G>A and B) rs1045642 G>A, are shown for the SA BCS cohort in comparison to the global populations. P values describe the comparison in frequency distribution between the SA BCS cohort, and the global and respective super populations.

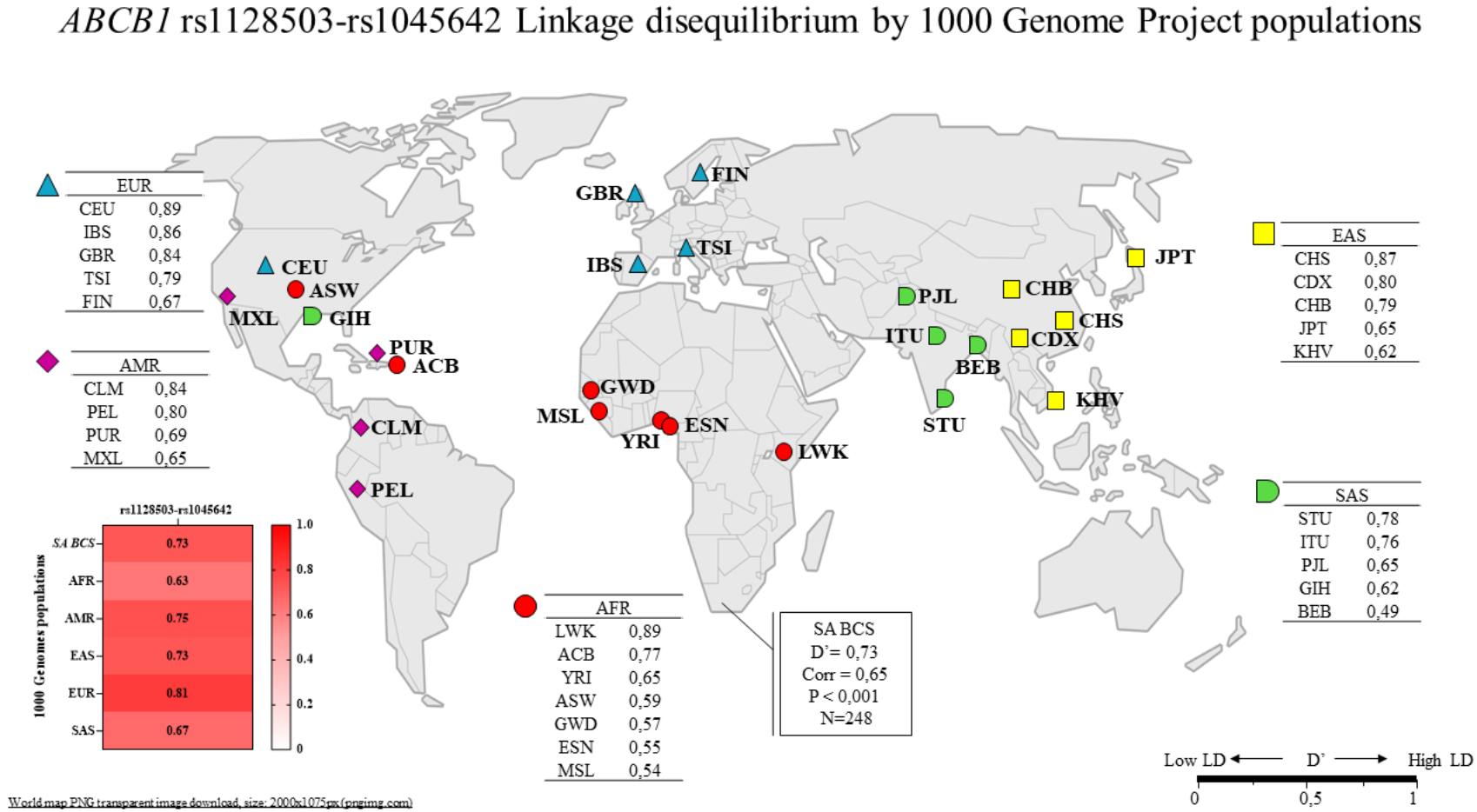


Figure 3.12: Linkage disequilibrium (LD) plot showing the pairwise analysis of the *ABCB1* rs1128503 G>A and rs1045642 G>A SNPs, within the SA BCS cohort. Data of the superpopulations AFR, AMR, EAS, EUR and SAS used to construct this map was obtained from www.ensembl.org (accessed on 1 February 2024). Shown are the │D'│ values, correlation coefficient (Corr), P value (P) and sample size (n) values, with │D'│ values > 0.7 indicating moderate to strong LD. A tabulated summary shows the LD scores for each *COMT* SNP pair

across the 1000 Genomes Projects Populations: AFR, African; AMR, Ad Mixed American; ACA, African Caribbeans in Barbados; ASW, Americans of African Ancestry USA; BEB, Bengali from Bangladesh; GBR, British in England and Scotland; CDX, Chinese Dai in Xishuangbanna, China; CLF, Colombians from Medellin, Colombia; EAS, East Asian; ESN, Esan in Nigeria; EUR, European ; FIN, Finnish in Finland; GWD, Gambian in Western Divisions, Gambia; GIH, Gujarati Indian from Houston, Texas; CHB, Han Chinese in Beijing, China; IBS, Iberian Population in Spain; ITU, Indian Telugu from the UK; JPI, Japanese in Tokyo, Japan; KHV, Kinh in Ho Chi Minh City, Vietnam; LWK, Luhya in Webuye, Kenya; MSI, Mende in Sierra Leone; MXL, Mexican Ancestry from Los Angeles; PEL, Peruvians from Lima, Peru; PUR, Puerto Ricans from Puerto Rico; PJL, Punjabi from Lahore, Pakistan; SAS, South Asian; CHS, Southern Han Chinese; STU, Sri Lankan Tamil from the UK;TSI, Toscani in Italia; CEU, Utah Residents with Northern and Western European Ancestry; YRI, Yoruba in Ibadan, Nigeria.

3.4.3 *OPRM1* rs1799971 A>G, and rs540825 T>A Minor Allele Frequencies

The *OPRM1* rs1799971 A>G SNP MAF in the SA BCS were noted to be comparable to the global populations reported in the 1000 Genomes project, $p>0.05$. The G MAF for rs1799971 A>G was underrepresented in the SA BCS cohort, whereas the major A allele was common. A frequency of 18.5% was observed for the G allele in the SA BCS, compared to 22.3% in the global population (**Figure 3.13**A). The highest and lowest MAFs were noted in the East Asian (39.3%), and African (0.9%) populations.

The MAF distribution pattern for *OPRM1* rs540825 T>A was significantly different between the SA BCS cohort compared to the global population reported in the 1000 Genomes project, $p<0.001$. The rs540825 SNP were noted to have an overrepresented MAF in the SA BCS (22.2%) compared to the reported global population (11.8%) (**Figure 3.13**B). The highest and lowest MAF was reported in the European (22.9%) and African (4.8%) populations respectively. Linkage Disequilibrium analyses noted a weak LD between rs1799971 A>G and rs540825 T>A with a D' =0.04 ($p<0.001$) for the SA BCS and a for the global population (**Figure 3.14**). For *OPRM1*, the global reported LD (1000 Genomes Project) scores were obtained from the LDpop tool, [LDlink | An Interactive Web Tool for Exploring Linkage Disequilibrium in Population Groups (nih.gov), updated version LDlink 5.6.4 (7/6/2023), accessed on 7 July 2023], using the GRCh37 genome build (**Figure 3.14**).

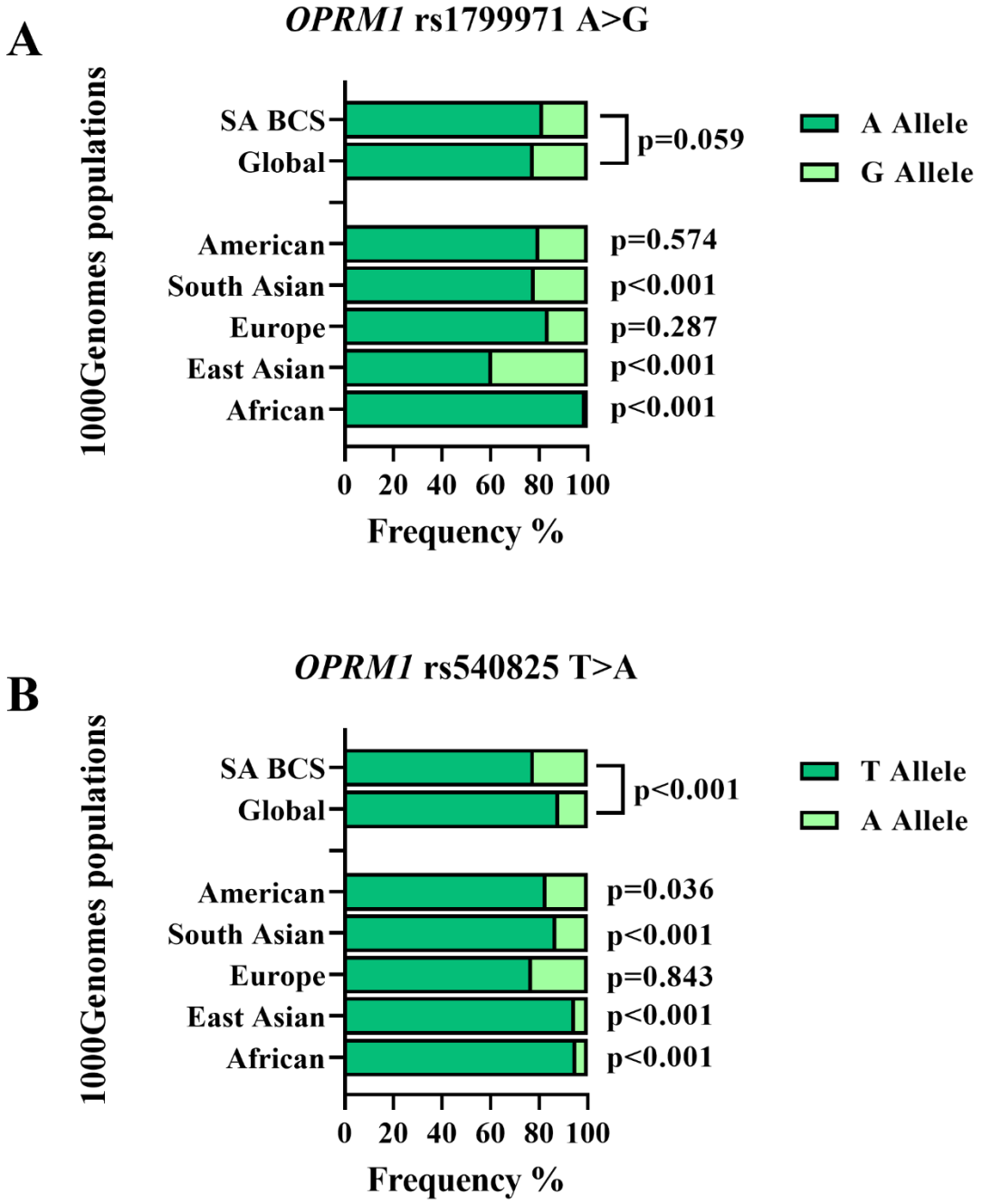


Figure 3.13: The global distribution and prevalence of allele frequencies for the *OPRM1* SNPs. Frequencies were obtained from the public database, NCBI-1000Genomes project- (https://www.ncbi.nlm.nih.gov/snp/). Major (green) and minor (white) allele frequencies for A) rs1799971 A>G and B) rs540825 T>A, are shown for the SA BCS cohort in comparison to the global populations. P values describe the comparison in frequency distribution between the SA BCS cohort, and the global and respective super populations.

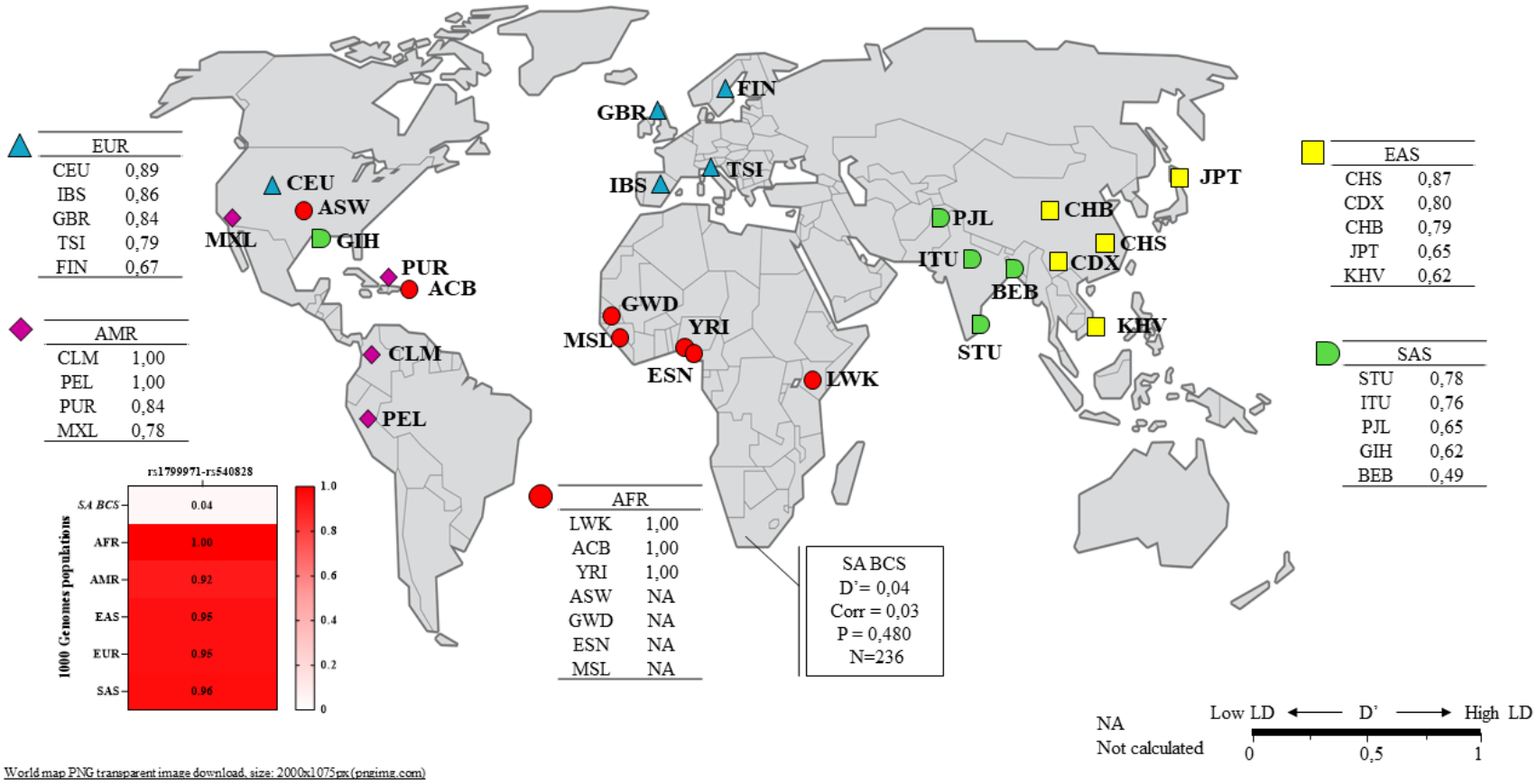


Figure 3.14: Linkage disequilibrium (LD) plot showing the pairwise analysis of *OPRM1* rs1799971 A>G and rs540825 T>A S, within the SA BCS cohort. Data of the superpopulations AFR, AMR, EAS, EUR and SAS used to construct this map was obtained from www.ensembl.org (accessed on 1 February 2024). Shown are the |D'| values, correlation coefficient (Corr), P value (P) and sample size

(n) values, with D'| values > 0.7 indicating moderate to strong LD. A tabulated summary shows the LD scores for each *COMT* SNP pair across the 1000 Genomes Projects Populations: AFR, African; AMR, Ad Mixed American; ACA, African Caribbeans in Barbados; ASW, Americans of African Ancestry USA; BEB, Bengali from Bangladesh; GBR, British in England and Scotland; CDX, Chinese Dai in Xishuangbanna, China; CLF, Colombians from Medellin, Colombia; EAS, East Asian; ESN, Esan in Nigeria; EUR, European ; FIN, Finnish in Finland; GWD, Gambian in Western Divisions, Gambia; GIH, Gujarati Indian from Houston, Texas; CHB, Han Chinese in Beijing, China; IBS, Iberian Population in Spain; ITU, Indian Telugu from the UK; JPI, Japanese in Tokyo, Japan; KHV, Kinh in Ho Chi Minh City, Vietnam; LWK, Luhya in Webuye, Kenya; MSI, Mende in Sierra Leone; MXL, Mexican Ancestry from Los Angeles; PEL, Peruvians from Lima, Peru; PUR, Puerto Ricans from Puerto Rico; PJL, Punjabi from Lahore, Pakistan; SAS, South Asian; CHS, Southern Han Chinese; STU, Sri Lankan Tamil from the UK;TSI, Toscani in Italia; CEU, Utah Residents with Northern and Western European Ancestry; YRI, Yoruba in Ibadan, Nigeria.

3.4.4 *COMT* rs6269 A>G, rs4633 C>T, rs4818 C>G and rs4680 G>A Minor Allele Frequencies

The MAF distribution pattern of the *COMT* (rs6269 A>G, rs4633 C>T, rs4818 C>G and rs4680 G>A) SNPs revealed distinct differences between the SA BCS cohort and the reported global population (**Figure 3.15).** The *COMT* rs6269 (G) and rs4633 (T) MAF were overrepresented in the SA BCS cohort compared to the global populations (1000 Genomes project). The rs6269 G and rs4633 T frequencies were 61.0% and 55.6% in the SA BCS, compared to 39.0% and 44.4% in the global population (**Figure 3.15**A and B). Discounting the SA BCS cohort, the highest and lowest rs6269 MAF were reported in the European (41.0%) and American (31.0%) populations, respectively. The highest and lowest MAF for rs4633 were reported in the European (49.9%) and East Asian (27.0%) populations, respectively.

In contrast, the *COMT* rs4818 G and rs4680 A, MAFs were similar between the SA BCS (26.2% and 39.8%) cohort and the global population (29.7% and 36.9%) respectively, $p>0.05$ (**Figure 3.15**C and D). The highest and lowest MAFs for rs4818 were reported in the European (40.3%) and African (17.0%) populations, respectively. Whereas the rs4680 highest and lowest MAF for rs4680 were reported in the European (50.0%), and East Asian (28.0%) populations, respectively. Linkage disequilibrium analysis noted a strong LD for the *COMT* rs4818 - rs4680 pair ($D' = 0.99$), whereas an LD decay ($D' < 0.9$) was noted for the remaining *COMT* SNP pairs **Figure 3.16**. Similar LD patterns were noted in the global populations obtained from the LDpop tool, [LDlink | An Interactive Web Tool for Exploring Linkage Disequilibrium in Population Groups (nih.gov), updated version LDlink 5.6.4 (7/6/2023), accessed on 7 July 2023], using the GRCh38 genome build (**Figure 3.16**).

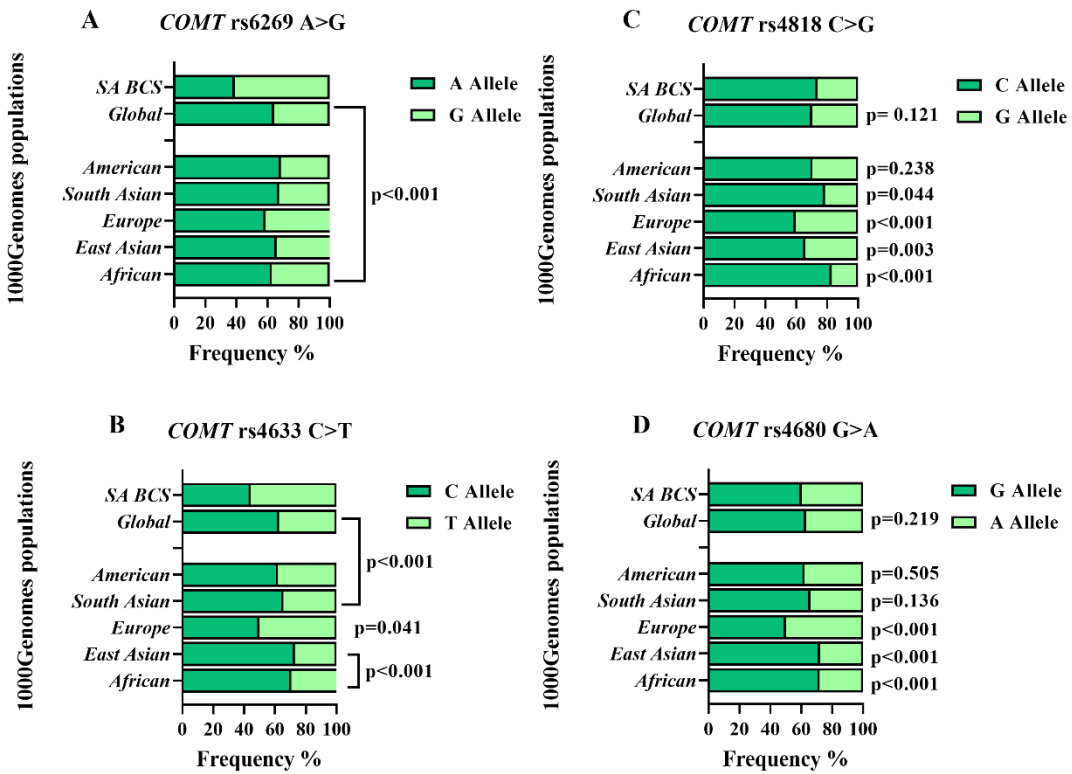
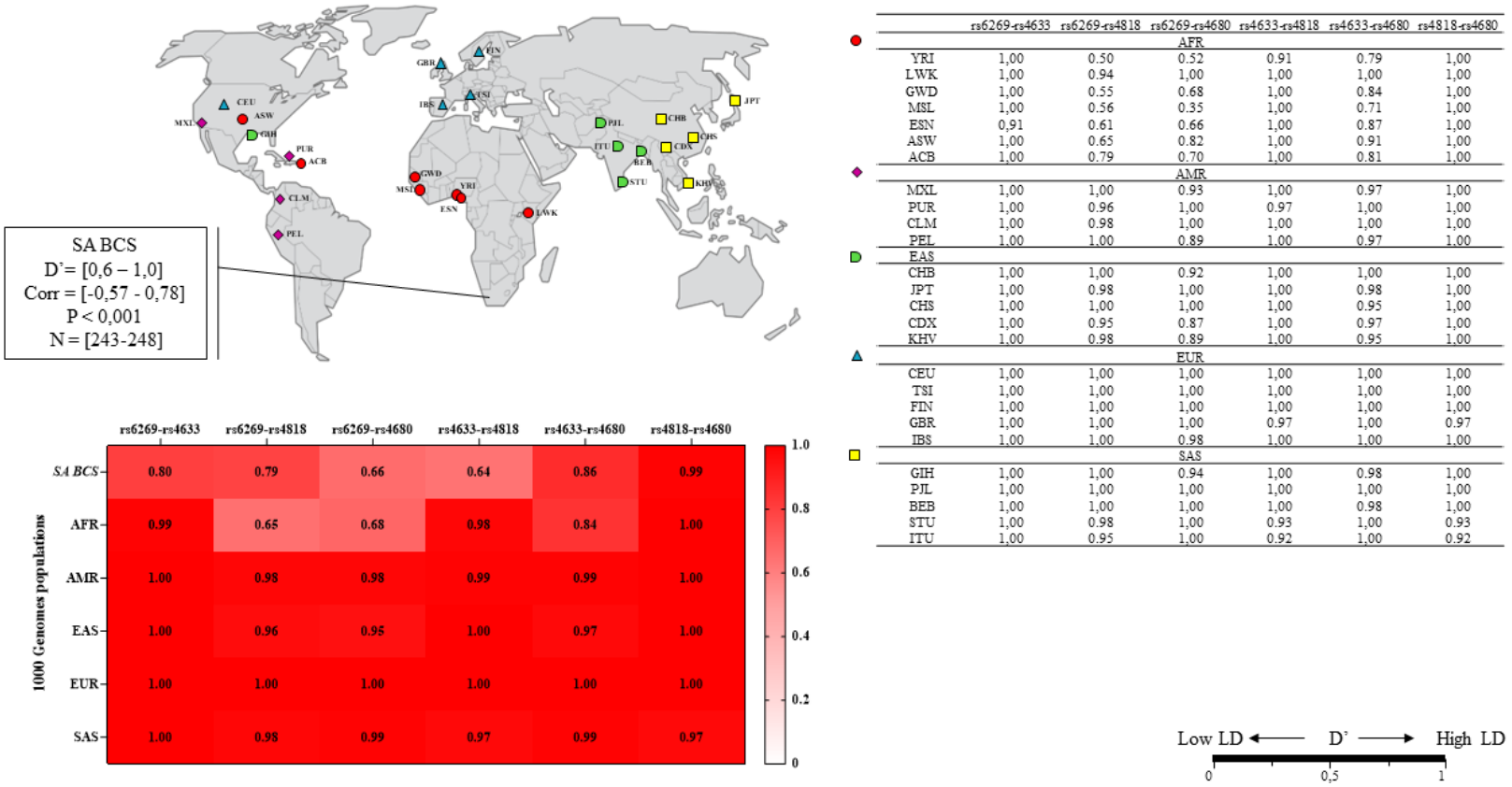


Figure 3.15: The global distribution and prevalence of allele frequencies for the *COMT* SNPs (rs6269 A>G, rs4633 C>T, rs4818 C>G, and rs4680 G>A). Frequencies were obtained from the public database, NCBI-1000Genomes project- (https://www.ncbi.nlm.nih.gov/snp/). Major (green) and minor (white) allele frequencies for A) rs6269 A>G, B) rs4633 C>T, C) rs4818 C>G, and D) rs4680 G>A are shown for the SA BCS cohort in comparison to the global populations. P values describe the comparison in frequency distribution between the SA BCS cohort, and the global and super populations.



	rs6269-rs4633	rs6269-rs4818	rs6269-rs4680	rs4633-rs4818	rs4633-rs4680	rs4818-rs4680
			AFR			
YRI	1,00	0.50	0.52	0.91	0.79	1,00
LWK	1,00	0.94	1,00	1,00	1,00	1,00
GWD	1,00	0.55	0.68	1,00	0.84	1,00
MSL	1,00	0.56	0.35	1,00	0.71	1,00
ESN	0,91	0.61	0.66	1,00	0.87	1,00
ASW	1,00	0.65	0.82	1,00	0.91	1,00
ACB	1,00	0.79	0.70	1,00	0.81	1,00
			AMR			
MXL	1,00	1,00	0.93	1,00	0.97	1,00
PUR	1,00	0.96	1,00	0.97	1,00	1,00
CLM	1,00	0.98	1,00	1,00	1,00	1,00
PEL	1,00	1,00	0.89	1,00	0.97	1,00
EAS						
CHB	1,00	1,00	0.92	1,00	1,00	1,00
JPT	1,00	0.98	1,00	1,00	0.98	1,00
CHS	1,00	1,00	1,00	1,00	0.95	1,00
CDX	1,00	0.95	0.87	1,00	0.97	1,00
KHV	1,00	0.98	0.89	1,00	0.95	1,00
			EUR			
CEU	1,00	1,00	1,00	1,00	1,00	1,00
TSI	1,00	1,00	1,00	1,00	1,00	1,00
FIN	1,00	1,00	1,00	1,00	1,00	1,00
GBR	1,00	1,00	1,00	0.97	1,00	0.97
IBS	1,00	1,00	0.98	1,00	1,00	1,00
			SAS			
GIH	1,00	1,00	0.94	1,00	0.98	1,00
PJL	1,00	1,00	1,00	1,00	1,00	1,00
BEB	1,00	1,00	1,00	1,00	0.98	1,00
STU	1,00	0.98	1,00	0.93	1,00	0.93
ITU	1,00	0.95	1,00	0.92	1,00	0.92

Figure 3.16: Linkage disequilibrium (LD) plot showing the pairwise analysis of *COMT* SNPs, within the SA BCS cohort. Data of the superpopulations AFR, AMR, EAS, EUR and SAS used to construct this map was obtained from www.ensembl.org (accessed on 1 February 2024). Shown are the │D'│values, correlation coefficient (Corr), P value (P) and sample size (n) values, with │D'│ values > 0.7 indicating moderate to strong LD. A tabulated summary shows the LD scores for each *COMT* SNP pair across the 1000 Genomes Projects

Populations: AFR, African; AMR, Ad Mixed American; ACA, African Caribbeans in Barbados; ASW, Americans of African Ancestry USA; BEB, Bengali from Bangladesh; GBR, British in England and Scotland; CDX, Chinese Dai in Xishuangbanna, China; CLF, Colombians from Medellin, Colombia; EAS, East Asian; ESN, Esan in Nigeria; EUR, European ; FIN, Finnish in Finland; GWD, Gambian in Western Divisions, Gambia; GIH, Gujarati Indian from Houston, Texas; CHB, Han Chinese in Beijing, China; IBS, Iberian Population in Spain; ITU, Indian Telugu from the UK; JPI, Japanese in Tokyo, Japan; KHV, Kinh in Ho Chi Minh City, Vietnam; LWK, Luhya in Webuye, Kenya; MSI, Mende in Sierra Leone; MXL, Mexican Ancestry from Los Angeles; PEL, Peruvians from Lima, Peru; PUR, Puerto Ricans from Puerto Rico; PJL, Punjabi from Lahore, Pakistan; SAS, South Asian; CHS, Southern Han Chinese; STU, Sri Lankan Tamil from the UK;TSI, Toscani in Italia; CEU, Utah Residents with Northern and Western European Ancestry; YRI, Yoruba in Ibadan, Nigeria.

3.5 GENETIC ASSOCIATIONS WITH DEMOGRAPHIC AND CLINICAL VARIABLES

Evaluation of the genotype effects on the quantitative and qualitative clinical variables described in **Chapter 2, Section 2.4.1**, are summarized in **Table 3.5**. No significant genotype frequency distribution differences were observed for the *ABCB1*, *OPRM1* and *COMT* SNPs across most of the participant's variables. Apart from the few associations noted for *ABCB1* rs1045642 G>A, rs1128503 G>A, *OPRM1* rs540825 T>A, and *COMT* rs6269A>G, rs4633 C>T and rs4818 C>G (**Table 3.5**).

No discernible differences in the genotype frequency distribution for the *ABCB1*, *OPRM1*, and *COMT* SNPs were found across most of the participants' variables. Except for the few correlations noted for the *ABCB1* rs1045642 G>A, rs1128503 G>A, *OPRM1* rs540825 T>A, and *COMT* rs6269A>G, rs4633 C>T, and rs4818 C>G SNPs, **Table 3.5**.

Table 3.5: Genotype Distribution Patterns Of The *ABCB1, OPRM1 And COMT* SNPs For The Participants' Clinical And Breast Cancer Treatment Variables.

Clinical and Breast Cancer Treatment	*ABCB1*		*OPRM1*		*COMT*			
	rs1128503 G>A	rs1045642 G>A	rs1799971 A>G	rs540825 T>A	rs6269 A>G	rs4633 C>T	rs4818 C>G	rs4680 G>A
Age at surgery	**0,020**	0,503	0,422	0,841	0,921	0,606	0,324	0,212
Time since surgery	0,209	**0,023**	0,896	0,167	0,460	**0,014**	0,404	0,413
Total number of nodes examined	0,886	0,191	0,722	0,707	0,409	0,864	0,760	0,768
Total number of nodes involved	0,670	0,687	0,696	0,258	**0,008**	0,958	0,450	0,610
Side of primary (Left vs Right)	0,916	0,976	0,859	0,803	0,818	0,786	0,243	0,917
Invasive ductal carcinoma	0,984	0,579	0,899	0,573	0,539	0,575	0,121	0,505
Lymphovascular invasion	0,137	0,775	0,411	0,605	0,385	0,954	0,463	0,446
Tumour grade (I, II, III and IV)	0,802	0,733	0,960	0,300	0,129	0,508	0,299	0,850
Type of surgery (MTX vs WLE)	0,207	0,190	0,413	0,399	0,673	0,917	0,736	0,822
Neo CT (Yes vs No)	0,647	0,587	0,511	0,292	**0,009**	0,788	0,925	0,736
Adjuvant CT (Yes vs No)	0,520	0,400	0,791	0,440	0,207	0,750	0,534	0,977
Adjuvant HT (Yes vs No)	0,832	0,673	0,844	0,831	0,689	0,823	0,152	0,823
Adjuvant RT (Yes vs No)	0,093	0,098	0,628	0,712	**0,002**	0,627	0,400	0,534
Lymph node surgery (ALND vs SLNB vs None)	0,706	0,241	0,920	**0,015**	0,135	0,296	**0,002**	0,202

Notes: **Bold** p-values indicate significant differences ($p<0.05$) between the genotype groups. Abbreviations: MTX, Mastectomy; WLE, Wide local excision; ALND, Axillary lymph node dissection; SLNB, Sentinel lymph node biopsy. Tests used for comparative analysis includes Kruskal Wallis H test, Fisher's exact test (*when a sample was <10); Chi-squared test.

3.5.1 *ABCB1* SNP Genotypes and Participants' Variables

Evaluation of the genotype effects of *ABCB1* SNPs on the quantitative and qualitative clinical variables noted a few significant associations (**Figure 3.17**). The *ABCB1* rs1128503 G>A polymorphism was associated with "age at surgery", where the median (IQR) age was 52 (22,74) yrs. for A/G carriers, compared to 56 (33,71) and 55 (36,71) yrs. for G/G and A/A carriers respectively, rendering a possible sample bias (**Figure 3.17**A). The *ABCB1* rs1045642 G>A polymorphism was associated with "Time since surgery", where the median (IQR) time that had passed was 2 (0.42,10) and 2 (0.42,9) yrs. for G/G and A/A carriers compared to 3 (0.42,10) yrs. for A/G carriers (**Figure 3.17**B). No associations were further observed between the SNPs, rs1128503 G>A, and rs1045642 G>A, and the remaining clinical variables, and breast cancer treatment modalities received, p>0.05.

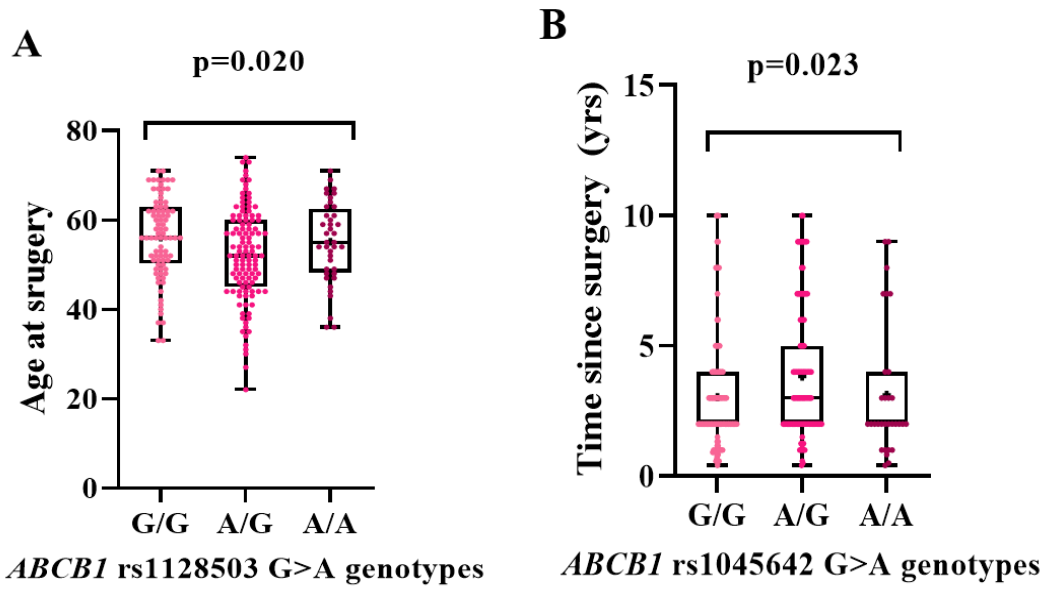


Figure 3.17: The *ABCB1* rs1045642 G>A and rs1128503 G>A genotype frequency distribution for the clinical characteristic's times since surgery, and age at surgery.

3.5.2 *OPRM1* SNP Genotypes and Participants' Variables

For the *OPRM1* rs1799971 A>G polymorphism, no significant differences were observed between the A/A, A/G, and G/G genotypes, and participants' variables were assessed in this study (**Figure 3.18**). Evaluation of the genotype distribution of the *OPRM1* rs540825 T>A SNP showed no differences in the quantitative variables between the T/T, T/A and A/A genotypes. On the other hand, of the categorical variables, lymph node surgery was noted to be significantly different between the genotypes for rs540825 T>A, p=0.015 (**Figure 3.18**). The *OPRM1* rs540825 A/A (3.6% and 3.2%) genotype was less frequently observed in participants who received lymph node (ALND and SLNB) surgery, compared to the T/T (59.2% and 51.6%) and A/T (36.9% and 45.2%) genotypes, respectively.

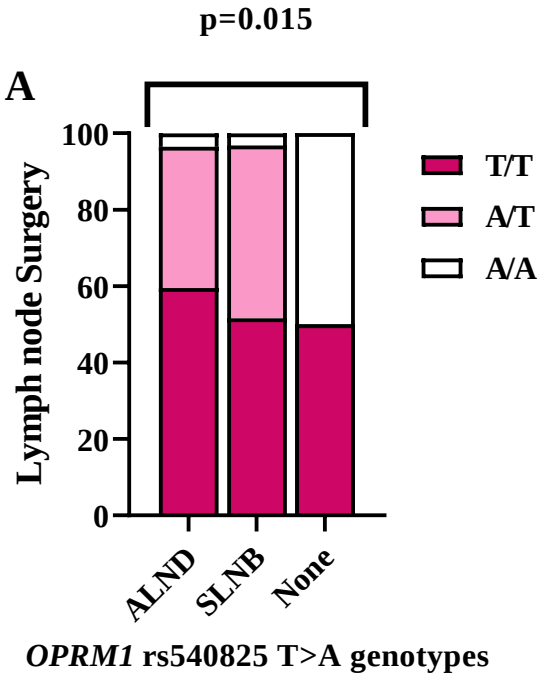


Figure 3.18: The *OPRM1 rs540825 T>A* genotype frequency distribution patterns for participants receiving the different lymph node surgeries.

3.5.3 *COMT* SNP Genotypes and Participants' Variables

Significant differences in the genotype distributions of the *COMT* rs6269 A>G, rs4633 C>T and rs4818 C>G polymorphisms were noted when the clinical variables were evaluated. (**Table 3.5**). Differences in the genotype distributions for *COMT* rs6269 A>G were noted between participants and the total number of nodes involved (p=0.008), however, the medians and IQRs were comparable between the genotypes (**Figure 3.19**A). It was interesting to note that for rs6269 A>G, fewer A/A (10.3% and 13%) genotype carriers received Neo CT (p=0.009), and RT (p=0.002) treatments, compared to G/G (44.8% and 39.6%) and A/G (44.8% and 47.4%) genotype carriers (**Figure 3.19**B and C). Evaluation of the rs4633 C>T also showed significant differences in the distribution of the genotypes (p=0.014) such that C/C [3 (2,4)] genotype carriers had a greater median (IQR) of time (yrs.) since surgery, in comparison to C/T [2 (2,4)] and T/T [2 (2,4)] genotype carriers (**Figure 3.19**D). Evaluation of participants with lymph node surgery with rs4818 showed that the C/C (48.6% and 74.2%) and C/G (46.9% and 22.6%) genotype carriers (Figure 3.19E) received more ALND and SLNB treatment (p=0.002) in comparison to the G/G (4.5% and 3.2%) genotype carriers (**Figure 3.19**E). No associations were noted between rs4680 G>A, and clinical variables assessed.

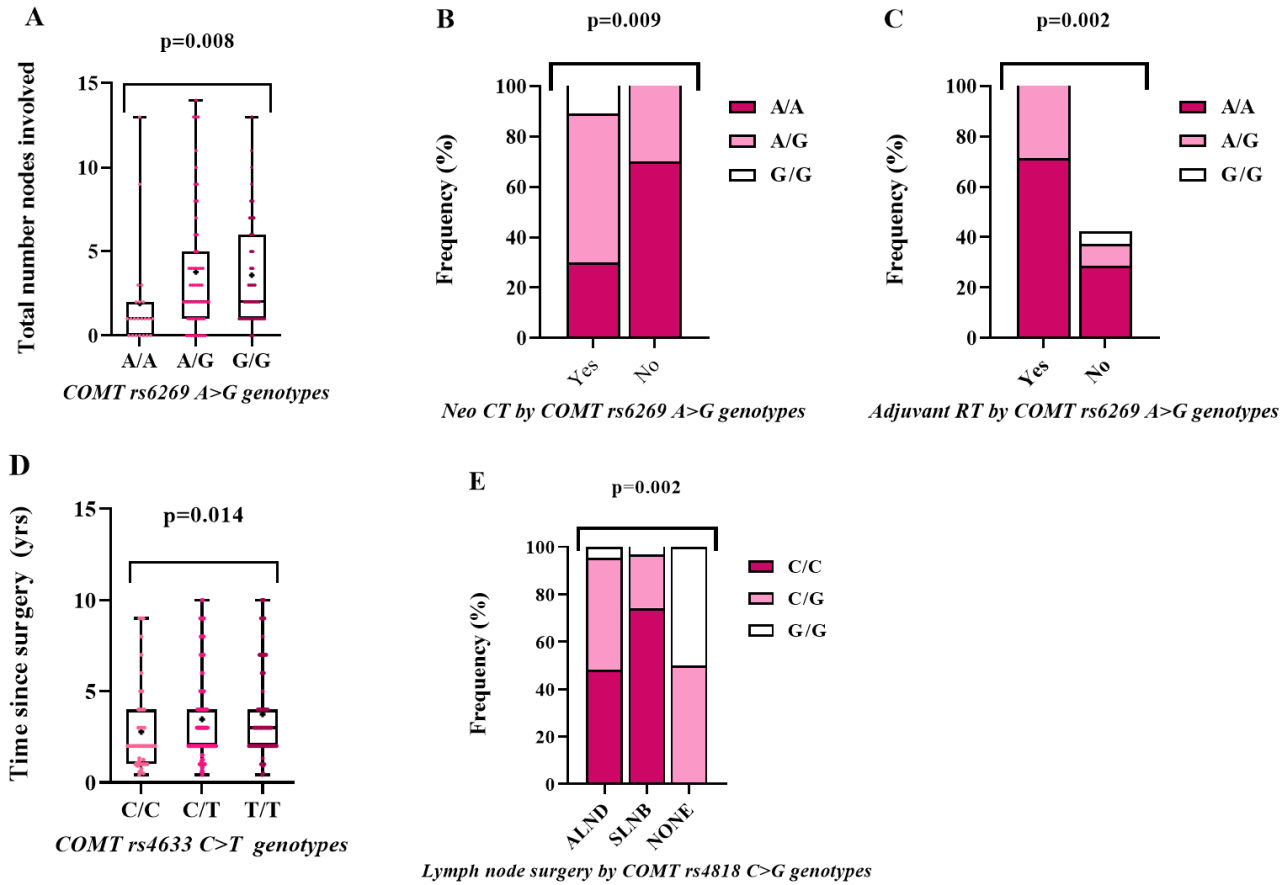


Figure 3.19: The genotype frequency distribution patterns of *COMT* rs6269 A>G, rs4633 C>T and rs4818 C>G between participants for clinical variables: the total number of nodes involved, Neo-chemotherapy and Radiation Therapy adjuvant received, time since surgery, and lymph node surgery received.

3.6 GENOTYPE AND ALLELE FREQUENCY DISTRIBUTIONS

3.6.1 *ABCB1* rs1128503 G>A and rs1045642 G>A

Table 3.6 summarises the adjusted (age at surgery) genotype and allele frequency distribution patterns between the no-low and moderate-high pain, disability, and combined (pain and disability) groups. The analyses noted no significant frequency differences for rs1128503 G>A between groups (no-low and moderate-high) in the pain, disability and combined categories, p>0.05 (**Table 3.6**).

For the *ABCB1* rs1045642 G>A SNP [(adjusted age at surgery), **Table 3.6**] analyses no significant differences between the groups (no-low vs moderate-high) of pain were observed.

In the disability category the A/A (p=0.028, OR:0.21, 95% CI:0.05-0.93, AIC: 228.5) genotype was significantly overrepresented in the no-low (14.9%) group, compared to the moderate-high (4.3%) group. In the dominant (p=0.022, OR: 0.46, 95% CI: 0.24-0.90, AIC:228.4) and the recessive (p=0.045, OR: 0.28, 95% CI: 0.06-1.21, AIC: 229.6) models, the A/A genotypes displayed significant differences in the frequency distribution patterns between groups. Based on AIC scores for rs1045642 G>A, the dominant model exhibited the most significant genetic model in the adjusted disability category (**Table 3.6**). In line with this finding, the A (p=0.015, OR:0.52, 95% CI:0.29-0.89) allele noted similar frequency distributions in the no-low (37.9%) and moderate-high (23.9%) groups. The *ABCB1* rs1045642 A/A genotype, and A allele, were therefore associated with a reduced likelihood of reporting moderate-high disability in SA BCS.

In the combined (pain and disability) category, significant differences in frequency distribution for the rs1045642 A/A (p=0.011, OR:0.25, 95% CI:0.07-0.89, AIC: 243.5) genotype were noted. The A/A genotype was overrepresented in the no-low (15.0%) group compared to the moderate-high (5.7%) group (Table 3.6). In the dominant model, the rs1045642 A/A-A/G vs G/G (p=0.004, OR: 0.40, 95% CI: 0.21-0.75, AIC: 242.3) genotype was significantly overrepresented in the no-low group compared to the moderate-high group. Based on the AIC score, this model was the most significant model for rs1045642 in the combined (pain and disability) category (**Table 3.6**). The A (p=0.003, OR: 0.48, 95% CI: 0.28-0.80) allele was also overrepresented in the no-low (38.5%) group compared to the moderate-high (23.6%) group, and therefore associated with a reduced likelihood of reporting moderate-high combined pain and disability (**Table 3.6**).

The *ABCB1* SNPs rs1128503 G>A and rs1045642 G>A both adhered to Hardy Hardy-Weinberg equilibrium in the no-low and moderate-high groups of pain, disability, and combination (pain and disability) categories, p>0.05 (**Table 3.6**).

Table 3.6: Adjusted genotype and minor allele frequency distributions of the *ABCB1* (rs1128503 G>A, rs1045642 G>A) polymorphisms between pain, disability, and combined (pain and disability) categories.

	Pain		Disability				Pain and Disability			
Polymorphism	**No-Low**	**Mod -High**	**No-Low**	**Mod-High**			**No-Low**	**Mod-High**		
SA BCS	**(n=184)**	**(n=68)**	**(n= 204)**	**(n=48)**		**AIC**	**(n=197)**	**(n=55)**		**AIC**
rs1128503 G>A	(n=174)	(n=66)	(n=194)	(n=46)			(n=187)	(n=53)		
G/G	35,1 (61)	34,8 (23)	35,6 (69)	32,6 (15)			35,3 (66)	34,0 (18)		
A/G	47,1 (82)	51,5 (34)	46,9 (91)	54,3 (25)			46,5 (87)	54,7 (29)		
A/A	17,8 (31)	13,6 (9)	17,5 (34)	13,0 (6)			18,2 (34)	11,3 (6)		
A allele	41.4 (144)	39.4 (52)	41.0(159)	40.2 (37)			41.4 (155)	38.7 (41)		
P value[1]	0.783		0.812				0.552			
A Allele P value[2]	0.755		0.907				0.655			
HWE	0.758	0.765	0.660	0.545			0.551	0.390		
rs1045642 G>A	(n=174)	(n=66)	(n=194)	(n=46)			(n=187)	(n=53)		
G/G	40,2 (70)	48,5 (32)	39,2 (76)	56,5 (26)	**0,022[a]**	228,4	38,0 (71)	58,5 (31)	**0,004[a]**	242,3
A/G	46,6 (81)	39,4 (26)	45,9 (89)	39,1 (18)	0,275[b]	232,5	47,1 (88)	35,8 (19)	0,076[b]	247,3
A/A	13,2 (23)	12,1 (8)	14,9 (29)	4,2 (2)	**0,045[c]**	229,6	15,0 (28)	5,7 (3)	0,076[c]	247,3
A allele	36.5 (127)	31.8 (42)	37.9 (147)	23.9 (22)			38.5 (144)	23.6 (25)		
P value[1]	0.367		**0.028**			228,5	**0.011**			243,5
A Allele P value[2]	0.392		**0.015**				**0.003**			
HWE	1.000	0.405	0.762	0.709			0.879	1.000		

Notes: Genotype and allele frequencies are expressed as a percentage (%) with the number of participants (n) in parentheses. Global P values[1] for genotype between groups and P values[2] for allele between groups; P-values in **bold** indicate significance ($P<0.05$). P values for logistic regression analysis are listed for the dominant [a], over-dominant [b], and recessive [c] models. P-values for the exact test of Hardy–Weinberg equilibrium for each of the categories are included in the Table; Abbreviations: AIC: Akaike information criterion score; Mod-High: Moderate-High; HWE: Hardy–Weinberg equilibrium.

3.6.2 *OPRM1* rs1799971 A>G, and rs540825 T>A

Evaluation of the adjusted (age at surgery) genotype and allele frequency distribution for *OPRM1* rs1799971 A>G and rs540825 T>A, noted no significant differences in distribution patterns between the no-low and moderate-high groups for pain, disability and combined (pain and disability), $p>0.05$ (**Table 3.7**). Hardy-Weinberg equilibrium analyses for *OPRM1* rs1799971 A>G and rs540825 T>A showed both SNPs were in HWE, in the pain, disability, and combined (pain and disability) categories (**Table 3.7**).

Table 3.7: Adjusted genotype and minor allele frequency distributions, of *OPRM1* rs1799971 A>G, rs540825 T>A polymorphisms between pain, disability, and combined (pain and disability) categories.

	Pain		Disability		Pain and Disability	
Polymorphisms	**No-Low**	**Mod -High**	**No-Low**	**Mod-High**	**No-Low**	**Mod-High**
SA BCS	**(n=184)**	**(n=68)**	**(n= 204)**	**(n=48)**	**(n=197)**	**(n=55)**
rs1799971 A>G	(n=174)	(n=66)	(n=194)	(n=46)	(n=187)	(n=53)
A/A	64,4 (112)	75,8 (50)	68,0 (132)	65,2 (30)	66,8 (125)	69,8 (37)
A/G	30,5 (53)	22,7 (15)	27,3 (53)	32,6 (15)	28,3 (53)	28,3 (15)
G/G	5,2 (9)	1,5 (1)	4,6 (9)	2,2 (1)	4,8 (9)	1,9 (1)
G allele	20.4 (71)	12.9 (17)	18,3 (71)	18.5(17)	19.0 (71)	16.0 (17)
P value[1]	0.199		0.497		0.587	
G Allele P value[2]	0.064		1.000		0.570	
HWE	0.496	1.000	0.244	0.663	0.346	1.000
rs540825 T>A	(n=165)	(n=63)	(n=184)	(n=44)	(n=177)	(n=51)
T/T	60,0 (99)	60,3 (38)	61,4 (113)	54,5 (24)	61,0 (108)	56,9 (29)
A/T	35,8 (59)	34,9 (22)	35,3 (65)	36,4 (16)	35,6 (63)	35,3 (18)
A/A	4,2 (7)	4,8 (3)	3,3 (6)	9,1 (4)	3,4 (6)	7,8 (4)
A allele	22,1 (73)	22.2 (28)	20.9 (77)	27.3 (24)	21,2 (75)	25,5 (26)
P value[1]	0.990		0.275		0.435	
A Allele P value[2]	1.000		0.201		0.347	
HWE	0.660	1.000	0.385	0.464	0.381	0.481

Notes: Genotype and allele frequencies are expressed as a percentage (%) with the number of participants (n) in parentheses. Global P values[1] for genotype between groups and P values[2] for allele between groups; P-values in **bold** indicate significance (P<0.05). P values for logistic regression analysis are listed for the dominant [a], over-dominant [b], and recessive [c] models (not shown). P-values for the exact test of Hardy–Weinberg equilibrium for each of the categories are included in the Table; Abbreviations: AIC: Akaike information criterion score; Mod-High: Moderate-High; HWE: Hardy–Weinberg equilibrium.

3.6.3 *COMT* rs6269 A>G, rs4633 C>T, rs4818 C>G and rs4680 G>A

The adjusted (age at surgery) analyses of genotype and allele frequency distribution for the *COMT* SNPs, rs6269 A>G, rs4633 C>T and rs4818 C>G SNPs, noted no significant associations for each of the categories (**Table 3.8**).

For the *COMT* SNP, rs4680 G>A, the A/A (p=0.024, OR: 3.23, 95% CI: 1.33-7.81: 268.7) genotype was significantly underrepresented in the no-low (12.7%) group compared to the moderate-high (21.5%) pain group (**Table 3.8**). In the dominant model, the rs4680 A/A (p=0.015, OR: 2.19, 95% CI: 1.14-4.21, AIC: 268.3) genotype was significantly underrepresented in the no-low group, compared to the moderate-high group. In the recessive model, the A/A (p=0.050, OR: 2.17, 95% CI: 1.01-4.67, AIC: 270.4) genotype displayed the same distribution pattern, however, only a trend of association was noted. Based on the AIC scores, the dominant model exhibited the most significant model for *COMT* rs4680 G>A. In alignment with this finding, the *COMT* rs4680 A (p=0.035, OR: 1.58, 95% CI: 1.03-2.43) allele was significantly underrepresented in the no-low (35.8%) group, compared to the moderate-high (46.9%) group. The *COMT* rs4680 A/A genotype and A allele were therefore associated with an increased likelihood of reporting moderate-high pain (**Table 3.8**).

No significant associations were noted between *COMT* rs4680 G>A, and the disability category (**Table 3.8**), p>0.05.

In the combined (pain and disability) category, the *COMT* rs4680 A/A (p=0.015, OR: 3.81, 95% CI: 1.47-9.85, AIC: 240.3) genotype was significantly underrepresented in the no-low (12.9%) group compared to the moderate-high (23.1%) group (**Table 3.8**). The dominant (p=0.009, OR: 2.51, 95% CI: 1.22-5.17, AIC: 240.0) and recessive (p=0.041, OR: 2.36, 95% CI: 1.06-5.24, AIC: 241.6) models displayed a significant association for the rs4680 A/A genotype. The A/A-A/G (dominant) and A/A (recessive) genotypes were significantly underrepresented in the no-low group compared to the moderate-high group. Based on the AIC score, the dominant model exhibited the most significant model for the *COMT* rs4680 G>A SNP. Similarly, the *COMT* rs4680 (A) (p=0.017, OR: 1.71, 95% CI: 1.07-2.71) allele was significantly underrepresented in the no-low (36.0%) compared to the moderate-high (49.0%) group. The *COMT* rs4680 A/A genotype and A allele were once more associated with an increased likelihood of reporting moderate-high combined pain and disability (**Table 3.8**).

Table 3.8: Adjusted genotype and minor allele frequency distributions, of the *COMT* (rs6269 A>G; rs4633 C>T; rs4818 C>G; rs4680 G>A) polymorphisms between pain, disability and combined (pain and disability) categories.

Polymorphism	Pain				Disability		Pain and Disability			
	No-Low (n=184)	Mod - High (n=68)		AIC	No-Low (n= 204)	Mod-High (n=48)	No-Low (n=197)	Mod-High (n=55)		AIC
rs6269 A>G	(n=175)	(n=65)			(n=195)	(n=45)	(n=188)	(n=52)		
G/G	38,9 (68)	35,4 (23)			38,5 (75)	35,6 (16)	37,8 (71)	38,5 (20)		
A/G	45,1 (79)	46,2 (30)			45,1 (88)	46,7 (21)	46,3 (87)	42,3 (22)		
A/A	16 (28)	18,5 (12)			16,4 (32)	17,8 (8)	16 (30)	19,2 (10)		
G allele	61,4 (215)	58,5 (76)			61,0 (238)	58,9 (53)	60,9 (229)	59,6 (62)		
P value[1]	0,848				0,937		0,787			
G Allele P value[2]	0,600				0,721		0,821			
HWE	0,532	0,617			0,457	1,000	0,651	0,406		
rs4633 C>T	(n=175)	(n=65)			(n=195)	(n=45)	(n=188)	(n=52)		
T/T	34,3 (60)	29,2 (19)			34,9 (68)	24,4 (11)	35,1 (66)	25 (13)		
C/T	46,9 (82)	47,7 (31)			44,6 (87)	57,8 (26)	45,7 (86)	51,9 (27)		
C/C	18,9 (33)	23,1 (15)			20,5 (40)	17,8 (8)	19,1 (36)	23,1 (12)		
T allele	57,7 (202)	53,1 (69)			57,2 (223)	53,3 (48)	58,0 (218)	51,0 (53)		
P value[1]	0,557				0,178		0,261			
T Allele P value[2]	0,407				0,556		0,220			
HWE	0,546	0,628			0,154	0,389	0,307	1,000		
rs4818 C>G	(n=171)	(n=65)			(n=192)	(n=44)	(n=185)	(n=51)		
C/C	52 (89)	52,3 (34)			51,6 (99)	54,5 (24)	50,8 (94)	56,9 (29)		
C/G	43,3 (74)	43,1 (28)			43,8 (84)	40,9 (18)	44,3 (82)	39,2 (20)		
G/G	4,7 (8)	4,6 (3)			4,7 (9)	4,5 (2)	4,9 (9)	3,9 (2)		
G allele	26,3 (90)	26,2 (34)			26,6 (102)	25,0 (22)	27,0 (100)	23,5 (24)		
P value[1]	0,480				0,880		0,618			
G Allele P value[2]	1.000				0,893		0,527			
HWE	0,247	0,526			0,201	0,702	0,199	0,707		
rs4680 G>A	(n=173)	(n=65)			(n=193)	(n=45)	(n=186)	(n=52)		
G/G	41 (71)	27,7 (18)	0,015[a]	268.3	39,9 (77)	26,7 (12)	40,9 (76)	25 (13)	**0,009[a]**	240.0
A/G	46,2 (80)	50,8 (33)	0,382[b]	273.4	45,6 (88)	55,6 (25)	46,2 (86)	51,9 (27)	0,342[b]	245.9

A/A	12,7 (22)	21,5 (14)	0,050[c]	270.4	14,5 (28)	17,8 (8)	12,9 (24)	23,1 (12)	**0,041[c]**	242.6
A allele	35,8 (124)	46,9 (61)			37,3 (144)	45,6 (41)	36,0 (134)	49,0 (51)		
P value[1]	**0,024**			268.7	0,113		**0,015**			240.3
A Allele P value[2]	**0,035**				0,152		**0,017**			
HWE	0,874	1,000			0,550	0,564	0,877	1,000		

Notes: Genotype and allele frequencies are expressed as a percentage (%) with the number of participants (n) in parentheses. Global P values[1] for genotype between groups and P values[2] for allele between groups; P-values in **bold** indicate significance ($P<0.05$). P values for logistic regression analysis are listed for the dominant [a], over-dominant [b], and recessive [c] models. P-values for the exact test of Hardy–Weinberg equilibrium for each of the categories are included in the Table; Abbreviations: AIC: Akaike information criterion score; Mod-High: Moderate-High; HWE: Hardy–Weinberg equilibrium.

3.7 INFERRED HAPLOTYPE ANALYSES

Haplotypes were statistically inferred and constructed for each of the genes using the individual genotype data for the *ABCB1* (**Supplemental Table 11**), *OPRM1* (**Supplemental Table 13**) and *COMT* (**Supplemental Table 15, Supplemental Table 16, Supplemental Table 17)** SNPs. All data presented here is adjusted for the confounder "age at surgery", with unadjusted data presented in the supplementary **Section 7.4.1**.

3.7.1 Inferred *ABCB1* (rs1128503 G>A - rs1045642 G>A) Haplotypes

The inferred *ABCB1* rs1128503 G>A- rs1045642 G>A haplotype analyses yielded the combinations G-G, A-A, A-G and G-A (**Figure 3.20**). No significant associations were evident for the inferred rs1128503-rs1045642 haplotype in the pain category p=0.798 (**Figure 3.20**A). In the disability (p=0.027) category, the inferred G-A haplotype was overrepresented in the no-low (6.9%) group and absent in the moderate-high (0.0%) group (**Figure 3.20**B). The inferred G-A(p=0.029, OR: 0.00, 95% CI: 0.00-0.00, haploscore= -2.38) haplotype was associated with a reduced likelihood of reporting moderate-high disability. In the combined (pain and disability);(p=0.019) category, the inferred A-A haplotype was overrepresented in the no-low (30.9%) group compared to the moderate-high (22.5%) group (**Figure 3.20**C). The inferred A-A (p=0.029, OR:0.63, 95% CI: 0.37-1.06, haploscore = -1.86) haplotype was also associated with a reduced likelihood of reporting moderate-high combined (pain and disability).

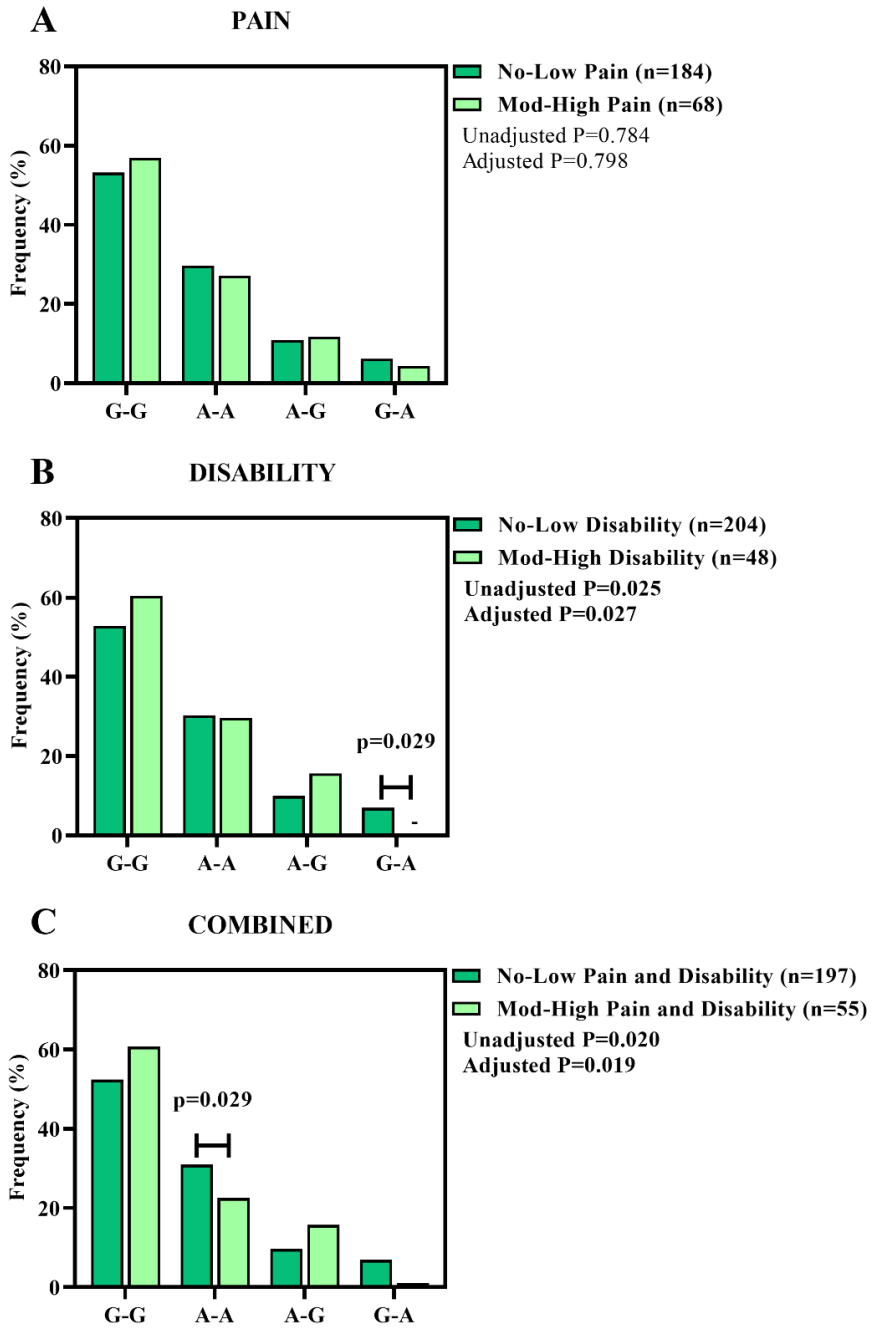


Figure 3.20: The inferred frequency distributions for the *ABCB1* (rs1128503 G>A-rs1045642 G>A) haplotypes. Shown are the no-low (green) and moderate-high (lime) groups for A) pain, B), disability and C) combined (pain and disability) in South African BCS. Depicted are statistically significant differences in the inferred haplotype frequencies between the two groups with the number of participants in parenthesis (n) and (-) presenting no frequency detected for a haplotype. All haplotype p-values shown are adjusted for age at surgery.

3.7.2 Inferred *OPRM1* (rs1799971 A>G-rs540825 T>A) Haplotypes

The inferred *OPRM1* rs1799971 A>G-*OPRM1* rs540825 T>A haplotype analyses generated the A-T, A-A, G-T and G-A combinations (**Figure 3.21**). In the pain (p=0.040) category, the inferred G-T haplotype was overrepresented in the no-low (16.4%) group compared to the moderate-high (6.9%) group. The inferred G-T (p=0.019, OR:0.33, 95% CI: 0.14-0.75, haploscore = -2.30) haplotype was therefore associated with a reduced likelihood of reporting moderate-high pain (**Figure 3.21**A). No significant associations were further noted for this haplotype in the disability or combined (pain and disability) categories, p>0.05 (**Figure 3.21**B and C).

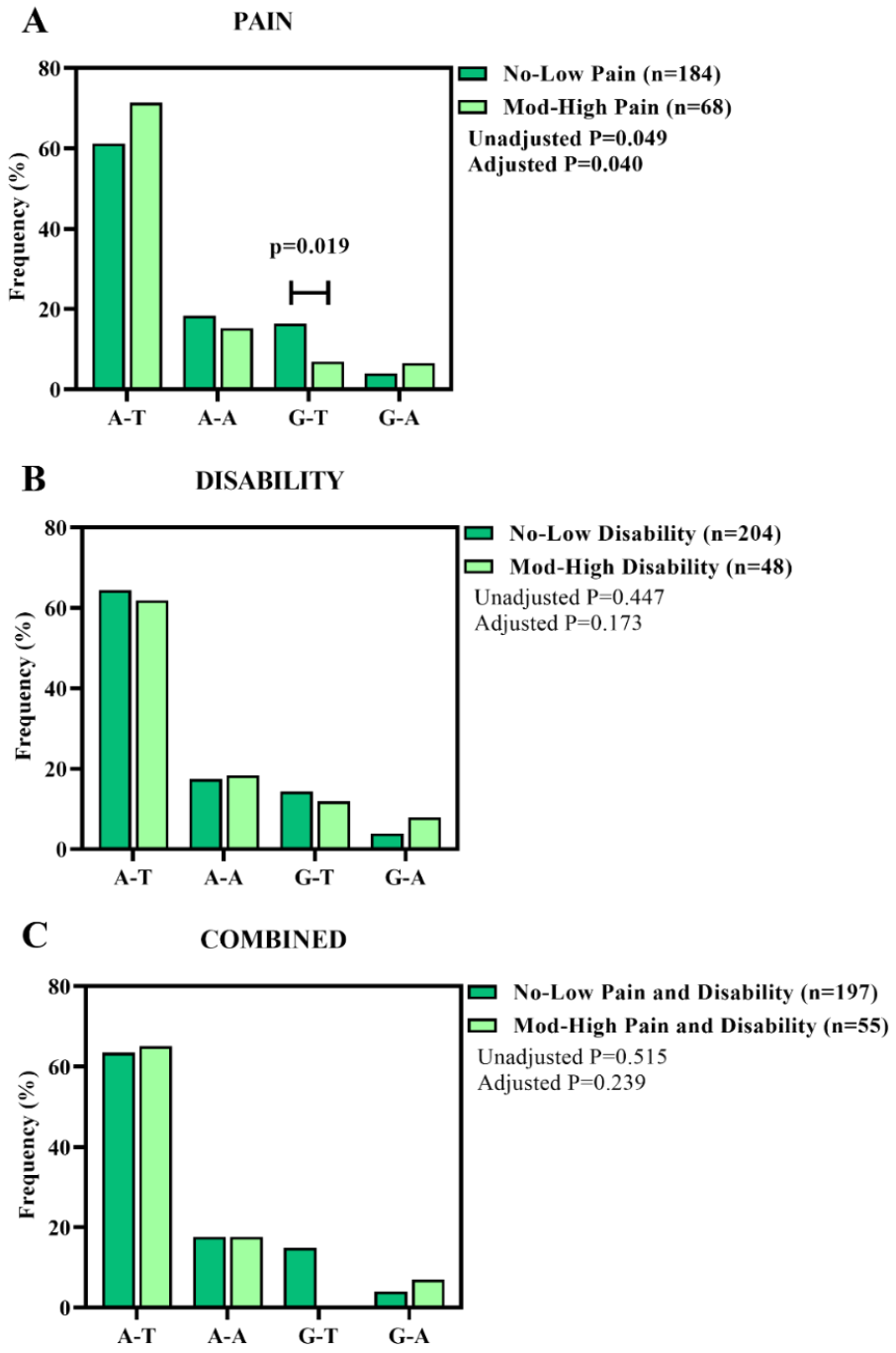


Figure 3.21: The inferred frequency distributions for the *OPRM1* (rs1799971 A>G-rs540825 T>A) haplotypes. Shown are the no-low (green) and moderate-high (lime) groups for A) pain, B), disability and C) combined (pain and disability) in South African BCS. Depicted are statistically significant differences in the inferred haplotype frequencies between the two groups with the number of participants in parenthesis (n) and (-) presenting frequency detected for a haplotype. All haplotype p-values shown are adjusted for age at surgery.

3.7.3 Inferred *COMT* (rs6269 A>G, rs4633 C>T, rs4818 C>G-rs4680 G>A) Haplotypes

Five inferred haplotypes were generated using *COMT* rs6269 A>G, rs4633 C>T, rs4818 C>G and rs4680 G>A SNPs that allowed assessment of the genomic region spanning the central haploblock for *COMT* (**Table 2.1**). The first inferred haplotype H1 (*COMT* rs6269 A>G - rs4633 C>T- rs4818 C>G - rs4680 G>A), yielded twelve haplotype combinations with seven reporting frequencies of < 3% in the no-low groups (**Supplemental Table 15**). No significant frequency distribution differences were, however, noted for the inferred H1 haplotypes, between the no-low and moderate-high groups for pain, disability and combined (pain and disability) categories, p>0.05 (**Figure 3.22**).

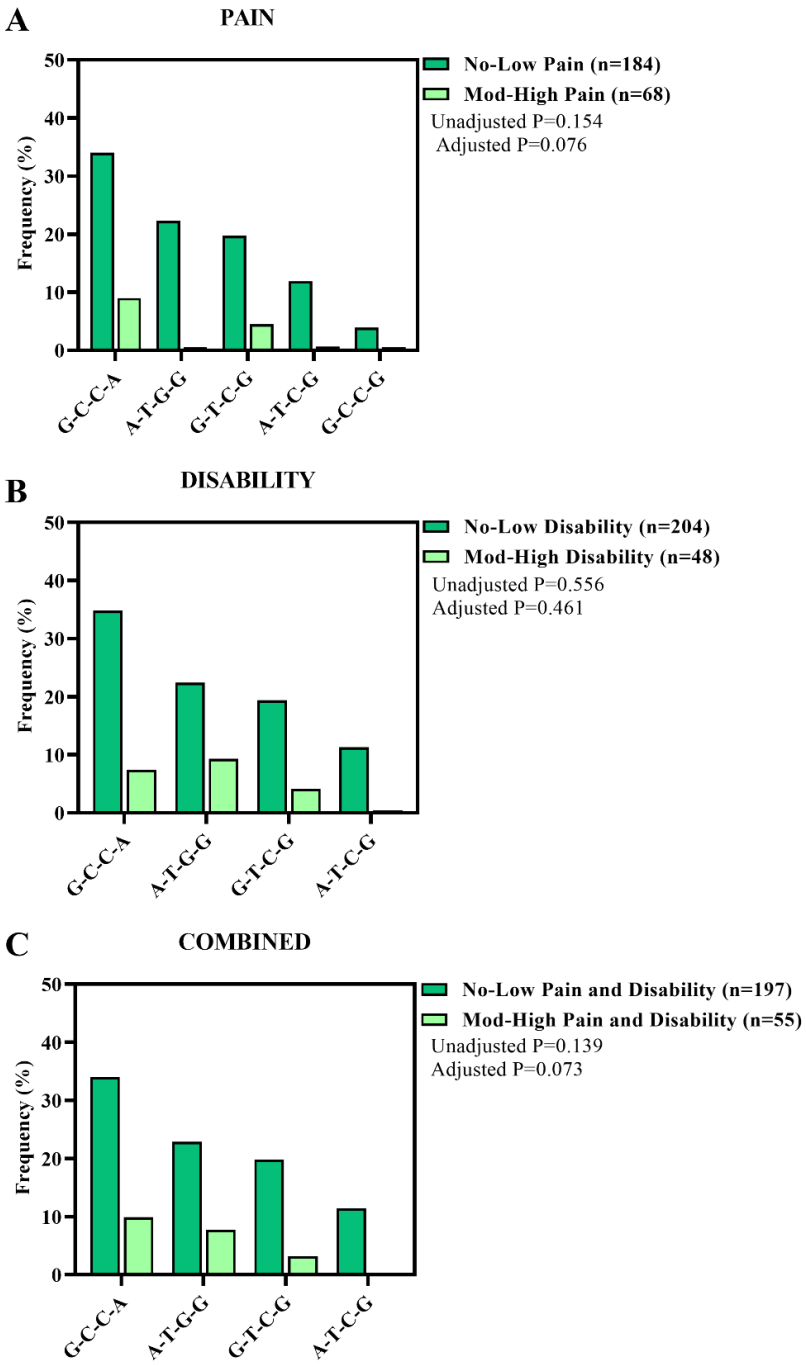


Figure 3.22: The frequency distribution patterns for the inferred *COMT* (rs6269 A>G-rs4633 C>G -rs4818 C>G -rs4680 G>A) haplotype. This illustration represents the frequencies in the no-low (green) and moderate-high (lime) groups for (A) pain, (B), disability and (C) combined (pain and disability) in South African BCS. Depicted are statistically significant differences for the inferred haplotype frequencies between the two groups with the number of participants in parenthesis (n) and all haplotype p-values shown as adjusted for age at surgery.

Evaluation of the inferred *COMT* (rs4633 C>T-rs4818 C>G-rs4680 G>A) haplotypes, H2, detected seven haplotype combinations, with only four (C-C-A, T-C-G, T-G-G and C-C-G) yielding frequencies of >3% (**Figure 3.23**). The pain (p=0.018) category noted the inferred T-C-G haplotype was significantly overrepresented in the no-low (31.5%) group compared to the moderate-high (3.7%) group. The *COMT* (rs4633-rs4818-rs4680) T-C-G (p=0.046, OR:0.65, 95% CI:0.38-1.09, haploscore = -1.72) haplotype was associated with reduced likelihood of reporting moderate-high pain (**Figure 3.23**A). No significant associations were noted for this haplotype in the disability category p> 0.05. Furthermore, for the combined (pain and disability) category, an association, p=0.023 was noted, however, no specific haplotype was significantly different between the no-low and moderate-high groups (**Figure 3.23**C).

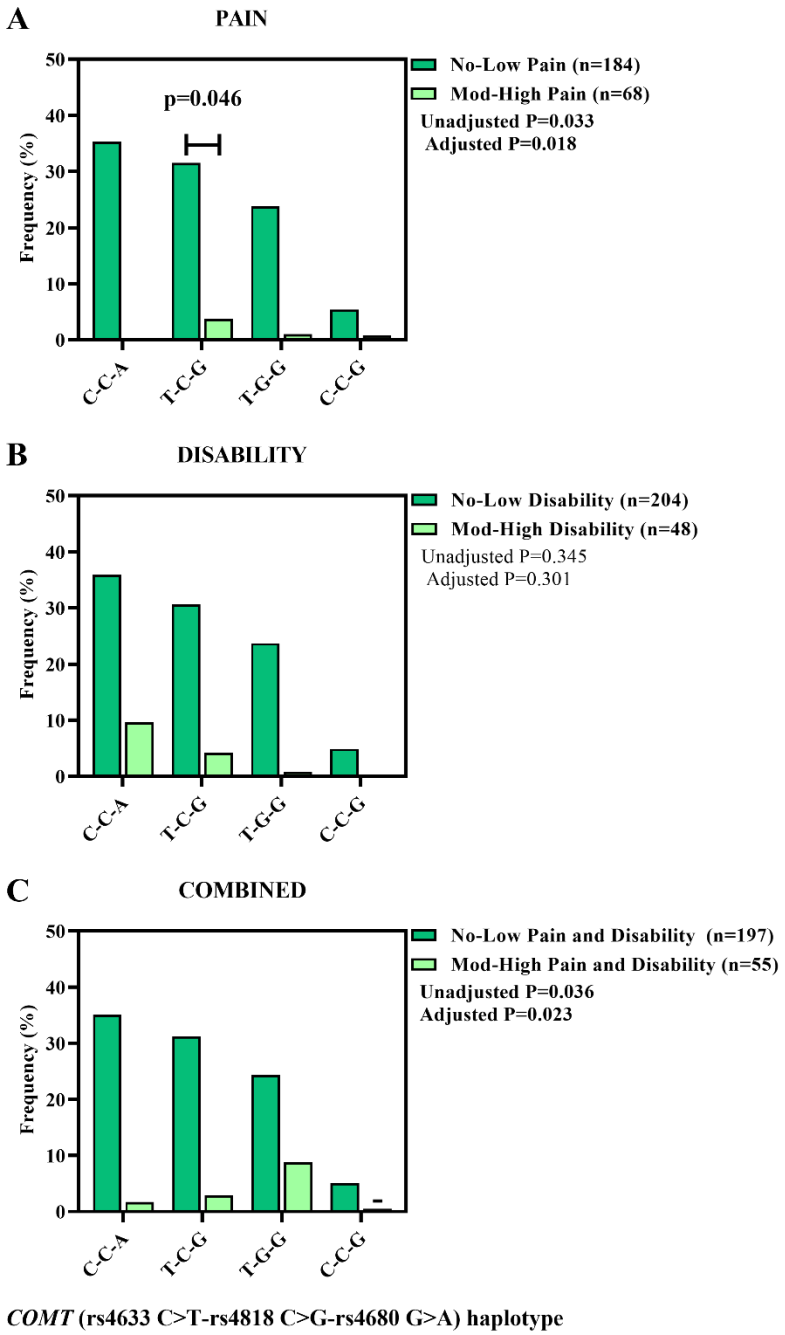


Figure 3.23: The frequency distribution patterns for the inferred *COMT* (rs4633 C>T – rs4818 C>G - rs4680 G>A) haplotype. This illustration represents the frequencies in the no-low (green) and moderate-high (lime) groups for (A) pain, (B), disability and (C) combined (pain and disability) in South African BCS. Depicted are statistically significant differences for the inferred haplotype frequencies between the two groups with the number of participants in parenthesis (n) and specific haplotype p-values shown as adjusted for age at surgery.

The inferred *COMT* (rs4818 C>G - rs4680 G>A) haplotype, H3 yielded four combinations, C-A, C-G, G-G, and G-A, of which three haplotypes noted frequencies >3% in the no-low group (**Figure 3.24**). In the pain (p=0.013) category, the inferred C-A haplotype was overrepresented in the no-low (36.9%) group, compared to the moderate-high (7.7%) group. The inferred C-G haplotype was overrepresented in the no-low (36.8%) group, compared to the moderate-high (6.5%) group. The inferred *COMT* (rs4818- rs4680) C-A(p=0.007, OR: 1.00, haploscore = 2.16) and C-G (p=0.009, OR: 0.56, 95% CI: 0.34-0.90, haploscore = -2.17) haplotypes were associated with equal and reduced likelihoods of reporting moderate-high pain, respectively (**Figure 3.24**A).

No significant associations were noted for the inferred H3 haplotype in the disability category p>0.05 (**Figure 3.24**B).

Evaluation of the combined (pain and disability) category (p=0.014), noted the inferred C-A haplotype was overrepresented in the no-low (36.9%) group, and absent in the moderate-high (0.0%) group. The inferred C-G haplotype, was significantly overrepresented in the no-low (36.2%) group, compared to the moderate-high (6.4%) group. The inferred *COMT* (rs4818-rs4680) C-A (p=0.004, OR: 1.00, haploscore = 2.44) and C-G (p=0.032, OR: 0.54, 95% CI: 0.32-0.91, haploscore = -1.92) haplotypes were therefore associated with equal and reduced likelihoods of reporting moderate-high combined pain and disability, respectively (**Figure 3.24**C).

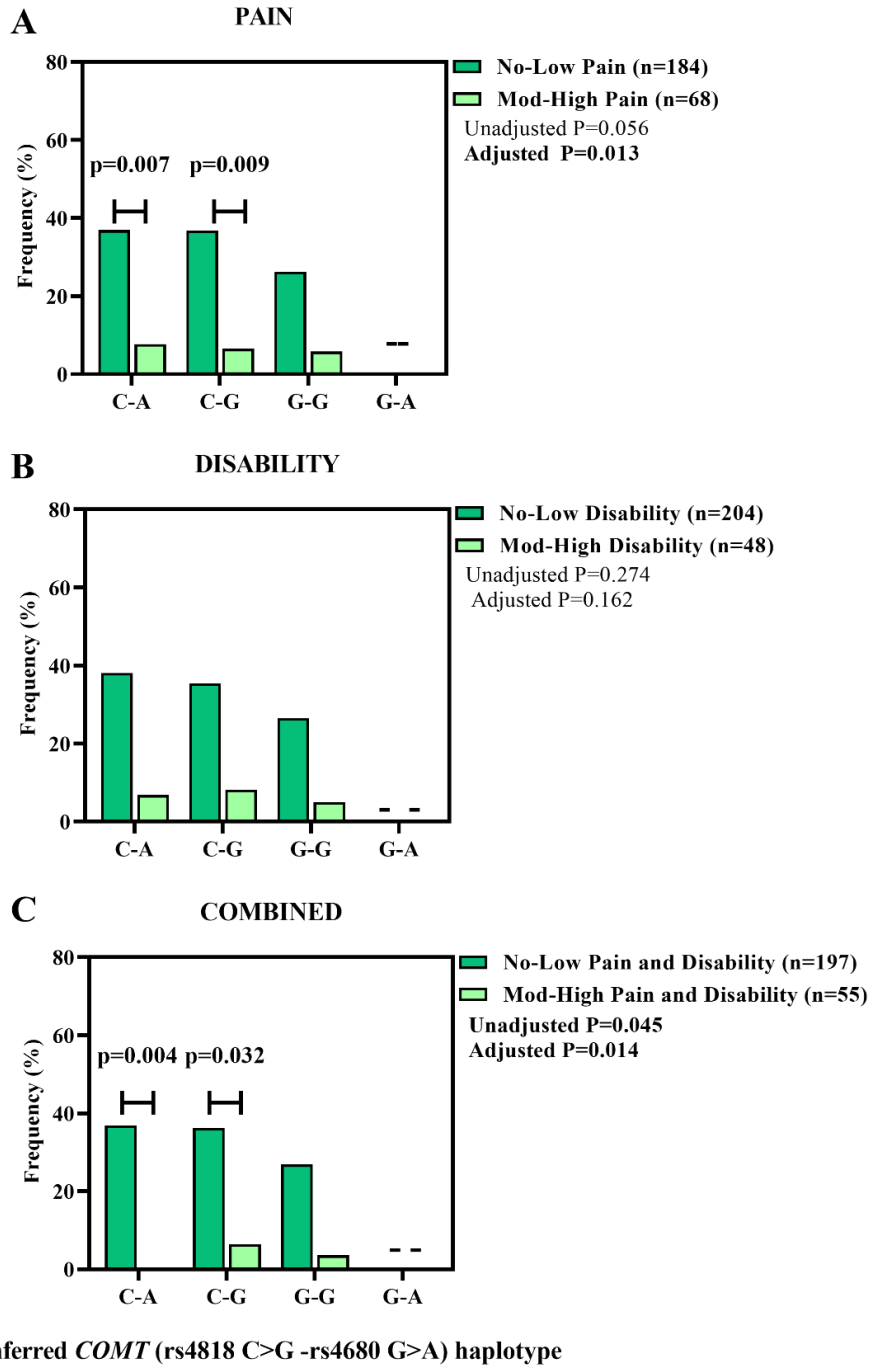


Figure 3.24: The frequency distribution patterns for the inferred *COMT* (rs4818 C>G – rs4680 G>A) haplotypes. This illustration represents the frequencies in the no-low (green) and moderate-high (lime) groups for (A/D) pain, (B/E), disability and (C/F) combined (pain and disability) in South African BCS. Depicted are statistically significant differences for the inferred align haplotype frequencies between the two groups with the number of participants in parenthesis (n) and specific haplotype p-values shown as adjusted for age at surgery.

The inferred *COMT* rs4633 C>T - rs4680 G>A haplotype H4, yielded four combinations, T-G, C-A, C-G, and T-A, of which three noted frequencies >3% in the no-low groups (**Figure 3.25**). Evaluation of the pain (p=0.014) category noted inferred T-G haplotype was overrepresented in the no-low (55.3%) group, compared to the moderate-high (44.8%) group. The *COMT* rs4633-rs4680 T-G (p=0.033, OR:1.00, haploscore = -1.91) haplotype was therefore associated with an equal likelihood of reporting moderate-high pain (**Figure 3.25**D).

The inferred H4 haplotype showed no significant differences in frequency distribution patterns between the no-low and moderate-high groups for disability, (p=0.133) (**Figure 3.25**). In the combined (pain and disability) category, the inferred T-G haplotype was overrepresented in the no-low (55.5%) group, compared to the moderate-high (41.7%) group. The inferred *COMT* (rs4633- rs4680) T-G (p=0.012, OR: 1, haploscore = -2.33) haplotype was once more associated with an equal likelihood of reporting moderate-high combined pain and disability, p=0.007 (**Figure 3.25**F).

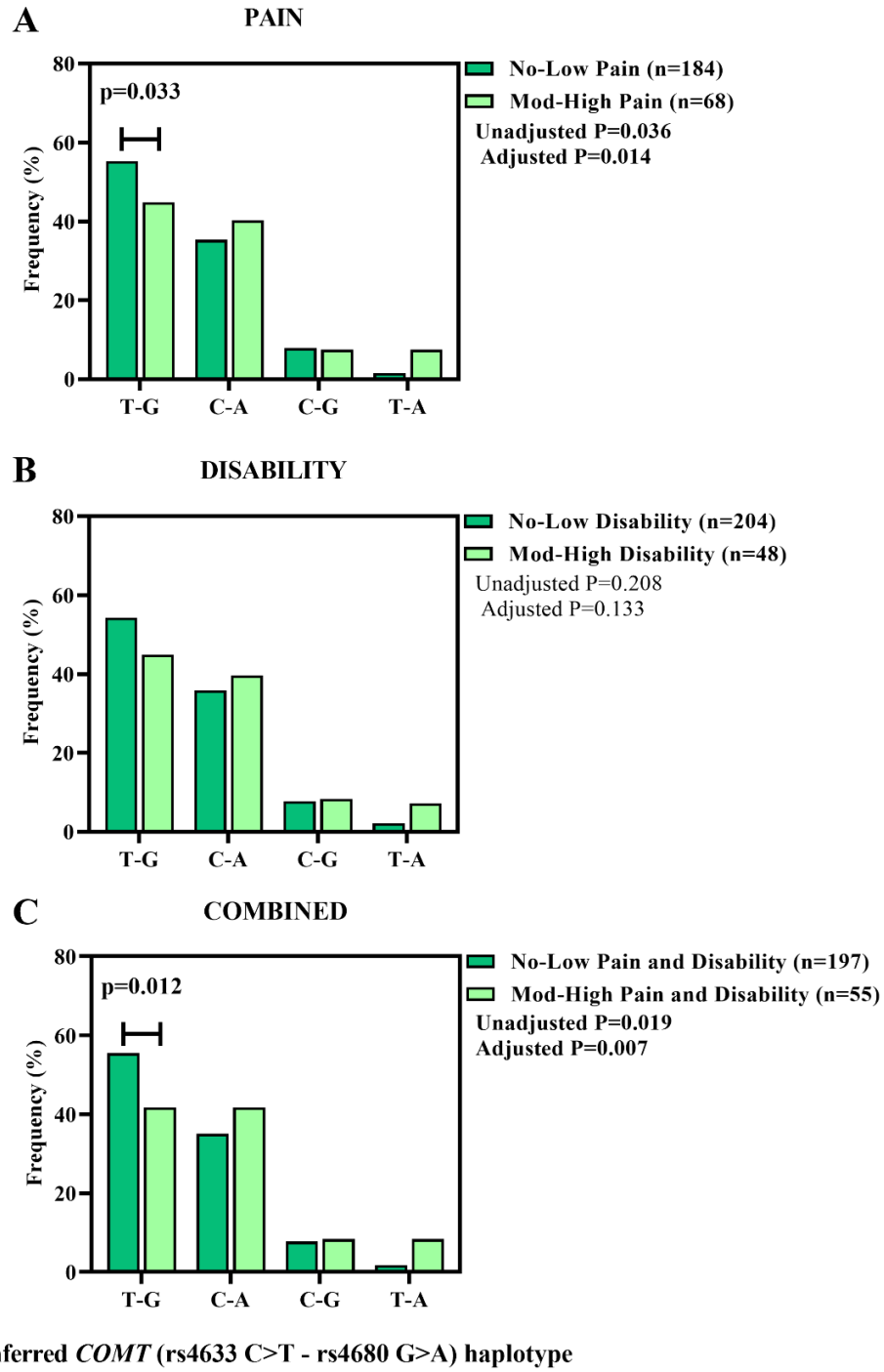


Figure 3.25: The frequency distribution patterns for the inferred *COMT* (rs4633 C>T – rs4680 G>A) haplotypes. This illustration represents the frequencies in the no-low (green) and moderate-high (lime) groups for (A/D) pain, (B/E), disability and (C/F) combined (pain and disability) in South African BCS. Depicted are statistically significant differences for the inferred align haplotype frequencies between the two groups with the number of participants in parenthesis (n) and specific haplotype p-values shown as adjusted for age at surgery.

Evaluation of the inferred *COMT* (rs6269 A>G-rs4680 G>A) haplotypes, H5, yielded four haplotype combinations A-G, G-A, G-G, and A-A, all noting frequencies of >3% (**Figure 3.26)**. Evaluation of the pain category showed the inferred G-G haplotype was significantly overrepresented in the no-low (27.7%) group, compared to the moderate-high (22.1%) group. While the inferred A-A haplotype was significantly underrepresented in the no-low (3.1%) group, compared to the moderate-high (10.7%) group. The inferred G-G (p=0.026, OR: 0.67, 95% CI: 0.38-1.18, haploscore = -1.64) haplotype was associated with a reduced likelihood of reporting moderate-high pain. Whereas the inferred A-A (p=0.007, OR: 2.09, 95% CI: 0.89-4.88, haploscore = 2.60) haplotype was associated with an increased likelihood of reporting moderate-high pain, p=0.010, (**Figure 3.26**A).

No significant differences in distribution patterns were noted when the disability groups were compared, p=0.068 (**Figure 3.26**B).

In the combined (pain and disability) category, the inferred A-A haplotype was underrepresented in the no-low (3.2%) group, compared to the moderate-high (12.4%) group (**Figure 3.26**C). The inferred *COMT* (rs6269-rs4680) A-A (p=0.003, OR:2.18, 95% CI: 0.92-5.17, haploscore = 2.83) haplotype was once more associated with an increased likelihood of reporting combined pain and disability, p=0.005 (**Figure 3.26**C).

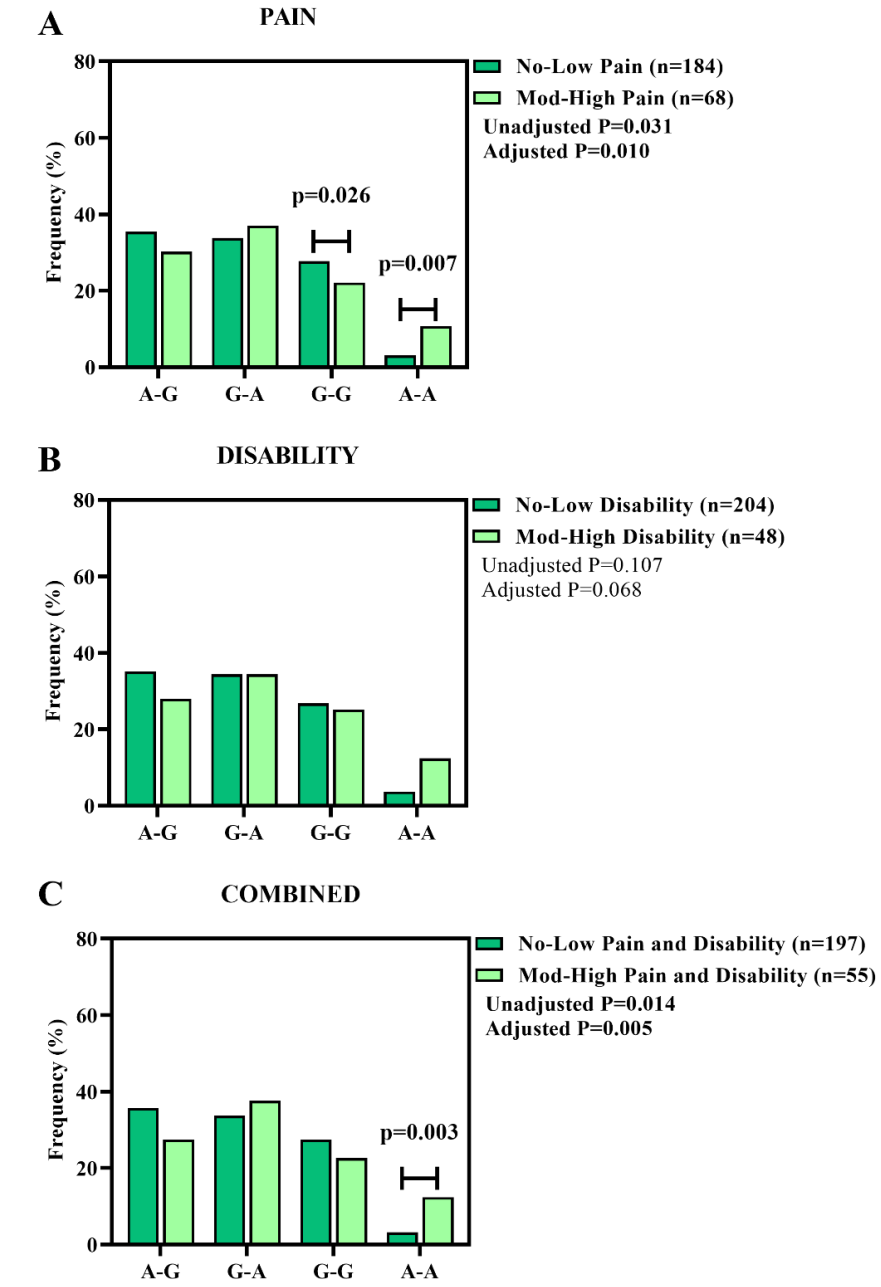


Figure 3.26: The frequency distribution patterns for the inferred *COMT* (rs6269 A>G-rs4680 G>A) haplotypes. This illustration represents the frequencies in the no-low (green) and moderate-high (lime) groups for (A) pain, (B), disability and (C) combined (pain and disability) in South African BCS. Depicted are statistically significant differences for the inferred haplotype frequencies between the two groups with the number of participants in parenthesis (n) and specific haplotype p-values shown as adjusted for age at surgery.

3.8 INFERRED ALLELE-ALLELE COMBINATIONS

The individual genotype data for *ABCB1*, *OPRM1* and *COMT* SNPs were used to construct specific allele-allele combinations, to serve as a proxy for gene-gene interactions. Analyses of the allele-allele combinations were performed and evaluated for pain, disability and combined (pain and disability) which included specifically the significantly associated and functional SNPs for *ABCB1*, *OPRM1*, and *COMT*. All data presented here is adjusted for the confounder "age at surgery", with unadjusted data presented in the supplementary **Section 7.4.1**.

3.8.1 *ABCB1* and *OPRM1* SNPs Interaction Analyses

Evaluation of the *ABCB1* and *OPRM1* gene-gene interaction analyses noted several associations for pain, disability and combined (pain and disability) (**Supplemental Table 18** and **Supplemental Table 19**). The *ABCB1* (rs1045642 G>A) - *OPRM1* (rs1799971 A>G-rs540825 T>A) allele-allele construct generated six (G-A-T, A-A-T, G-G-T, G-A-A, A-A-A and A-G-T) combinations with frequencies >3% (**Figure 3.27**). No significant differences in the frequency distribution patterns were noted for these allele-allele combinations when pain (p=0.106) and disability (p=0.053) scores were evaluated (**Figure 3.27**A and B). In the combined (pain and disability, p=0.027) category, the inferred A-A-T allele-allele combination was noted to be overrepresented in the no-low (24.4%) compared to the moderate-high (16.7%) group. The *ABCB1* (rs1045642) - *OPRM1* (rs1799971- rs540825) A-A-T (p=0.029, OR: 0.58, 95% CI: 0.18-1.45, haploscore = -2.08) allele-allele combination was significantly associated with reduced likelihood of reporting moderate-high combined pain and disability (**Figure 3.27**C).

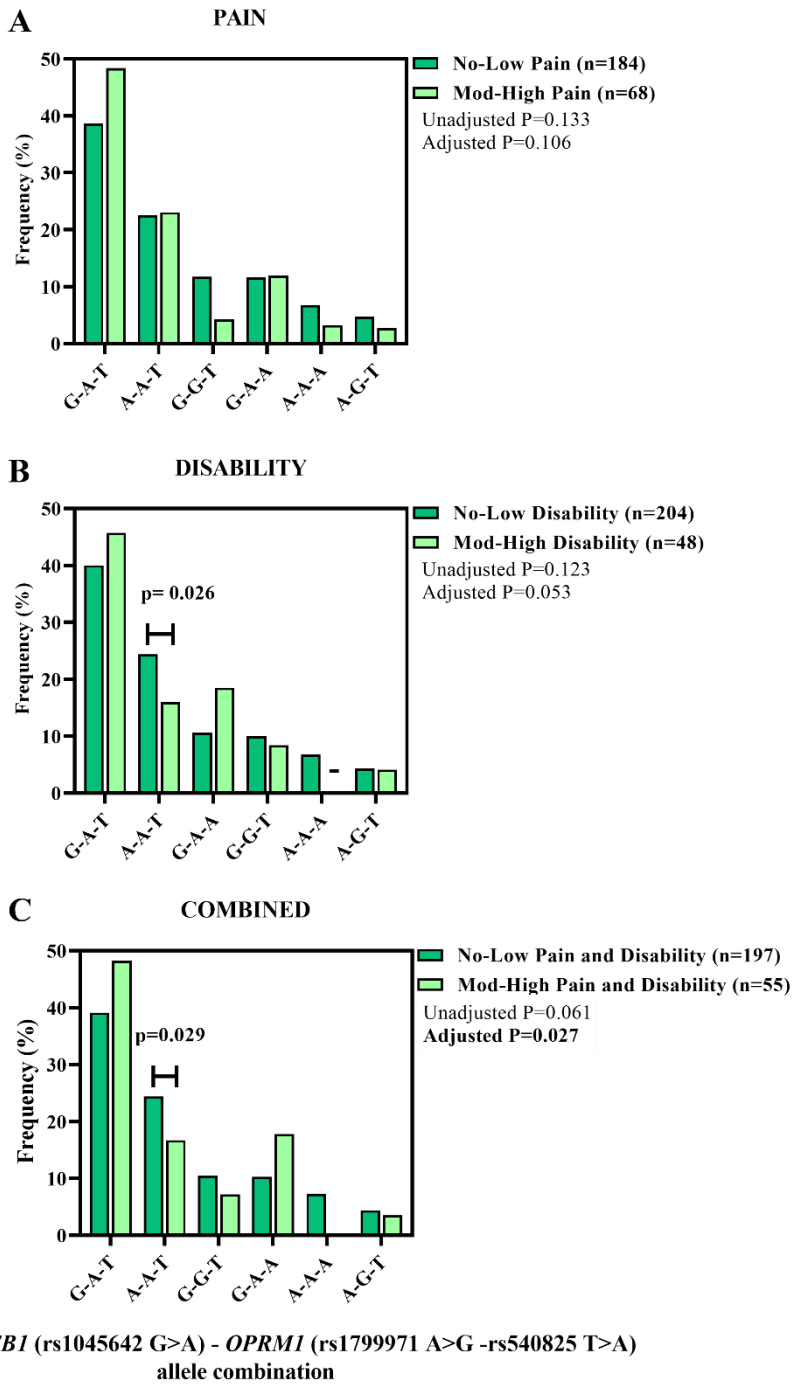


Figure 3.27: The frequency distribution patterns for the inferred *ABCB1* (rs1045642 G>A)- *OPRM1* (rs1799971 A>G-rs540825 T>A) allele-allele combinations. This illustration represents the frequencies in the no-low (orange) and moderate-high (peach) groups for the shoulder phenotypes (A) pain, (B), disability and (C) combined (pain and disability) in South African BCS. Depicted are statistically significant differences for frequencies of the allele-allele combinations between the two groups with the number of participants in parenthesis (n), and all specific haplotype p-values shown as adjusted for age at surgery.

Evaluation of *ABCB1* (rs1045642 G>A) - *OPRM1* (rs1799971 A>G) generated four combinations, G-A, G-G, A-G and A-A (**Figure 3.28**A-C). No significant differences were noted for the pain (p=0.244) or disability (p=0.077), categories (**Figure 3.28**A and B). In the combined (pain and disability, p=0.028) category, however, the inferred G-A allele combination was significantly underrepresented in the no-low (49.3%) compared to the moderate-high (65.2%) group. The inferred A-A allele-allele combination was significantly overrepresented in the no-low (31.8%) compared to the moderate-high (17.6%) group. The inferred *ABCB1* (rs1045642)-*OPRM1* (rs1799971) G-A (p=0.005, OR:1.00, haploscore = 2.64) and A-A (p=0.008, OR: 0.44, 95% CI:0.24-0.80, haploscore = -2.59) allele-allele combinations were therefore associated with equal and reduced likelihood of reporting moderate-high combined pain and disability, respectively (**Figure 3.28**C).

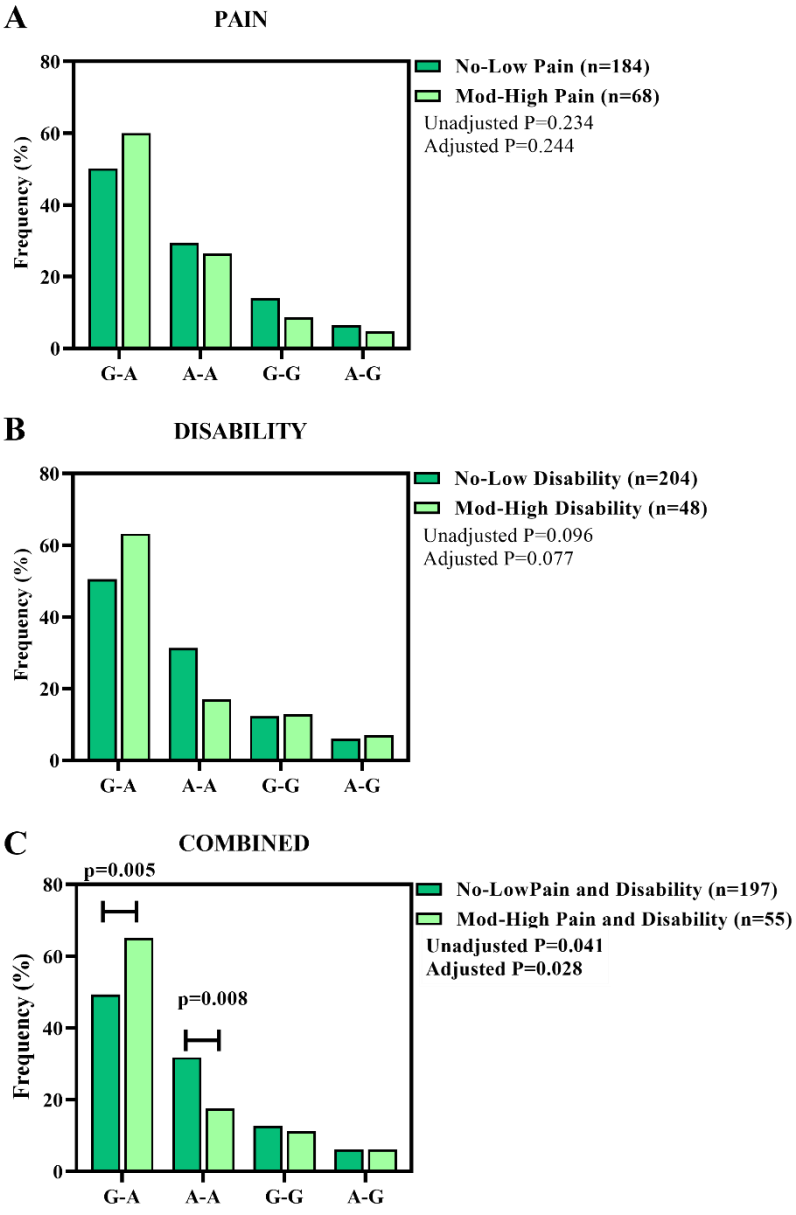


Figure 3.28: The frequency distribution patterns for the inferred *ABCB1* (rs1045642 G>A)- *OPRM1* (rs1799971 A>G) allele-allele combinations. This illustration represents the frequencies in the no-low (orange) and moderate-high (peach) groups for the shoulder phenotypes (A) pain, (B), disability and (C) combined (pain and disability) in South African BCS. Depicted are statistically significant differences for frequencies of the allele-allele combinations between the two groups with the number of participants in parenthesis (n), and all specific haplotype p-values shown as adjusted for age at surgery.

Evaluation of the *ABCB1* (rs1045642 G>A) - *OPRM1* (rs540825 T>A) construct, identified four allele-allele combinations G-T, A-T, G-A, and A-A (**Figure 3.29**). No significant differences in allele frequencies were noted between the no-low and moderate-high groups for pain, p=0.684 (**Figure 3.29**A). Evaluation of the disability (p=0.026) category noted the inferred A-T allele-allele combination was significantly overrepresented in the no-low (28.8%) group compared to the moderate-high (19.3%) group. Further, the inferred G-A allele-allele combination was significantly underrepresented in the no-low (12.9%) compared to the moderate-high (21.4%) group, (**Figure 3.29**B). The *ABCB1* (rs1045642)-*OPRM1* (rs540825) A-T (p=0.019, OR: 0.62, 95% CI: 0.33-1.16, haploscore = -2.16) and the alternate G-A (p=0.021, OR: 1.57, 95% CI: 0.30-3.10, haploscore = 2.00) allele combinations were significantly associated with reduced and increased likelihood of reporting moderate-high disability (p=0.026).

For the combined (pain and disability) (p=0.014) category, the analysis detected a significant association, globally, between the no-low and moderate-high groups (**Figure 3.29**C). The A-T allele-allele combination was once more significantly overrepresented in the no-low (28.9%) group, compared to the moderate-high (19.8%) group (**Figure 3.29**C). Additionally, the G-A allele-allele combination was also significantly underrepresented in the no-low (12.7%) group, compared to the moderate-high (20.7%) group. The *ABCB1* (rs1045642)-*OPRM1* (rs540825) A-T (p=0.014, OR:0.62, 95% CI:0.35-1.10, haploscore = -2.24) and G-A (p=0.030, OR: 1.50, 95% CI: 0.78-2.86, haploscore = 1.86) allele-allele combinations were associated with reduced and increased likelihood of reporting moderate-high combined pain and disability (**Figure 3.29**C).

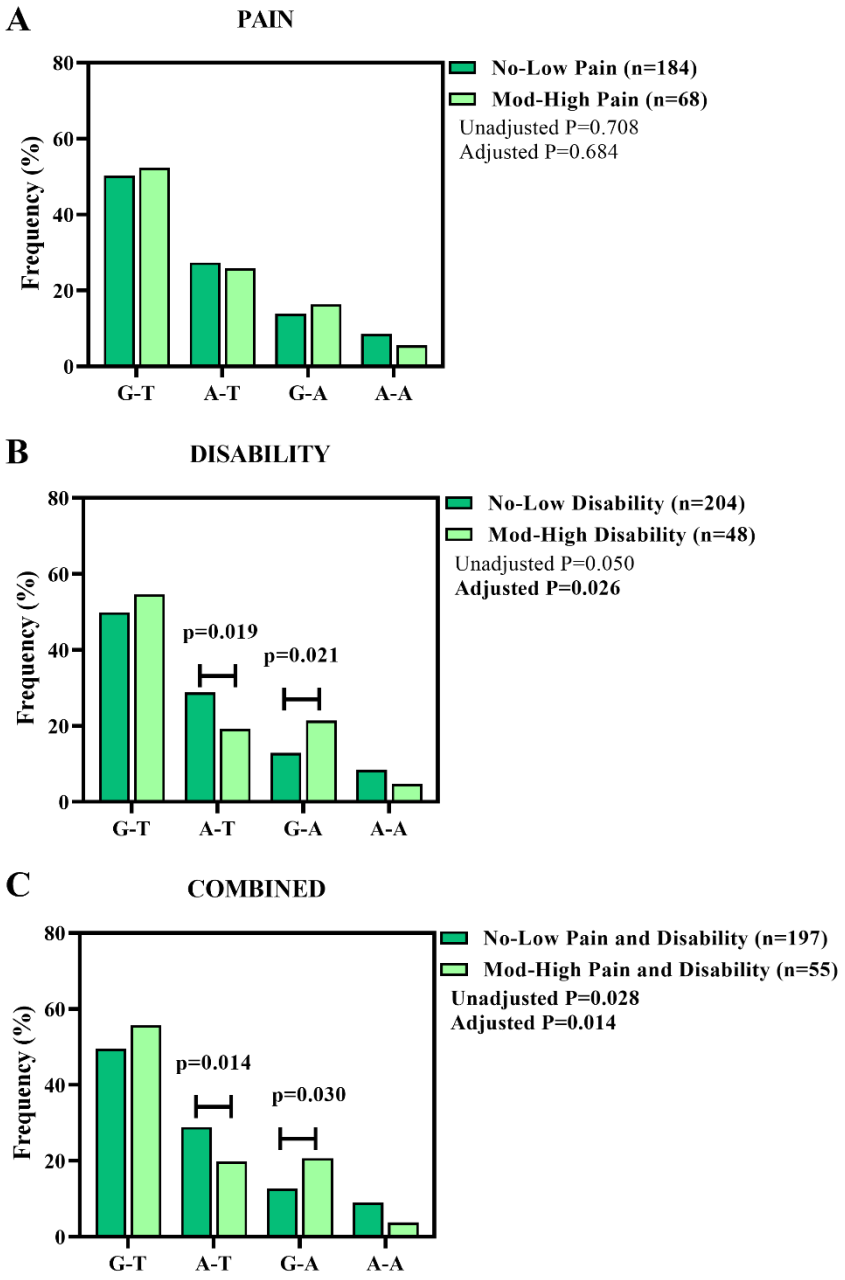


Figure 3.29: The frequency distribution patterns for the inferred *ABCB1* (rs1045642 G>A)- *OPRM1* (rs540825 T>A) allele-allele combinations. This illustration represents the frequencies in the no-low (orange) and moderate-high (peach) groups for the shoulder phenotypes (A) pain, (B), disability and (C) combined (pain and disability) in South African BCS. Depicted are statistically significant differences for frequencies of the allele-allele combinations between the two groups with the number of participants in parenthesis (n), and all specific haplotype p-values shown as adjusted for age at surgery.

3.8.2 *OPRM1* and *COMT* SNPs Interaction Analyses

The gene-gene interaction analyses between the *OPRM1* and *COMT* SNPs revealed a few significant associations in the pain, disability and combined (pain and disability) categories (**Supplemental Table 20** and **Supplemental Table 21**). The *OPRM1-COMT* construct, including the SNPs rs1799971 A>G-rs540825 T>A, and rs4680 G>A, yielded six combinations (A-T-G, A-T-A, A-A-G, G-T-G, G-T-A, and A-A-A) with frequencies >3% (**Figure 3.30**).

Evaluation of the pain (p=0.014) category detected a significant association between the no-low and moderate-high groups, globally. The inferred A-T-A allele-allele combination was significantly underrepresented in the no-low (21.5%) group, compared to the moderate-high (30.7%) group. The inferred G-T-G allele-allele combination, was also significantly overrepresented in the no-low (9.6%) group, while being absent in the moderate-high (0.0%) group. The inferred *OPRM1* (rs1799971-rs540825)-*COMT* (rs4680) A-T-A (p=0.008, OR: 1.36, 95% CI: 0.77-2.41, haploscore = 2.44) and G-T-G (p=0.004, OR: 0.00, 95% CI: 0.00-0.00, haploscore = -3.02) allele-allele combinations were therefore associated with increased and reduced likelihoods of reporting moderate-high pain, respectively (**Figure 3.30**A).

No significant associations were further noted for the *OPRM1* (rs1799971 A>G-rs540825 T>A)-*COMT* (rs4680 G>A) allele-allele combinations, in the disability (p=0.231) and the combined (pain and disability, p=0.063) categories (**Figure 3.30**B and C).

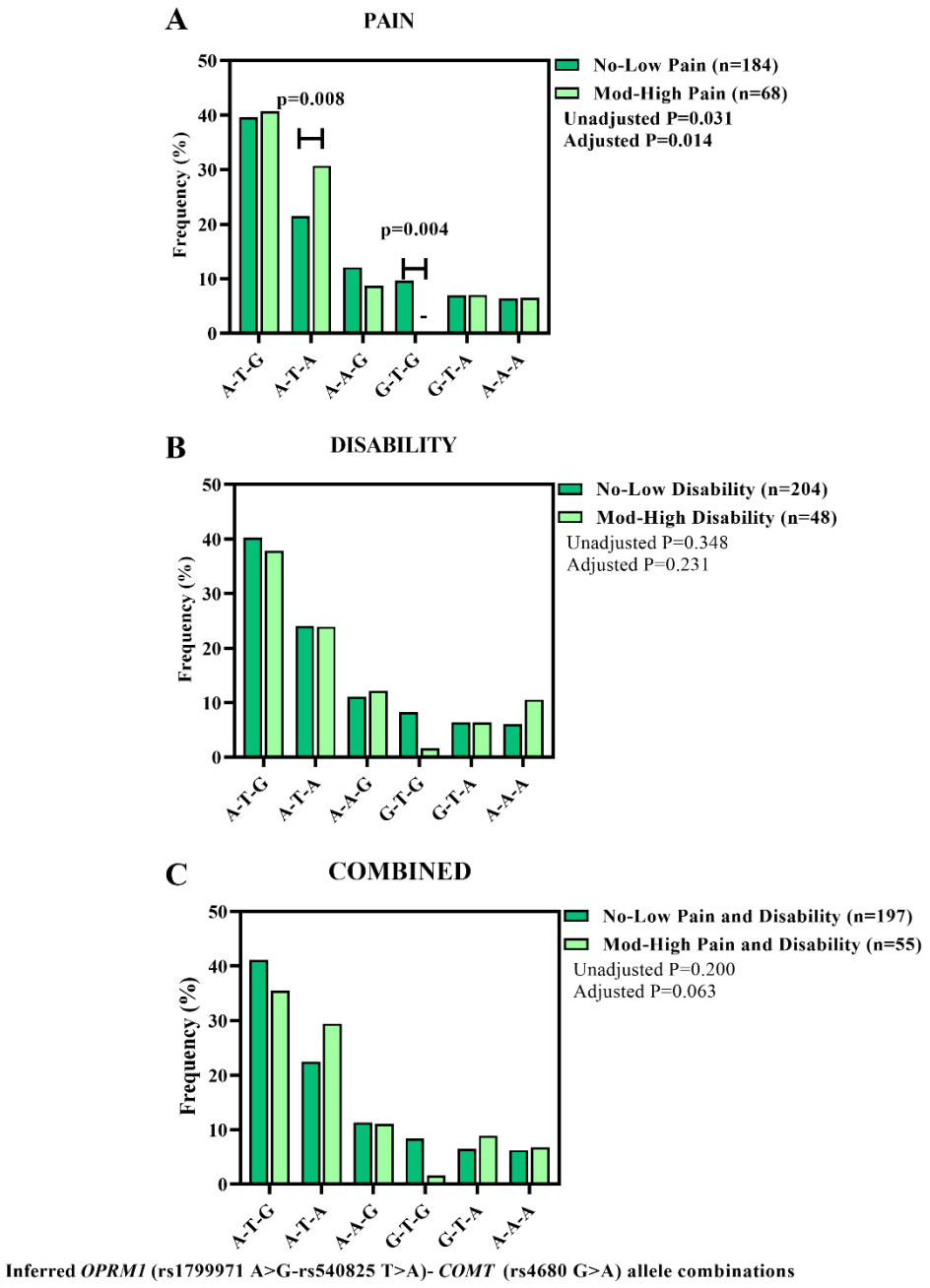


Figure 3.30: The frequency distribution patterns for the inferred *OPRM1* (rs1799971 A>G-rs540825 T>A)- *COMT* (rs4680 G>A) allele-allele combinations. This illustration represents the frequencies in the no-low (orange) and moderate-high (peach) groups for the shoulder phenotypes (A) pain, (B), disability and (C) combined (pain and disability) in South African BCS. Depicted are statistically significant differences for frequencies of the allele-allele combinations between the two groups with the number of participants in parenthesis (n), haplotype frequencies of 0% as (-), and all specific haplotype p-values shown as adjusted for age at surgery.

Evaluation of the *OPRM1* (rs1799971 A>G)-*COMT* (rs4680 G>A) construct, yielded four combinations, namely G-A, A-A, G-G and A-G (**Figure 3.31**). Evaluation of the pain (p=0.011) category noted the inferred A-A allele combination was significantly underrepresented in the no-low (27.9%) group, compared to the moderate-high (36.9%) group. The inferred G-G allele combination was significantly overrepresented in the no-low (11.4%) group, compared to the moderate-high (2.6%) group. The inferred *OPRM1* (rs1799971)-*COMT* (rs4680) A-A(p=0.004, OR: 1.35, 95% CI: 0.85-2.15, haploscore = 2.40) and G-G (p=0.010, OR: 0.23, 95% CI: 0.05-1.03, haploscore = -2.60) allele-allele combinations were associated with increased and reduced likelihoods or reporting moderate-high pain (**Figure 3.31**A).

No significant associations were noted between this construct and disability (p=0.135) as noted in **Figure 3.31**B.

In the evaluation for the combined (pain and disability) (p=0.027) category, the inferred A-A was underrepresented in the no-low (28.7%) group compared to the moderate-high (36.0%) group (**Figure 3.31**). Further, the A-G allele-allele combination was significantly overrepresented in the no-low (52.4%) group compared to the moderate-high (46.7%) group. The inferred *OPRM1* (rs1799971)-*COMT* (rs4680) A-A (p=0.010, OR: 1.42, 95% CI: 0.85-2.35, haploscore = 2.00) and A-G (p=0.046, OR: 1.00, haploscore = -1.55) allele-allele combinations were associated with increased and equal likelihoods of reporting moderate-high combined (pain and disability), respectively (**Figure 3.31**C).

Evaluation of the inferred *OPRM1* (rs540825 T>A) - *COMT* (rs4680 G>A) allele-allele combinations yielded the combinations T-G, T-A, A-G and A-A. In the category of pain (p=0.052) and disability (p=0.079), the analysis detected no significant associations (**Figure 3.31**D and E).

For combined (pain and disability, p=0.016), the analysis detected the inferred T-G (p=0.008,) allele combination was significantly overrepresented in the no-low (49.5%) group, compared to the moderate-high (36.6%) group. (**Figure 3.31**F). The inferred A-A allele combination was significantly underrepresented in the no-low (8.0%) group, compared to the moderate-high (11.2%) group (**Figure 3.31**F). The *OPRM1* (rs540825)–*COMT* (rs4680) T-G (p=0.008, OR: 1.00, haploscore = -2.38) and A-A (p=0.012, OR: 1.89, 95% CI: 0.81-4.38, haploscore = 1.48) allele-allele combinations were associated with equal and increased likelihood of reporting moderate-high combined (pain and disability), respectively.

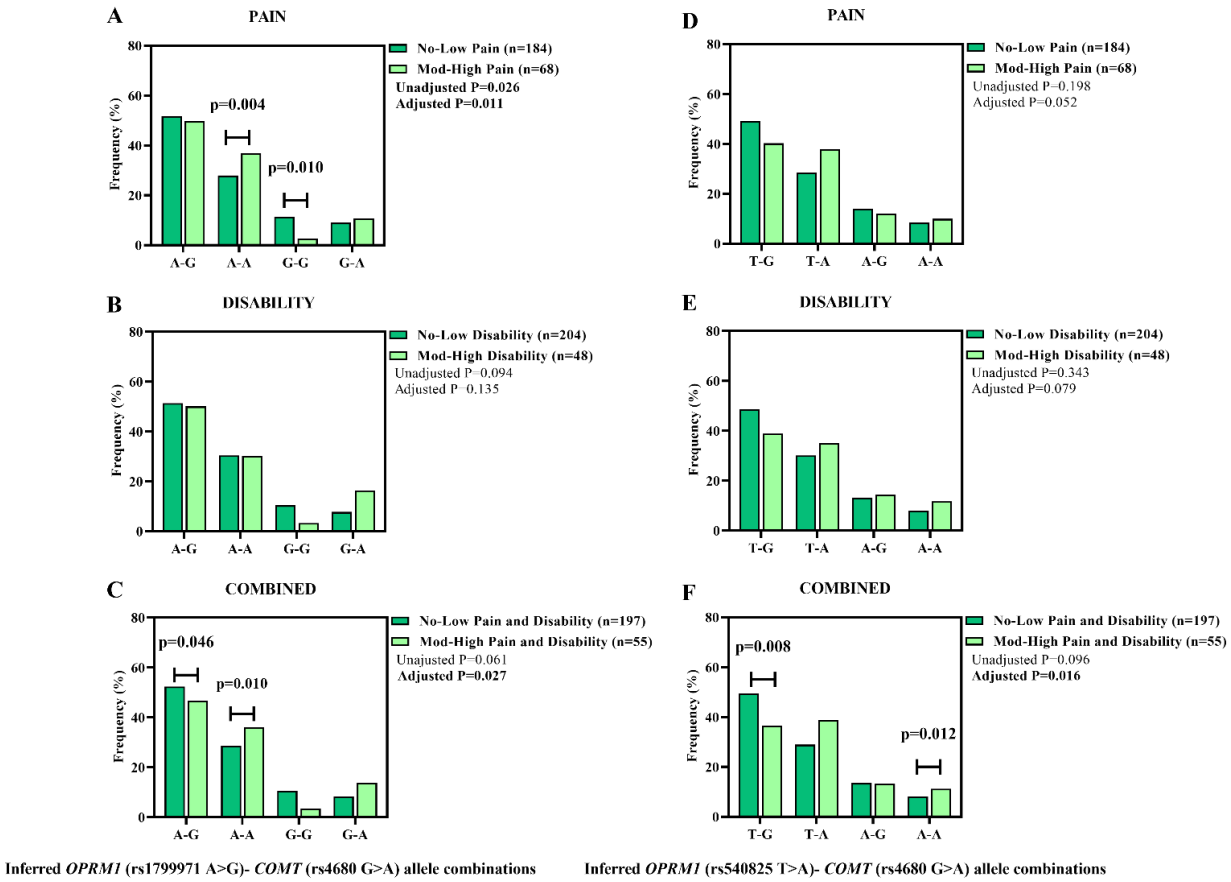


Figure 3.31: The frequency distribution patterns for the inferred *OPRM1* (rs1799971 A>G) – *COMT* (rs4680 G>A) and *OPRM1* (rs540825 T>A)- *COMT* (rs4680 G>A) allele-allele combinations. This illustration represents the frequencies in the no-low (orange) and moderate-high (peach) groups for the shoulder phenotypes (A) pain, (B), disability and (C) combined (pain and disability) in South African BCS. Depicted are statistically significant differences for frequencies of the allele-allele combinations between the two groups with the number of participants in parenthesis (n), and all specific haplotype p-values shown as adjusted for age at surgery.

3.8.3 *ABCB1* and *COMT* SNPs Interaction Analyses

The gene-gene interaction analysis between the *ABCB1* and *COMT* genes also revealed a few significant associations (**Supplemental Table 22** and **Supplemental Table 23**). The *ABCB1* and *COMT* construct containing the rs1128503 G>A, rs1045642 G>A and rs4680 G>A SNPs, yielded the six allele-allele combinations, G-G-G, G-G-A, A-A-G, A-A-A, A-G-G, and G-A-G, reporting frequencies of >3% (**Figure 3.32**). No significant differences in the frequency distribution patterns were noted between the no-low and moderate groups in the pain (adjusted p=0.102) and disability (adjusted p=0.087) categories (**Figure 3.32**A and B).

In the combined (pain and disability) (p=0.008) category, a significant association was detected globally between the no-low and moderate-high groups (**Figure 3.32**C). The inferred A-A-G allele-allele combination was significantly overrepresented in the no-low (19.0%) group, compared to the moderate-high (10.5%) group. The *ABCB1* (rs1128503-rs1045642)- *COMT* (rs4680) A-A-G (p=0.006, OR:0.68, 95% CI: 0.27-1.71, haploscore = -2.26) allele-allele combination was associated with reduced likelihood of reporting moderate-high combined (pain and disability); (**Figure 3.32**C).

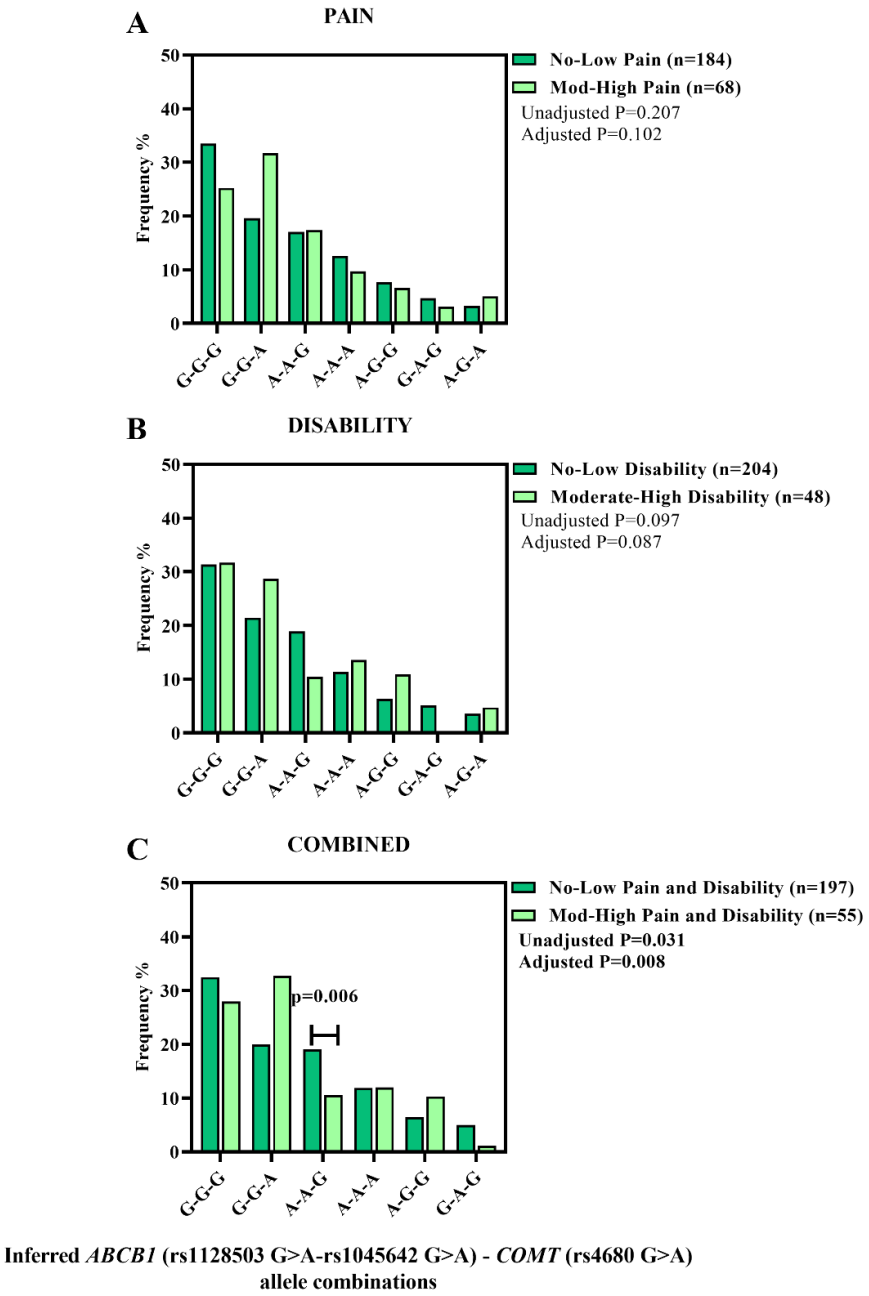


Figure 3.32: The frequency distribution patterns for the inferred *ABCB1* (rs1128503 G>A-rs1045642 G>A) - *COMT* (rs4680 G>A) allele-allele combinations. This illustration represents the frequencies in the no-low (orange) and moderate-high (peach) groups for the shoulder phenotypes (A) pain, (B), disability and (C) combined (pain and disability) in South African BCS. Depicted are statistically significant differences for frequencies of the allele-allele combinations between the two groups with the number of participants in parenthesis (n), and all specific haplotype p-values shown as adjusted for age at surgery.

The next construct *ABCB1* (rs1128503 G>A) – *COMT* (rs4680 G>A), generated four allele-allele combinations, G-G, A-G, G-A and A-A (**Figure 3.33**). In the pain (p=0.030) category, the inferred G-A allele-allele combination was significantly underrepresented in the no-low (21.1%) group compared to the moderate-high (32.6%) group. The *ABCB1* (rs1128503) – *COMT* (rs4680) G-A (p=0.005, OR: 2.08, 95% CI: 1.12-3.84, haploscore = 2.33) allele-allele combination was associated with an increased likelihood of reporting moderate-high pain (p=0.030); (**Figure 3.33**A).

No significant differences were noted in the frequency distribution patterns for *ABCB1* (rs1128503 G>A)- *COMT* (rs4680 G>A), between the no-low and moderate-high groups for disability category (p=0.311); (**Figure 3.33**B).

In the combined (pain and disability, p=0.028) category, the inferred G-A allele combination was significantly underrepresented in the no-low (21.8%) group compared to the moderate-high (32.6%) group (**Figure 3.33**C). The inferred G-A (p=0.008, OR: 1.94, 95% CI: 1.02-3.69, haploscore = 2.29) allele-allele combination was associated with an increased likelihood of reporting combined (pain and disability).

Evaluation of the *ABCB1* (rs1045642 G>A) – *COMT* (rs4680 G>A) construct generated four inferred allele-allele combinations, G-G, G-A, A-G and A-A (**Figure 3.33**). Evaluation of the pain (p=0.008) category noted the inferred G-A allele-allele combination was significantly underrepresented in the no-low (23.0%) group compared to the moderate-high (36.8%) group (**Figure 3.33**D). The inferred G-A (p=0.001, OR: 2.27, 95% CI: 1.25-4.10, haploscore = 2.85)

allele-allele combination was associated with an increased likelihood of reporting moderate-high pain (**Figure 3.33**D).

In the disability (p=0.019) category, the inferred G-A allele-allele combination was significantly underrepresented in the no-low (25.1%) group, compared to the moderate-high (33.2%) group. While the alternate A-G allele-allele combination was significantly overrepresented in the no-low (24.2%) group, compared to the moderate-high (10.2%) group. The inferred G-A (p=0.018, OR: 1.16, 95% CI: 0.62-2.15, haploscore = 2.09) and A-G (p=0.003, OR: 0.38, 95% CI: 0.15-0.98, haploscore = -2.72) allele-allele combinations were associated with increased and reduced likelihoods of reporting moderate-high disability (p=0.019), respectively (**Figure 3.33**E).

Equally, evaluation of the combined (pain and disability) (p=0.001) category, also noted significant differences in the distribution patterns of the G-A and A-G allele-allele combinations (**Figure 3.33**F). The inferred G-A allele-allele combination was significantly underrepresented in the no-low (23.3%) group, compared to the moderate-high (38.2%) group. While the alternate A-G allele-allele combination was significantly overrepresented in the no-low (24.2%) group compared to the moderate-high (11.8%) group (**Figure 3.33**F). The inferred G-A (p<0.001, OR: 1.75, 95% CI: 0.97-3.18, haploscore = 3.35) and A-G (p=0.002, OR: 0.53, 95% CI: 0.24-1.20, haploscore = -2.85) allele-allele combinations were once more associated with an increased and reduced likelihood of reporting moderate-high combined (pain and disability), respectively (**Figure 3.33**F).

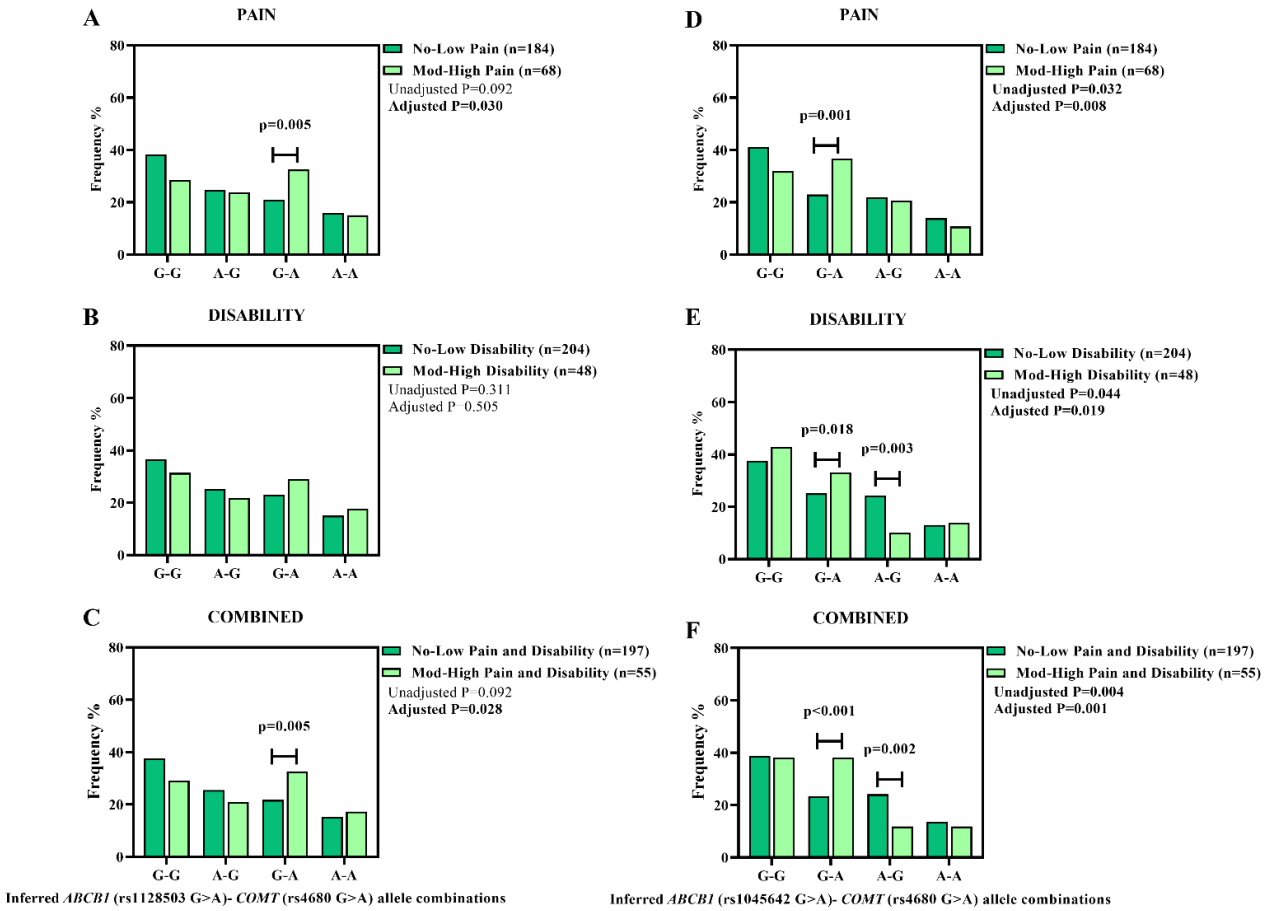


Figure 3.33: The frequency distribution patterns for the inferred *ABCB1* (rs1045642 G>A)- *COMT* (rs4680 G>A) and *ABCB1* rs1128503 G>A)- *COMT* (rs4680 G>A) allele-allele combinations. This illustration represents the frequencies in the no-low (orange) and moderate-high (peach) groups for the shoulder phenotypes (A) pain, (B), disability and (C) combined (pain and disability) in South African BCS. Depicted are statistically significant differences for frequencies of the allele-allele combinations between the two groups with the number of participants in parenthesis (n), and all specific haplotype p-values shown as adjusted for age at surgery.

3.8.4 *ABCB1*, *OPRM1*, and *COMT* SNPs Interaction Analyses

A 3-way gene-gene interaction analysis for the candidate genes, *ABCB1*, *OPRM1* and *COMT* were conducted, which included the three most widely studied SNPs reported for each gene (**Supplemental Table 24**). The construct *ABCB1* (rs1045642 G>A) - *OPRM1* (rs1799971 A>G) - *COMT* (rs4680 G>A) yielded six (G-A-G, A-A-G, G-A-A, A-A-A, G-G-G, and G-G-A) allele-allele combinations reporting frequencies of >3% (**Figure 3.34**).

Evaluation of the pain (p=0.011) category noted significant frequency differences for the G-A-A and G-G-G allele-allele combinations between the no-low and moderate-high groups. The inferred G-A-A allele-allele combination was significantly underrepresented in the no-low (16.8%) group compared to the moderate-high (28.8%) group. While the inferred G-G-G allele-allele combination was significantly overrepresented in the no-low (7.6%) and absent in the moderate-high (0%) group (**Figure 3.34**A). Moreover, the inferred G-A-A (p<0.001, OR: 1.93, 95% CI: 1.01-3.69, haploscore = 3.20) and G-G-G (p=0.019, OR: 0.00, 95% CI: 0.00-0.00, haploscore = -2.50) allele-allele combinations were associated with an increased and reduced likelihood of reporting moderate-high pain.

The analysis of the disability (p=0.047) category also detected a significant association between the no-low and moderate-high groups (**Figure 3.34**B). Here, the inferred A-A-G allele-allele combination was significantly overrepresented in the no-low (20.9%) group, compared to the moderate-high (9.3%) group. The *ABCB1* (rs1045642)- *OPRM1* (rs1799971)- *COMT* (rs4680) A-A-G (p=0.008, OR: 0.37, 95% CI: 0.13-1.01, haploscore = -2.43) allele-allele combination was associated with a reduced likelihood of reporting moderate-high disability (p=0.047). No

significant associations were further noted between the remaining allele-allele combinations and this category (**Figure 3.34**B).

The analysis of the combined (pain and disability, p=0.008) category also detected an association with significant differences in frequency distributions for the A-A-G and G-A-A allele-allele combinations. The inferred A-A-G allele-allele combination was significantly overrepresented in the no-low (20.7%) group compared to the moderate-high (11.1%) group. Whereas the G-A-A allele-allele combination was significantly underrepresented in the no-low (17.7%) group, compared to the moderate-high (29.3%) group. The inferred A-A-G (p=0.006, OR: 0.51, 95% CI: 0.22-1.20, haploscore = -2.47) and G-A-A (p<0.001, OR: 1.60, 95% CI: 0.81-3.19, haploscore = 3.17) allele-allele combinations were therefore associated with a reduced and increased likelihood of reporting moderate-high combined (pain and disability); (**Figure 3.34**C).

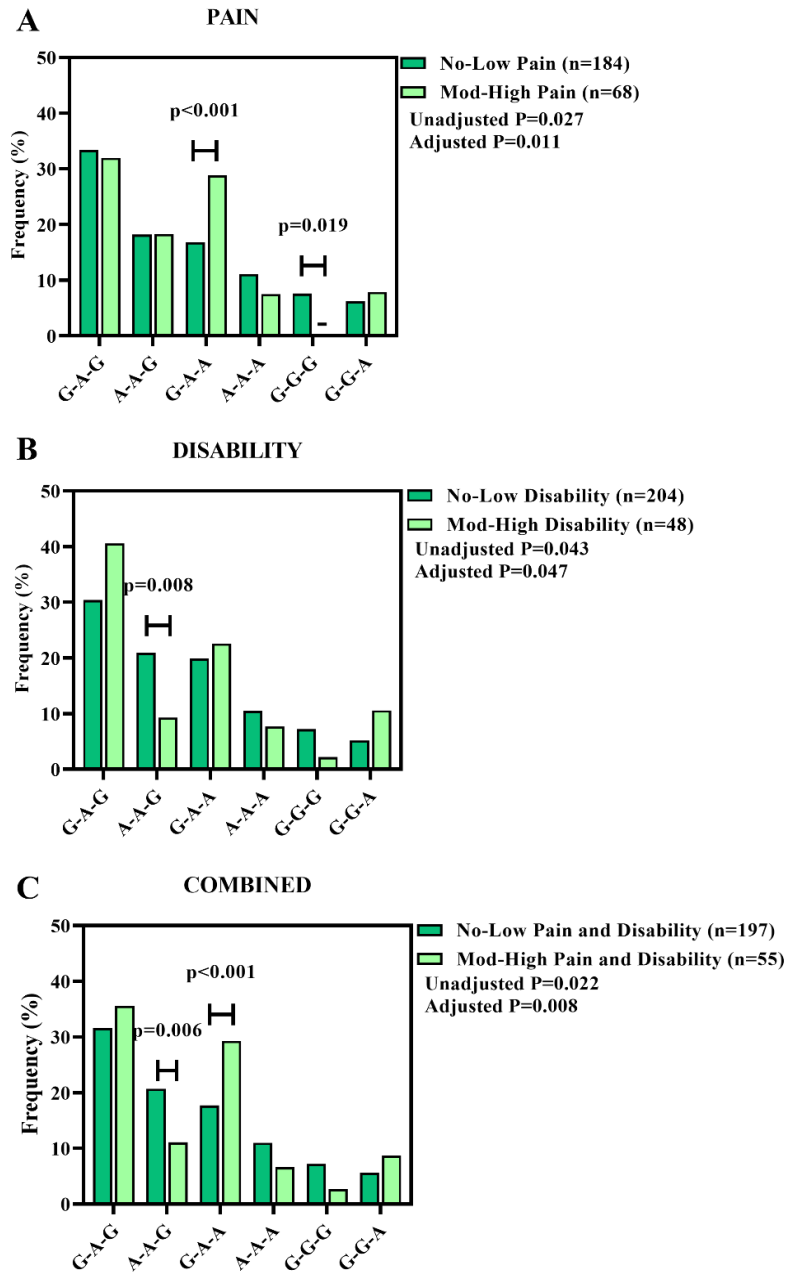


Figure 3.34: The frequency distribution patterns for the inferred *ABCB1* (rs1045642 G>A)- *OPRM1* (rs1799971 A>G) -*COMT* (rs4680 G>A) allele-allele combinations. This illustration represents the frequencies in the no-low (orange) and moderate-high (peach) groups for the shoulder phenotypes (A) pain, (B), disability and (C) combined (pain and disability) in South African BCS. Depicted are statistically significant differences for frequencies of the allele-allele combinations between the two groups with the number of participants in parenthesis (n), and all specific haplotype p-values shown as adjusted for age at surgery.

3.9 BIOINFORMATIC ANALYSES

3.9.1 SIFT, PolyPhen-2 and FATHMM Analyses

Computational methods were employed to evaluate the potential effects of the polymorphisms on the translated gene product. Polymorphisms were screened through the SIFT, PolyPhen-2 and FATHMM-MLK algorithms and the predicted scores are presented in **Table 3.9.**

Table 3.9: SIFT, PolyPhen-2, and Fathmm-MLK prediction scores for candidate *ABCB1, OPRM1* and *COMT* polymorphisms.

Chr	Gene	SNP ID	Position		Coding effect	Functional effect predicted by		
						SIFT	Polyphen2	Fathmm-MLK
7	***ABCB1***	rs1128503	87179601	G>A	Syn (G412G)	T (1.00)	-	B (0,03)
		rs1045642	87138645	G>A	Syn (I1145I)	T (1.00)	**D (0.98) ***	B (0,03)
6	***OPRM1***	rs1799971	154360797	A>G	Non-Syn (N40D)	T (0,11)	B (0.23)	B (0,03)
		rs540825	154414446	T>A	Non-Syn (Q402H)	T (0,45)	B (0,00) *	B (0,00)
22	***COMT***	rs6269	19949952	A>G	UTR	-	-	B (0,09)
		rs4633	19950235	C>T	Syn (H62H)	T (0,67)	-	B (0,08)
		rs4818	19951207	C>G	Syn (L136L)	T (1.00)	-	B (0,08)
		rs4680	19951271	G>A	Non-Syn (V158M)	T (0,23)	B (0.01)	B (0,25)

SIFT-scores: 0.0-0.05 deleterious, 0.05-1.0 tolerated; PP2- scores: 0.0 - 0.15 Benign, 0.15-1.0 possibly damaging, 0.85-1.0 damaging; FTHM scores- >0.5 is Deleterious, <0.5 Benign; (-) No available scores to be reported; * PP2 scores retrieved from ensemble. Abbreviations: Syn, Synonymous; Non-Syn, Non-synonymous; UTR, untranslated region. Significant effects are in **bold.**

The *in-silico* analyses of the *ABCB1* polymorphisms predicted that the substitution effects were mostly benign. However, Polyphen-2 prediction of the rs1045642 G>A SNPs predicted a damaging effect on the resulting protein product. In silico analysis for *OPRM1* by SIFT, Polyphen-2 Sorting Intolerant From Tolerant, Polyphen-2 and FATHMM-MKL, showed SNP predicted effects to be like *ABCB1*. For *COMT* polymorphisms, rs6269 A>G, rs4633 C>T,

rs4818 C>G, and rs4680 G>A in silico analyses showed benign effects on the encoded protein predicted.

3.9.2 Two-Dimensional RNA Structure Prediction for Candidate SNPs

Using the *S*FOLD tool, analysis of *ABCB1*, rs1045642 G>A indicated that compared to the G allele, the A allele resulted in the loss of a hydrogen bond at nucleotide position 44 in the 5'-3' direction and resulted in a predicted increased multibranch loop (**Figure 3.35**A and B). In addition, the substitution noted a 3-point (-20.10 to -17.10) change in the energy reaction representing protein stability (ΔG°_{37}). Analysis of the rs1128503 SNP-containing structure indicated no distinct changes, however, the reaction showed a change in energy between the G and A alleles (**Figure 3.35**C and D).

*S*FOLD analysis of *OPRM1* rs1799971 A>G showed that compared with the A allele, the G allele resulted in the loss of an internal loop (**Figure 3.36**A and B). Furthermore, the A>G substitution resulted in a predicted 5-point change (ΔG°_{37} = -33.20 to -38.10) in the energy reaction, with the G allele noting a more negative ΔG°_{37}, compared with the A allele. The secondary predicted changes for *OPRM1* rs540825 T>A, indicated no structural differences between these two alleles; however, a change in the energy reaction (ΔG°_{37} = -22.70 to -19.70) was noted (**Figure 3.36**C and D).

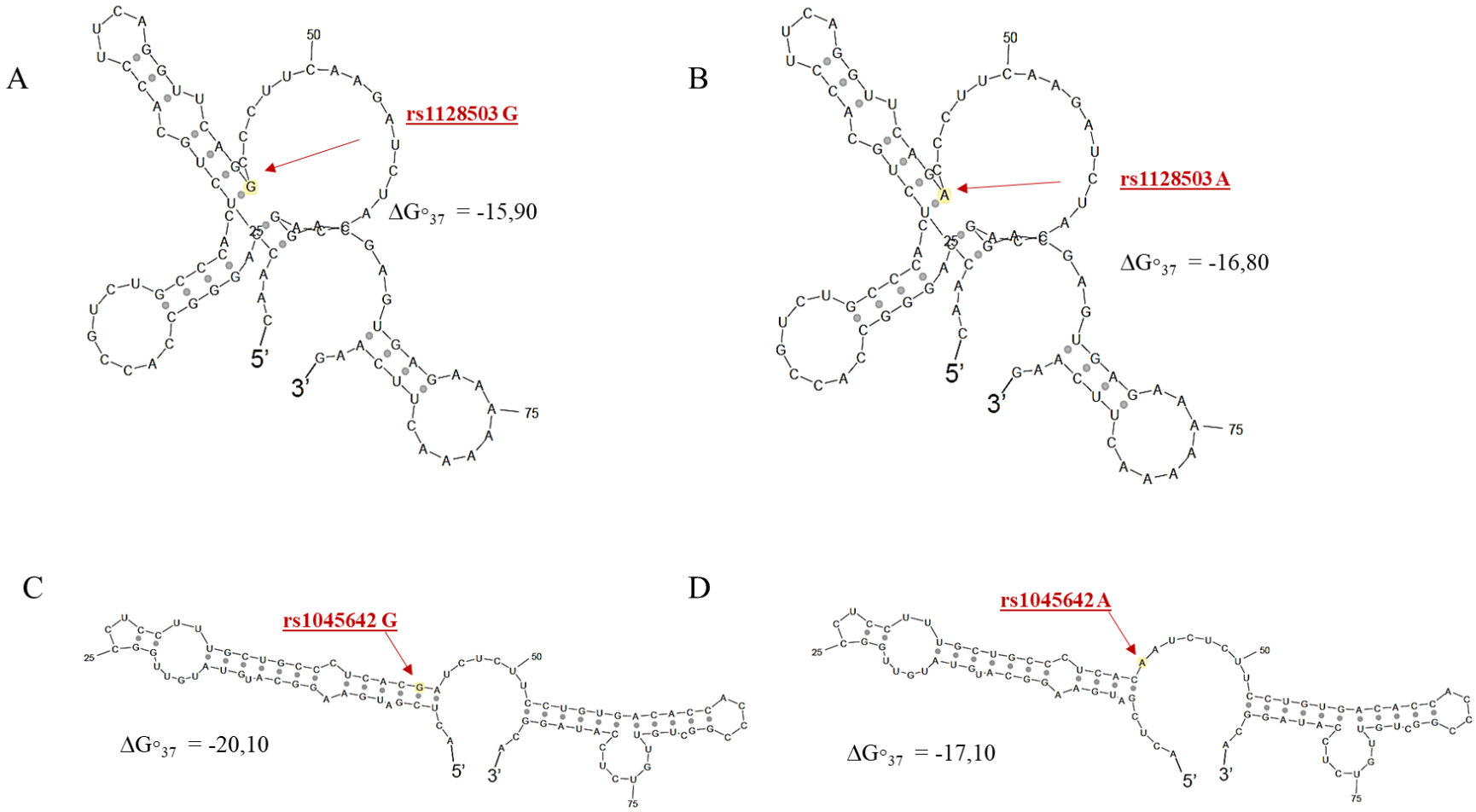


Figure 3.35: Computational prediction of the *ABCB1* RNA 2D structure using SFOLD. Shown are the top-scoring conformations for the *ABCB1* A) rs1128503- G, B) rs1128503- A, C) rs1045642 - G, and D) rs1045642 -A SNPS, with the corresponding Delta G scores (ΔG°37).

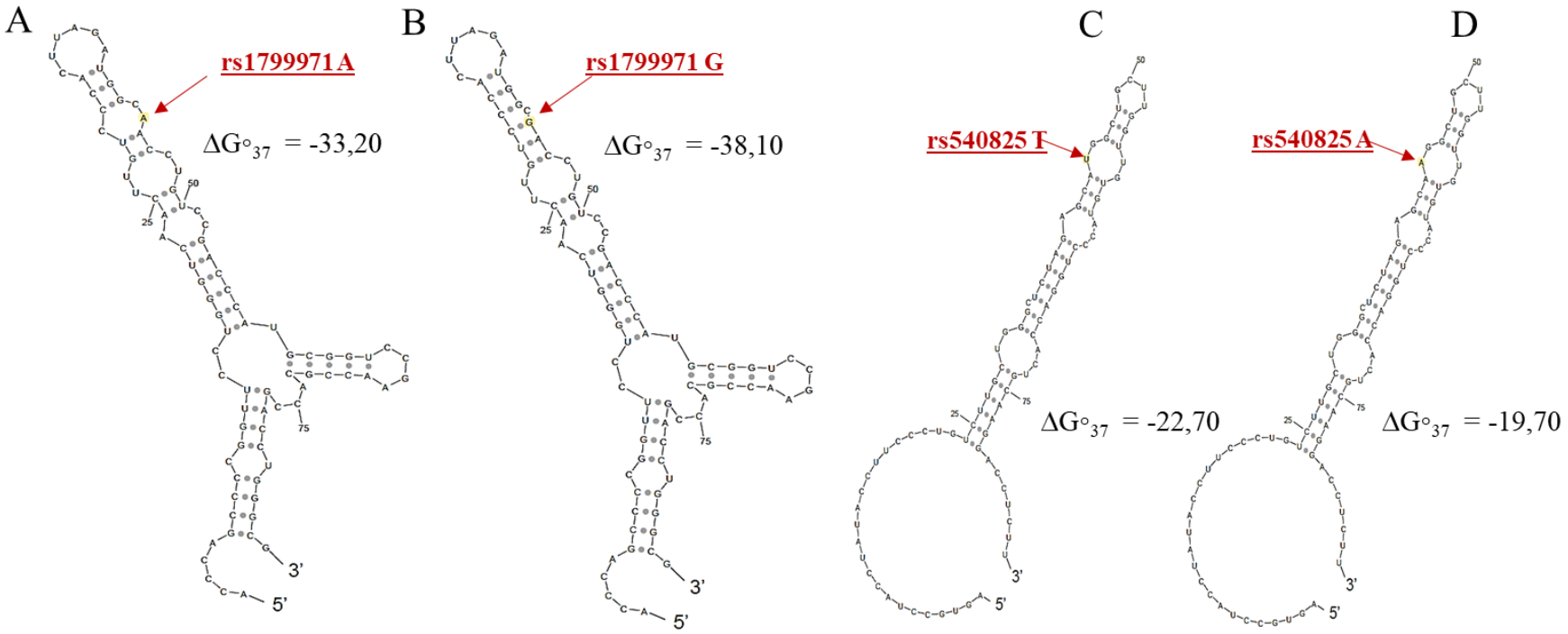


Figure 3.36: Computational prediction of the *OPRM1* RNA 2D structure using SFOLD. Shown are the top-scoring conformations for the *OPRM1* A) rs1799971-A, B) rs1799971-G, C) rs540825-T and D) rs540825-A SNPS, with the corresponding Delta G scores ($\Delta G°_{37}$).

*S*FOLD analysis for *COMT* indicated that rs6269 A>G SNP change did not affect protein structure, nor did the energy reaction note a change (**Figure 3.37**A and B). rs4633 noted distinct structural changes that resulted in the loss of an internal loop, and repositioning of the major multiloop branch (**Figure 3.37**C and D). The reaction also noted a reduction in the delta G score representing potential mRNA thermostability.

COMT rs4818 C>G also noted distinct structural changes, where the C to G allele substitution led to the shortening of an extending branch and the loss of an internal loop on that branch (**Figure 3.38**A). The substitution resulted in the restructuring of the major multibranch loop and the repositioned branch (**Figure 3.38**B). The substitution also noted a 3-point (C: -27.30 to G: -24.70) difference in the energy reaction noted between the alleles.

For rs4680 G>A, there were extreme structural and energy reaction changes noted (**Figure 3.38**C and D). The (G) to (A) allele substitution resulted in a complete change in predicted structure orientation. The substitution caused the loss of an extended loop, and the addition of three internal loops onto the structure upstream of the 3' region, as well as a 3-point change in the energy reaction between the G and A alleles (**Figure 3.38**D).

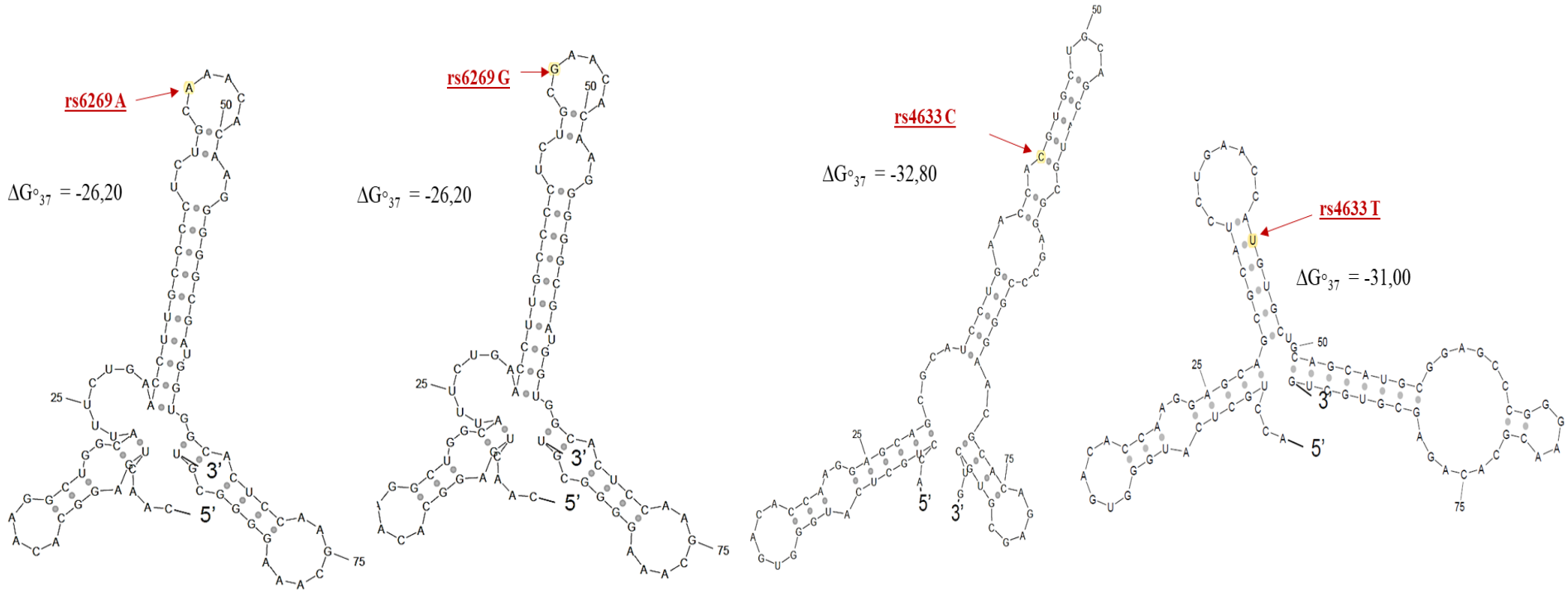


Figure 3.37: Computational prediction of the *COMT* RNA 2D structure using SFOLD. Shown are the top-scoring conformations for the *COMT* A) rs6269-A, B) rs6269-G, C) rs4633-C, and D) rs4633-T SNPS, with the corresponding Delta G scores (ΔG°_{37}).

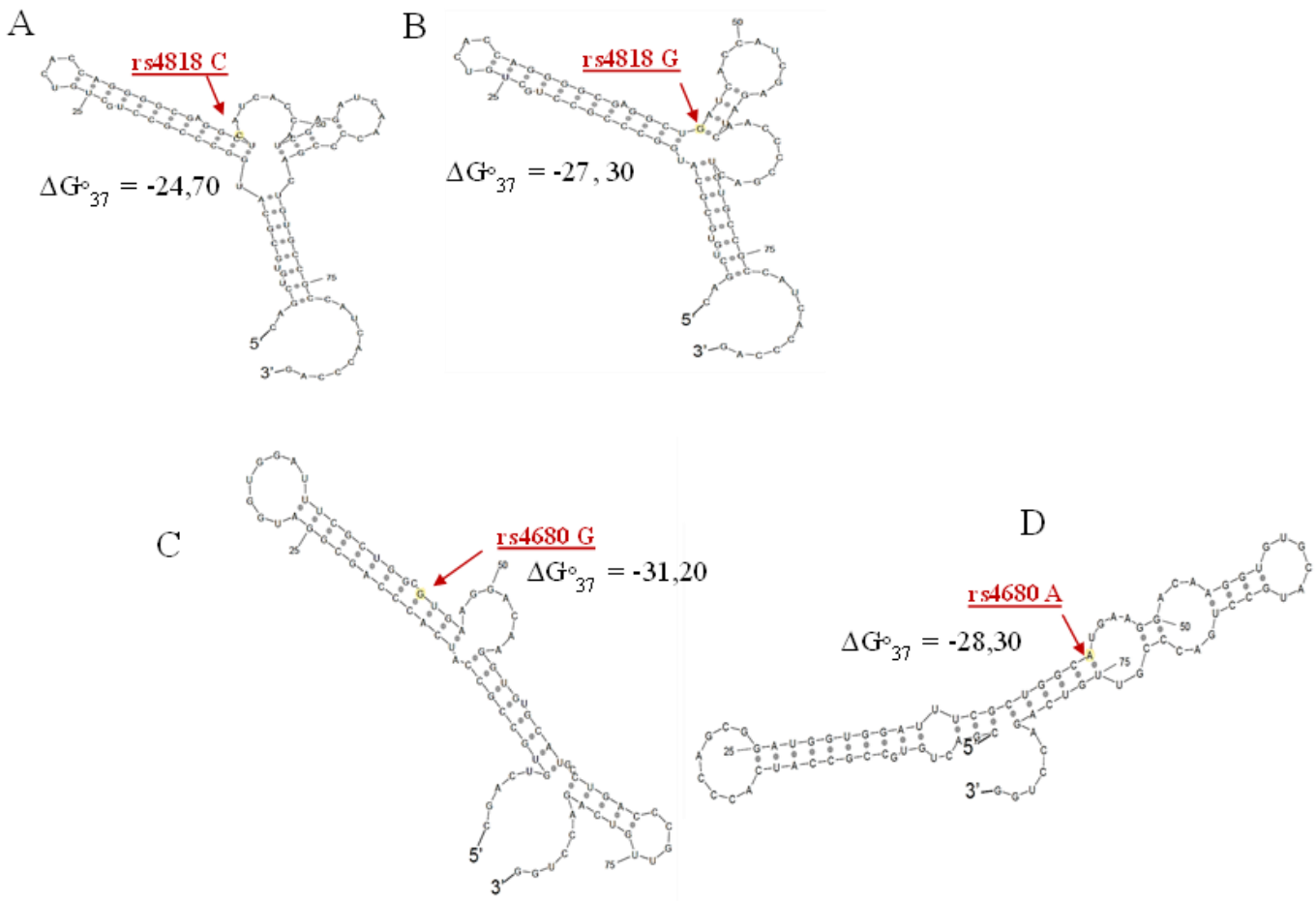


Figure 3.38: Computational prediction of the *COMT* RNA 2D structure using *S*FOLD. Shown are the top-scoring conformations for the *COMT* A) rs4818-C, B) rs4818-G, C) rs4680-G, and D) rs4680-A SNPs, with the corresponding Delta G scores (ΔG°_{37}).

3.9.3 GeneMANIA Gene-Associated Networks

GeneMANIA analyses were done using an automatically assigned weighted (presented in %) method that used basic gene ontology biological functions. Search parameters were to look at a maximum of twenty interactive genes with a total of ten shared attributes /functions.

Interaction analyses between *ABCB1* and *OPRM1* showed the genes phospholipase D2 (*PLD2*), ATP-Binding cassette subfamily B member 4 (*ABCB4)*, G-protein subunit β1 (*GNB1*) and solute carrier family 22 member 2 proteins (*SLC22A2*) directly links the *ABCB1* and *OPRM1* networks **(Figure 3.39**). The *PLD2* gene is co-expressed (1.98%) with *ABCB1* and shared physical interactions (4.19%) and predicted networks (12.04% and 33.03%) with *OPRM1* (Supplemental Table 18). *ABCB4* and *ABCB1* are family members and therefore co-expressed (2.06%, 3.93%, 1.11%, 1.87%), share predicted (70.53%) and shared protein domain (2.42% and 2.55%) networks, whereas *ABCB4* shares a genetic interaction (0.11%) with *OPRM1* (**Supplemental Table 25**). The *GNB1* protein shares a genetic interaction (0.09%) with *ABCB1*, and physical interactions (0.08%) and pathway (0.50% and 23.22%) networks with *OPRM1*. *SLC22A2*, a transporting protein shares physical interaction (16.64%) networks with *ABCB1*, while being co-expressed (1.89%) with *OPRM1*. Furthermore, the functional network analyses showed that *ABCB1* and *OPRM1* share transcriptional targets (**Supplemental Table 25**).

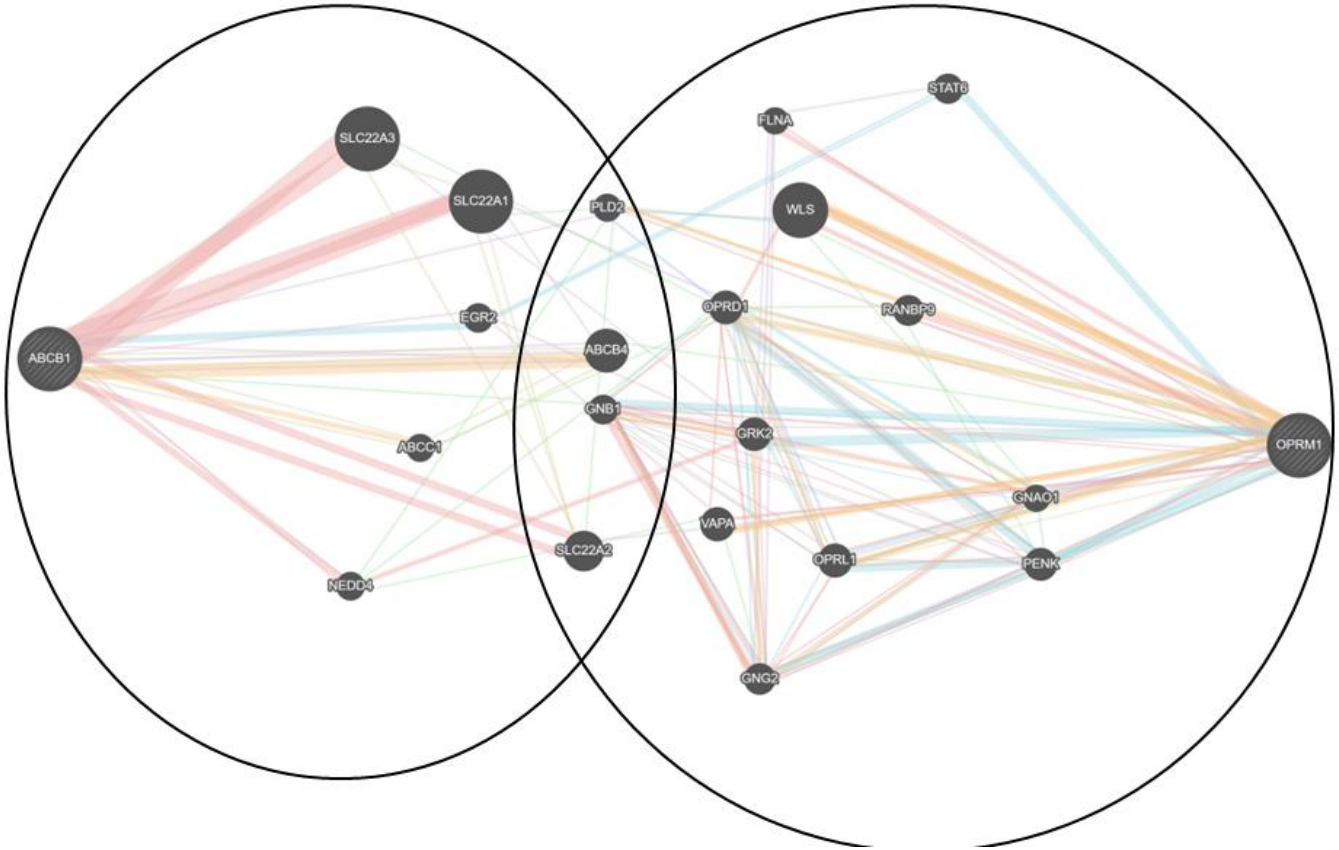


Figure 3.39: GeneMANIA Network analysis for the *ABCB1* and *OPRM1* genes. Shown here is the Venn diagram illustrating the shared gene-associated networks for *ABCB1* and *OPRM1*. The networks highlighted are the physical interaction (pink), co-expressed (purple), predicted (orange), co-localization (dark blue), genetic interactions (dark green), pathway (light blue) and shared protein domain (light green) networks of 20 genes.

However, no direct interactions were found between *OPRM1* and *COMT* in the GeneMANIA analyses (**Figure 3.40**). Several secondary gene-associated networks were noted that included the genes proenkephalin (*PENK*), opioid receptor-δ 1 (*OPRD1*), opioid-related nociceptin 1 (*OPRL1*), signal transducer and activator of transcription 6 (*STAT6*), fibroblast growth factor 2 (*FGF2*), adenosyl homocysteinase (*AHCY*), Ras and Rab interactor 1 (*RIN1*) and methionine adenosyl transferase 1A (*MAT1A*). The data showed that *OPRM1* and *COMT* share interactive networks that link through genetic and physical interactions, pathways, and co-expressed networks between *PENK*, *OPRD1*, *FGF2* and *AHCY* (**Supplemental Table 26**). Similarly, the *RIN1*, *STAT6, OPRD1* and *OPRL1* genes share co-expressed and shared protein domain networks that connects *OPRM1* and *COMT* network pathways. No functional networks were noted between *OPRM1* and *COMT*.

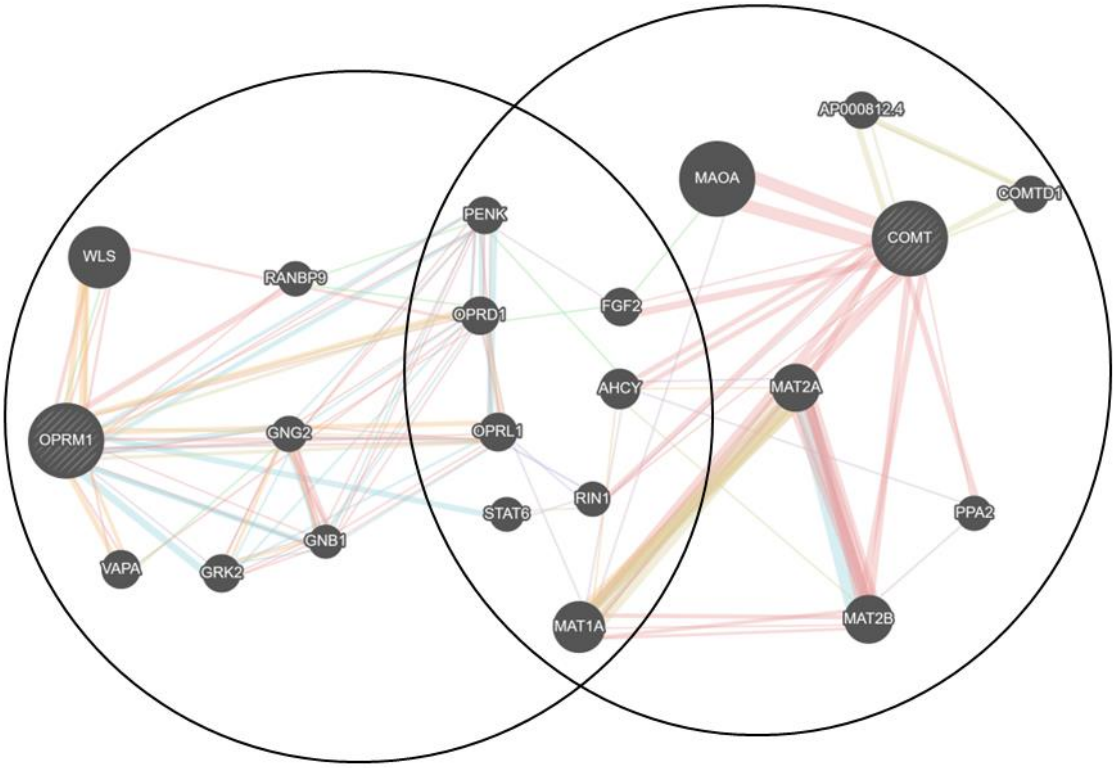


Figure 3.40: GeneMANIA Network analysis for the *OPRM1* and *COMT* genes. Shown here is the Venn diagram illustrating the shared gene-associated networks for *OPRM1* and *COMT*. The networks highlighted are the physical interaction (pink), co-expressed (purple), predicted (orange), co-localization (dark blue), genetic interactions (dark green), pathway (light blue) and shared protein domain (light green) networks of 20 genes.

For *ABCB1* and *COMT* interaction analyses, the genes monoamine oxidase A (*MAOA*) and catechol-O-methyltransferase domain containing 1 (*COMTD1*) were noted to directly connect the networks (**Figure 3.41**). *MAOA* shares co-expressed networks (1.28%) with *ABCB1*, and physical interaction (41.95%) networks with *COMT* (**Supplemental Table 27**). While *COMTD1* has a genetic interaction (0.10%) with *ABCB1* and shared protein domain (3.22% and 42.69%) networks with *COMT*. Several secondary gene-associated networks were also noted, but no functional networks were shared between *ABCB1* and *COMT* (**Supplemental Table 27**).

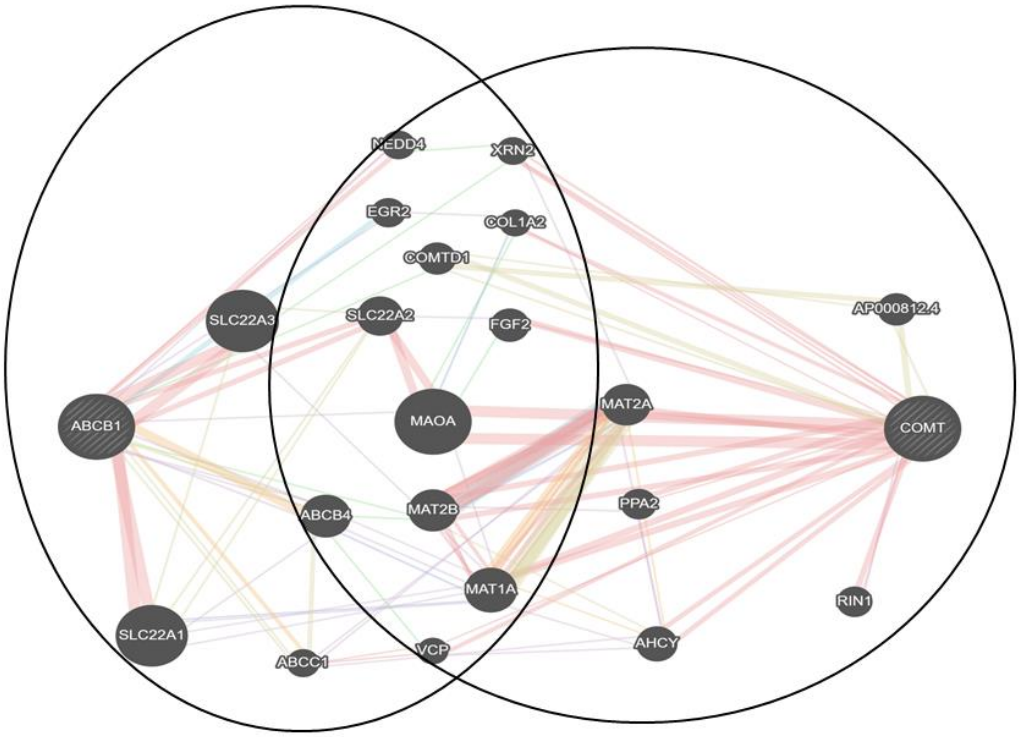


Figure 3.41: GeneMANIA Network analysis for the *ABCB1* and *COMT* genes. Shown here is the Venn diagram illustrating the shared gene-associated networks for *ABCB1* and *COMT*. The networks highlighted are the physical interaction (pink), co-expressed (purple), predicted (orange), co-localization (dark blue), genetic interactions (dark green), pathway (light blue) and shared protein domain (light green) networks of 20 genes.

Analyses of all three genes showed several interactive networks overlapping, with the closest interactions consisting of the *MAT1A* gene (**Figure 3.42**). Data output showed that *MAT1A* shares physical interactions (22.13%) with *COMT*, is co-expressed (1.42% and 0.48%) and co-localized (1.35%) with *ABCB4*. *MAT1A* is also co-expressed (0.87%) with *OPRL1* which shows *ABCB1*, *OPRM1* and *COMT* share an interaction through secondary gene-associated network pathways (**Figure 3.42**).

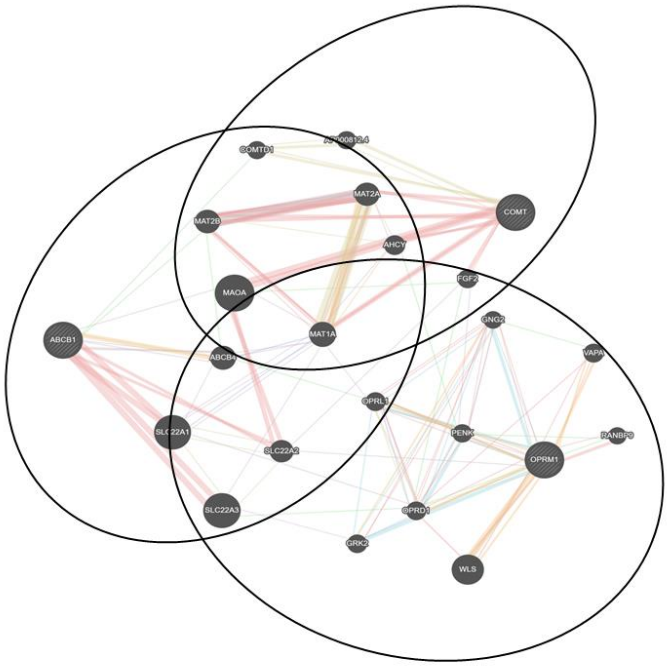


Figure 3.42: GeneMANIA Network analysis for the *ABCB1*, *OPRM1* and *COMT* genes. Shown here is the Venn diagram illustrating the shared gene-associated networks for *ABCB1, OPRM1* and *COMT*. The networks highlighted are the physical interaction (pink), co-expressed (purple), predicted (orange), co-localization (dark blue), genetic interactions (dark green), pathway (light blue) and shared protein domain (light green) networks of 20 genes.

3.9.4 EnrichR: Gene Set Enrichment Analyses.

The EnrichR web-based application was used to screen the genes against several libraries for transcriptional and regulatory factors, pathways, gene ontologies and phenotypes, diseases, and drugs. A significant association between the gene set and an enrichment term is reported using the adjusted p-value for multiple hypotheses (Benjamini-Hochberg method).

Transcription Library

Although no significant associations were noted for the gene set *ABCB1*, *OPRM1* and *COMT*, using a position-weighted matrix (PWMs) modelling from TRANSFAC and JASPAR, two transcription factor binding motifs were noted for the gene set, namely myocyte enhancer factor 2A (*MEF2A)* and forkhead box L1 (*FOXL1);* (**Table 3.10**).

Looking at computationally predicted mRNA targets, no significant associations were noted for *ABCB1*, *OPRM1* and *COMT* combined (TargetScan microRNA, 2017). For mRNA-targeted gene interactions from miRBase (2017), the miR-16-5p was associated with both *OPRM1* and *COMT* (adjusted p=0.036), (**Table 3.10**). In addition, screening the publication lists from the UCSC Genome Browser using the PWMs method, the results showed *ABCB1* and *OPRM1* are both associated with the TF binding site V$*STAT4_01* (adjusted p=0.009). This was noted in the GeneMANIA outputs earlier.

Table 3.10: EnrichR transcription library for *ABCB1*, *OPRM1 and COMT* gene set.

Genes	TERM	P-value	Adjusted P-value
	TRANSFAC and JASPAR PWMs		
ABCB1; OPRM1; COMT	MEF2A (human)	0,003	0,193
ABCB1; OPRM1; COMT	FOXL1 (human)	0,024	0,209
ABCB1; OPRM1	HMGA1 (human)	0,014	0,193
ABCB1; OPRM1	POU1F1 (human)	0,014	0,193
ABCB1; OPRM1	MYB (human)	0,014	0,193
OPRM1; COMT	PRDM1 (human)	0,015	0,193
ABCB1; OPRM1	HOXD9 (human)	0,017	0,193
	TargetScan MicroRNA 2017		
ABCB1; OPRM1	hsa-miR-875-5p	0,018	0,271
OPRM1; COMT	hsa-miR-296-3p	0,027	0,271
OPRM1; COMT	hsa-miR-324-5p	0,027	0,271
OPRM1; COMT	hsa-miR-3152-3p	0,028	0,271
	miRBase 2017		
OPRM1; COMT	hsa-miR-16-5p	0,017	**0,036**
	Genome Browser PWMs		
ABCB1; OPRM1	V$STAT4 01	0,001	**0,009**
ABCB1; OPRM1	CTTTAAR UNKNOWN	0,007	0,056

P-value was determined using Fisher's exact test, to correct for multiple hypotheses, the Benjamini-Hochberg method was employed.

Pathway Library

Screening the pathway databases, in the manually curated BioPlanet (2019) catalogue, *ABCB1* and *OPRM1* are associated with apoptosis, and *ABCB1* and *COMT* with metabolism ($p<0.05$). Additionally, in the Elsevier pathway collection, *ABCB1* and *COMT* are associated with Parkinson's, *OPRM1* and *COMT* with endometriosis, and *ABCB1* and *OPRM1* with Epilepsy ($p<0.05$); (**Table 3.11**).

Table 3.11: EnrichR pathways library for *ABCB1*, *OPRM1* and *COMT* gene set.

Genes	Term	P-value	Adjusted P-value
	BioPlanet 2019		
ABCB1; OPRM1	T cell receptor regulation of apoptosis	0,003	**0,010**
ABCB1; COMT	Metabolism	0,018	**0,031**
	Elsevier		
ABCB1; COMT	Proteins Involved in Parkinson's Disease	0,000	**0,002**
OPRM1; COMT	Proteins Involved in Endometriosis	0,000	**0,006**
ABCB1; OPRM1	Proteins Involved in Epilepsy	0,001	**0,006**

P-value was determined using Fisher's exact test, to correct for multiple hypotheses, the Benjamini-Hochberg method was employed.

Ontologies and Phenotype Library

Screening several databases, the results showed overlapping attributes for the study gene set, *ABCB1*, *OPRM1* and *COMT*. In the Gene Ontology database, specifically the biological and cellular components (2021), the gene set is noted to be associated with stress response regulation (*ABCB1* and *OPRM1*), and with localisations to the axon, dendrite, and neuron projections (*OPRM1* and *COMT*), ($p<0.05$). The gene set also showed an association in knockout mice with pharmacokinetic and movement-related phenotypes, $p<0.05$ (MGI Mammalian Phenotype level 4, 2021); (**Table 3.12**).

The text mining database JENSEN showed several associations between the study gene set, and various ontology terms in specific tissues, cellular localisation and human diseases, $p<0.05$ (**Table 3.12**). Interestingly, all three genes, *ABCB1*, *OPRM1* and *COMT* are noted to be associated with the adult-specific tissues, the *GCH1* complex, and plasma membrane regions and membrane components (**Table 3.12**).

Table 3.12: EnrichR ontologies library for *ABCB1*, *OPRM1* and *COMT* gene set.

Genes	Term	P-value	Adjusted P-value
	GO Biological Process 2021		
ABCB1; OPRM1	regulation of response to stress (GO:0080134)	0,000	**0,000**
	GO Cellular Component 2021		
OPRM1; COMT	axon (GO:0030424)	0,000	**0,001**
OPRM1; COMT	dendrite (GO:0030425)	0,001	**0,001**
OPRM1; COMT	neuron projection (GO:0043005)	0,002	**0,003**
	MGI Mammalian Phenotype level 4 2021		
ABCB1; COMT	abnormal xenobiotic pharmacokinetics MP:0008875	0,000	**0,000**
OPRM1; COMT	increased vertical activity MP:0002574	0,000	**0,007**
	JENSEN Tissues		
ABCB1; OPRM1; COMT	Adult	0,000	**0,010**
OPRM1; COMT	Ganglion	0,001	**0,025**
	JENSEN Compartments		
ABCB1; OPRM1; COMT	GCH1 complex	0,000	**0,000**
ABCB1; OPRM1; COMT	Plasma membrane region	0,000	**0,006**
ABCB1; OPRM1	Tic complex	0,000	**0,008**
ABCB1; OPRM1	Plastid membrane	0,000	**0,008**
ABCB1; OPRM1	Chloroplast membrane	0,000	**0,008**
ABCB1; OPRM1	Efflux pump complex	0,000	**0,008**
ABCB1; OPRM1	Chloroplast envelope	0,000	**0,008**
ABCB1; OPRM1	Plastid envelope	0,000	**0,008**
OPRM1; COMT	Post synapse	0,001	**0,013**
OPRM1; COMT	Dendrite	0,002	**0,017**
OPRM1; COMT	Cell body	0,002	**0,017**
ABCB1; OPRM1; COMT	Plasma membrane part	0,002	**0,018**
OPRM1; COMT	Synapse part	0,003	**0,019**
OPRM1; COMT	Somato dendritic compartment	0,003	**0,020**
OPRM1; COMT	Cell projection part	0,006	**0,028**
OPRM1; COMT	Neuron projection	0,006	**0,028**
OPRM1; COMT	Neuron part	0,012	**0,038**
ABCB1; OPRM1; COMT	Integral component of membrane	0,017	**0,040**
ABCB1; OPRM1; COMT	Intrinsic component of membrane	0,019	**0,040**
OPRM1; COMT	Cell projection	0,022	**0,046**
	JENSEN Diseases		
OPRM1; COMT	Substance abuse	0,000	**0,000**
OPRM1; COMT	Nicotine dependence	0,000	**0,000**
OPRM1; COMT	Pain agnosia	0,000	**0,001**
OPRM1; COMT	Alcohol dependence	0,000	**0,001**

P-value was determined using Fisher's exact test, to correct for multiple hypotheses, the Benjamini-Hochberg method was employed.

Diseases Library

Screening for human diseases, in the RARE disease GeneRIF/AutoRIF catalogue using PubMed searches, the gene set *ABCB1*, *OPRM1* and *COMT* were all associated with antisocial personality disorder, Dyskinesia drug-induced, a few headache and pain types as well as the mobility condition, myotonia congenita, p<0.001 (**Table 3.13**).

Table 3.13: EnrichR diseases library for *ABCB1*, *OPRM1* and *COMT* gene set.

Genes	Term	P-value	Adjusted P-value
RARE Disease GENERIF gene lists			
ABCB1; OPRM1; COMT	Antisocial personality disorder	0,000	**0,000**
ABCB1; OPRM1; COMT	Dyskinesia drug-induced	0,000	**0,000**
ABCB1; OPRM1; COMT	Cough headache	0,000	**0,000**
ABCB1; OPRM1; COMT	Thunderclap headache	0,000	**0,000**
ABCB1; OPRM1; COMT	Hypnic headache	0,000	**0,000**
ABCB1; OPRM1; COMT	Amyotonia congenita	0,000	**0,000**
ABCB1; COMT	Orthostatic intolerance	0,000	0,001
OPRM1; COMT	Grant syndrome	0,000	0,001
OPRM1; COMT	Mononeuritis multiplex	0,000	0,002
ABCB1; COMT	TAU syndrome	0,000	0,002
ABCB1; OPRM1	Primary biliary cirrhosis	0,000	0,002
ABCB1; COMT	Myxoma-spotty pigmentation-endocrine overactivity	0,000	0,002
ABCB1; COMT	Poland syndrome	0,000	0,002
ABCB1; COMT	Xeroderma pigmentosum	0,000	0,002
ABCB1; COMT	Alexander disease	0,000	0,003
OPRM1; COMT	Wisconsin syndrome	0,000	0,003
ABCB1; OPRM1	Epilepsy juvenile absence	0,000	0,003
ABCB1; COMT	Renal cell carcinoma 4	0,000	0,004
ABCB1; COMT	Neurotoxicity syndromes	0,000	0,004
ABCB1; OPRM1	Compartment syndrome	0,000	0,004
OPRM1; COMT	Basilar migraine	0,000	0,005
ABCB1; COMT	Thyroid cancer medullary	0,000	0,005
OPRM1; COMT	Lynch syndrome	0,000	0,005
ABCB1; COMT	Limb dystonia	0,000	0,005
ABCB1; COMT	Seminoma	0,000	0,005
ABCB1; OPRM1	Cholera	0,000	0,005

	Hypertrophic osteoarthropathy primary or		
ABCB1; COMT	idiopathic	0,000	0,005
ABCB1; OPRM1	Tuberous sclerosis	0,001	0,005
ABCB1; OPRM1	Bourneville syndrome	0,001	0,005
ABCB1; COMT	Narcolepsy	0,001	0,005
OPRM1; COMT	Testotoxicosis	0,001	0,007
OPRM1; COMT	Precocious puberty	0,001	0,007
ABCB1; COMT	Congenital nonhemolytic jaundice	0,001	0,007
OPRM1; COMT	Brown syndrome	0,001	0,007
ABCB1; OPRM1	Lymphocytes absent	0,001	0,009
	RARE diseases Autorif gene list		
ABCB1; OPRM1; COMT	Dystonia 13	0,000	**0,000**
ABCB1; OPRM1; COMT	Hereditary type 2 neuropathy	0,000	**0,000**
ABCB1; OPRM1; COMT	Hereditary type 1 neuropathy	0,000	**0,000**
OPRM1; COMT	Neuroleptic malignant syndrome	0,000	0,004
ABCB1; COMT	Neurotoxicity syndromes	0,000	0,004
ABCB1; COMT	Orthostatic intolerance	0,000	0,005
ABCB1; COMT	Amyloidosis cerebral	0,000	0,005
ABCB1; COMT	Diabetic mastopathy	0,000	0,010
ABCB1; COMT	Miura syndrome	0,001	0,020

P-value was determined using Fisher's exact test, to correct for multiple hypotheses, the Benjamini-Hochberg method was employed.

Drugs Library

Screening the drug signature database, several associations were noted between the study gene set and all three genes associated with morphine, p<0.001 (**Table 3.14**).

Table 3.14: EnrichR drugs library for *ABCB1*, *OPRM1* and *COMT* gene set.

Genes	Term	P-value	Adjusted P-value
	DsigDB		
ABCB1; OPRM1; COMT	morphine CTD 00006352	0,000	**0,000**
ABCB1; OPRM1; COMT	morphine BOSS	0,000	**0,000**
ABCB1; OPRM1	GBR 12909 dihydrochloride TTD 00008176	0,000	0,000
ABCB1; OPRM1	reserpine	0,000	0,001
ABCB1; OPRM1	Digitoxigenin TTD 00007578	0,000	0,001
ABCB1; OPRM1	danazol	0,000	0,001
ABCB1; OPRM1	mifepristone	0,000	0,001
ABCB1; OPRM1	Digitoxigenin CTD 00005821	0,000	0,001
ABCB1; OPRM1	Methylbenzethonium chloride CTD 00000197	0,000	0,001
ABCB1; COMT	methyldopa CTD 00006311	0,000	0,001
ABCB1; OPRM1	pimozide	0,000	0,001
ABCB1; COMT	estrone CTD 00005927	0,000	0,001
ABCB1; OPRM1	digoxin CTD 00005825	0,000	0,001
ABCB1; OPRM1	Naloxone hydrochloride BOSS	0,000	0,001
ABCB1; OPRM1	disulfiram	0,000	0,002
ABCB1; COMT	Adenosine triphosphate CTD 00005324	0,000	0,002
ABCB1; OPRM1	clotrimazole	0,000	0,002
ABCB1; COMT	okadaic acid CTD 00007275	0,000	0,002
OPRM1; COMT	METHAMPHETAMINE CTD 00006286	0,000	0,002
ABCB1; OPRM1	celecoxib CTD 00003448	0,000	0,003
ABCB1; COMT	clozapine CTD 00005693	0,000	0,003
ABCB1; OPRM1	NSC25485 CTD 00006443	0,000	0,004
OPRM1; COMT	dopamine BOSS	0,000	0,008
ABCB1; OPRM1	Capsaicin CTD 00005570	0,000	0,008
ABCB1; COMT	Fulvestrant CTD 00002740	0,001	0,011
ABCB1; OPRM1	cycloheximide CTD 00005731	0,001	0,011
ABCB1; COMT	3'-Azido-3'-deoxythymidine CTD 00007047	0,001	0,011
OPRM1; COMT	Dronabinol CTD 00006853	0,001	0,011
OPRM1; COMT	Phorbol 12-myristate 13-acetate CTD 00006852	0,002	0,011
ABCB1; COMT	tamoxifen CTD 00006827	0,005	0,011
ABCB1; COMT	genistein CTD 00007324	0,011	0,016
ABCB1; COMT	Bisphenol A CTD 00000312	0,011	0,016
ABCB1; OPRM1	metronidazole PC3 UP	0,012	0,017
ABCB1; COMT	resveratrol CTD 00002483	0,018	0,023
ABCB1; OPRM1	Decitabine CTD 00000750	0,023	0,029
ABCB1; COMT	progesterone CTD 00006624	0,026	0,032

3.10 KEY FINDINGS OVERVIEW

This chapter presented several findings, some of which are in alignment with previous reports as well as novel data. The study explored the clinical profile of South Africa Breast Cancer Survivors (SA BCS) highlighting the burden of pain, disability, and combined (pain and disability) symptoms along with other psychological effects despite pain management. The analyses conducted identified associations within the SA BCS cohort between a selection of prioritized single nucleotide polymorphisms (SNPs) and the different breast cancer (BC) treatments received. Furthermore, the prioritized SNPs were found to be associated with the burden of pain, disability, and combined (pain and disability) symptoms. Notably, the analyses revealed that certain combinations of alleles were linked to protective effects, while others were associated with harmful effects. Additionally, the bioinformatic analyses demonstrated the association of the candidate genes explored in the thesis, with specific pathways and networks. Particularly noteworthy were the results of enrichment analysis, which revealed a significant alignment between the genes, their functions, and disease/drug phenotypes. To summarise, the findings of this thesis provide insights into the broader implications and contextualization of the genetic findings within the scope of pathways and disease-related functions.

4 CHAPTER FOUR: DISCUSSION

4.1 INTRODUCTION

This thesis is the first study describing the evaluation of the associations between polymorphisms in genes involved in the opioid signalling and pain pathway, and chronic shoulder pain and disability in breast cancer survivors (BCS) of mixed ancestry from South Africa (SA). Using a candidate gene approach, the polymorphisms in genes *ABCB1, OPRM1* and *COMT* were evaluated. These genes encode proteins playing key roles in the opioid signalling pathway as described in **Chapter 1, Section 1.4** [464, 802].

The main novel findings included:

i. Independently, a significant association was noted for the *ABCB1* rs1045642 single-nucleotide polymorphism (SNPs), with the A/A genotype and A allele associated with reduced likelihood of reporting moderate-high disability and combined (pain and disability). The *COMT* rs4680 A/A genotype and A allele were also significantly associated with an increased likelihood of reporting moderate-high pain and combined (pain and disability).

ii. For the *ABCB1* (rs1128503 G>A-rs1045642 G>A) haplotype analysis, the inferred G-A and A-A haplotypes were significantly associated with reduced likelihoods of reporting moderate-high disability and combined (pain and disability), respectively. The *OPRM1* (rs1799971 A>G – rs540825 T>A) inferred G-T haplotype was significantly associated with reduced likelihood of reporting moderate-high pain. Inferred haplotype analysis of five *COMT* haplotypes noted significant associations for H2-H5, the most notable associations being for the rs6269 A>G -rs4680 G>A genetic

interval. This analysis revealed the G-G and A-A haplotypes were associated with reduced and increased likelihood of reporting moderate-high pain, respectively.

iii. Gene–gene interaction analyses demonstrated significant associations between the *ABCB1* (rs1045642 G>A) – *OPRM1* (rs1799971 A>G – rs540825 T>A) and the *ABCB1* (rs1045642 G>A) – *OPRM1* (rs1799971 A>G). The inferred A-A-T and A-A allele-allele combinations were associated with reduced likelihoods of reporting moderated-high combined (pain and disability). *ABCB1* (rs1045642 G>A) – *OPRM1* (rs540825 T>A) combination analyses demonstrated that the A-T combination was associated with reduced likelihood of reporting moderate-high disability/combined, and the alternate G-A combination was associated with increased likelihood of reporting moderate-high disability/combined.

iv. The *OPRM1* (rs1799971-rs540825) - *COMT* (rs4680) combination analyses demonstrated that the A-T-A and G-T-G were associated with increased and reduced likelihoods of reporting moderate-high pain. Similarly, the *OPRM1* (rs1799971 A>G)-*COMT* (rs4680 G>A) allele-allele combinations A-A, and G-G/A-G combinations were associated with increased and reduced likelihoods of reporting moderate-high pain and combined (pain and disability). The *OPRM1* (rs540825 T>A) - *COMT* (rs4680 G>A) A-A allele-allele combination was associated with an increased likelihood of reporting moderate-high combined (pain and disability).

v. Analyses of the *ABCB1* (rs1128503 - rs1045642) - *COMT* (rs4680) combination demonstrated that the A-A-G were significantly associated with reduced likelihood of reporting combined (pain and disability). In the 2-SNP pairing, the *ABCB1-COMT* (rs4680 G>A) G-A allele combinations were associated with an increased likelihood of reporting moderate-high pain, disability, and combined groups, $p<0.05$. In addition, the

alternate combination A-G was significantly associated with reduced likelihoods of reporting moderate-high disability and combined (pain and disability), $p<0.05$.

vi. The three-way gene-gene interactional analyses (*ABCB1* rs1045642- *OPRM1* rs1799971- *COMT* rs4680), demonstrated that the three allele-allele combinations were associated with increased and reduced likelihoods of reporting moderate-high scores, namely, (i) G-A-A in pain and combined pain and disability; (ii) A-A-G in disability and combined pain and disability; and (iii) G-G-G in pain only.

vii. Bioinformatic analyses supported the analyses by highlighting the functional and biological gene-associated networks.

4.2 SA BCS PROFILE: A 1-YEAR ANALYSIS OF PATTERNS RELATING TO PAIN, DISABILITY, ANXIETY, DEPRESSION, AFFECT AND PAIN MANAGEMENT

Understanding the development and prevalence of chronic shoulder pain and disability in SA BCS is a complex and multifaceted undertaking. In this thesis, a preliminary study was performed to provide an insight into the clinical status of BCS specifically during the first year commencing treatment for breast cancer. This was achieved through the examination of opioid prescriptions and clinical outcomes in a subset of participants for whom all of the data points were available throughout the one year (**Chapter 2**, **Sections 2.4.1** and **2.5**). Previous literature reported that older individuals are more likely to receive more opioid scripts compared to younger aged groups, opioids particularly prescribed for musculoskeletal pain [803]. Another reported that BMI is a potential factor for long-term opioid therapy and that increasing BMI is associated with increasing opioid scripts [804]. Our analysis of these few participants indicated that factors such as age, BMI or BC treatment types were not necessarily reliable predictors of opioid prescription.

In addition, significant changes in pain, disability, anxiety, depression, and affect among SA BCS during the study period were observed. As previously presented in the literature review (**Chapter 1**, **Section 1.3.1**), the 3-month mark is a significant change in diagnoses from where symptoms beyond this are regarded as chronic. Additionally, the levels of pain increasing up to 3 months postoperatively and decreasing slightly at 1 year postoperatively appear to be in line with the observations of many other studies [433, 805].

Anxiety and depression are well-described psychological side effects associated with postoperative pain and reportedly may be predictive of severe post-operative pain and analgesic requirements like tramadol [806-809]. The preliminary findings of this study, however, noted normal levels of both anxiety and depression outcomes that increased over the study period. In addition, tramadol and paracetamol dosage seem to only correlate with anxiety levels rather than depression. Positive and negative affect (mood) are also outcomes associated with pain conditions, and to a degree considered to include symptoms of anxiety and depression[810]. It is reported that positive and negative affect will decrease and increase, respectively, in chronic pain conditions over time, which is consistent with the trends noted in the subset of sample analyses [434]. Furthermore, it is suggested that this trend (increasing and decreasing affect) may not necessarily be indicative of pain levels [434]. In this thesis, the patterns of negative affect scores appear to mirror those observed for pain, disability and combined. Whereas positive affect scores exhibited consistent decreasing patterns.

Regarding the relationship between drug administration and clinical outcomes, it was noted that total prescription of tramadol appears to predict pain, disability, anxiety, and negative affect at 3 months and 1 year postoperatively. Whereas the total paracetamol prescribed was more predictive of pain/disability, combined (3 months), anxiety (3 months and 1 year), and negative

affect (3 months only). The finding that the total tramadol prescription, as opposed to the total paracetamol prescriptions, is predictive of pain/disability and combined symptoms at one year aligns with the drugs' mechanism of action (MoA). The literature extensively described the role of opioids and opioid receptors in the development of chronic pain conditions [460, 811] _ENREF_445[811, 812]. Conversely, the MoA of paracetamol involves the inhibition of prostaglandins which causes inflammation in the CNS, thereby activating pain receptors [411, 813]. Moreover, the anti-inflammatory effect of paracetamol is reportedly mild in comparison and may over time become tolerated [411, 814, 815].

The central concern motivating this thesis is driven by the observed increase in pain management without clear evidence of pain relief. As discussed in **Chapter 1**, **section 1.3,** it is proposed that inadequate pain relief during the acute postoperative period is predictive of the development of chronic pain and disability. It is also proposed that inadequate pain relief due to non-responsiveness to opioid analgesics could increase the likelihood of developing chronic pain and disability. Additionally, the clinical variables (for example anxiety and depression) of BCS may also play a role in the chronicity and the exacerbation of pain and disability. These results motivated the primary focus of this thesis which aimed to investigate the role of genetics in the prevalence of chronic shoulder pain and disability, specifically genes forming part of the opioid signaling and pain pathway.

4.3 THE GENETIC ASSOCIATION STUDY

Evaluation of chronic shoulder pain and disability in the full SA BCS cohort showed that 27%, 19% and 22% reported moderate-high pain, disability, and combined symptoms, respectively. Furthermore, we noted similar frequencies of pain following breast cancer treatments like the wide local excision (WLE), axillary lymph node dissection (ALND) and radiation therapy (RT)

in comparison to previously published data [7, 749, 775, 816]. The frequency of disability in the SA BCS cohort was, however, relatively lower in comparison to the literature as many studies reported a higher prevalence (>30%) in predominantly European populations (German n=314 [771]; German n=686 [776]; Danish n=132 [777] and NE Brazilian n=101[773]). In addition, participants in these cohorts received mastectomies in addition to RT and ALND [771, 773, 776, 777]. Earlier reports have shown that the different treatment modalities employed may impact the likelihood of developing pain and disability symptoms and have therefore been the basis for the present study.

Several clinical and demographical variables were evaluated in the BCS cohort; however, significant associations were observed for age at the time of surgery and the total number of nodes involved. Younger age was associated with an increased likelihood of reporting moderate-high pain, disability and combined (pain and disability). This result is in alignment with previous research reporting the association between younger age and persistent pain in BCS [20, 773, 817]. It is proposed that changes in pain perception are age-related; compared to older adults (> 65 years), younger adults (18–44 years) report greater pain because of psychological stress [818]. Younger adults with chronic pain experience physiological and mental health issues, and the disruption of daily routines, including the ability to work full-time can lead to a financial burden [819, 820]. However, the relationship between age and pain is complex and subject to debate. For instance, while evidence suggests that the threshold for perceiving pain may increase with age, indicating a need for greater stimulus, it has also been observed that the ability to tolerate maximum pain intensity may decline with age[821-824]. With more than 70% of participants having received an ALND, it was noteworthy to find no significant association between ALND and shoulder pain, disability or combined (pain and disability). There are two forms of lymph node surgeries, the sentinel lymph node biopsy (SLNB) procedure that is

specifically designed to remove isolated sentinel lymph nodes, and the axillary lymph node dissection (ALND) which involves the removal of a selective group of lymph nodes in the axilla [825, 826]. Moreover, it has been described as a risk factor for the development of persistent pain post-surgery for breast cancer [20].

4.3.1 *ABCB1* rs1128503 G>A and rs1045642 G>A SNP Associations

Evaluation of the genetic contribution of *ABCB1* gene polymorphisms in modulating shoulder disability and movement-related pain in this BCS's cohort showed that the *ABCB1* rs1045642 A/A genotype and A allele were significantly associated with 80% / 75% and 48% / 50% reduced likelihood of reporting moderate-high disability / combined (pain and disability), respectively. Haplotype analyses highlighted a defined *ABCB1* genetic interval in modulating pain and disability where the *ABCB1* (rs1128503 G>A-rs1045642 G>A) G-A/A-A haplotypes were associated with a reduced likelihood of reporting disability/combined (pain and disability) groups, respectively. It's noteworthy that disability symptoms were emphasized for this polymorphism, however, shoulder pain often leads to pain-related disability[827-829]. Reports indicate that there is a significant correlation between fear of movement and disability, with fear often resulting in altered range of motion patterns and affecting shoulder function [827-829]. These findings, therefore, correlate with other studies reporting lower pain, although data on postoperative pain is limited [471, 830, 831]. The rs1128503 A allele and the functional rs1045642 A allele have steadily been associated with requiring fewer opioids compared to G allele carriers and with rs1045642 A carriers regarded as "good responders" [496, 512, 515, 737, 832]. It is reported that the rs1045642 A/A genotype and A allele are associated with decreased protein functions and are at greater risk of opioid-related side effects that include sweating, muscular tension, stress, and sedation, compared to G/G and A/G carriers [495, 497, 509]. Furthermore, given its implication in opioid requirements and response studies, it is hypothesized that the A/A

genotype has lower P-gp expression which may result in less efflux of opioids at the BBB level, and therefore individuals with that genotype profile, may need fewer opioids to control pain. This hypothesis is supported by the findings of Hoffmeyer *et al.* (2000)[497], who showed a reduction in P-gp expression and altered activity related to the A/A genotype. More recently, allelic-specific expression analysis of liver autopsy samples noted the rs1045642 A allele was associated with lower mRNA levels compared to the G allele [833]. Additionally, research reports that *ABCB1* expression and function could also be influenced by epigenetic alterations, in particular DNA methylation and histone acetylation, as seen in response to chemotherapy [834-836]. Cancer-related studies reveal that increased expression of *ABCB1* increases resistance to anthracyclines and taxane drugs [834-836]. The increased expression can lead to modified transcription factor activity, as well as gene mutation, rearrangements, hypomethylation and potentially miRNA regulation [837-842].

Several SNPs have been investigated for *ABCB1*, this study focused on rs1128503 G>A and rs1045642 G>A and the evaluation of the SNP frequencies showed that the rs1128503 MAF in the SA BCS was similar to the global population. Rendering it as a suitable candidate for investigating symptoms of pain and disability across diverse populations. In addition, an evaluation of the LD structure for the three most widely studied SNPs using the populations of Africa is reported on Ensembl (Ensembl genome browser 108). The SNPS, rs11282503 G>A, rs2032582 G>A, and rs1045642 G>A, have been consistently investigated in haplotype analysis [22, 490, 501]. The LD structure review noted that the LD scores were similar for the populations between rs1045642-rs2032582, rs2032582-rs1128503, and rs1045642-rs1128503. On average the LD scores were strong between pairs. Furthermore, the LD scores in the BCS cohort were comparable (rs1045642-rs1128503) to the published scores reported for African populations. When considering the associations between the genotypes and

demographical/clinical variables, it was observed that A/G genotype carriers (rs1128503 and rs1045642) were associated with being younger at the time of surgery and reporting longer periods of experiencing pain. The latter qualifies the carriers as chronic, and the former is in alignment with previous literature on age.

4.3.2 *In Silico* Exploration of *ABCB1* rs1128503 G>A and rs1045642 G>A SNPs

In evaluating the potential effects of each SNP, SIFT, PP2 and FATHMM analysis indicated the synonymous *ABCB1* rs1128503 G>A SNP, had a tolerated/benign effect. However, research shows synonymous SNPs, or "silent" mutations could influence gene function through subtle changes in translational processes, e.g., co-translational folding [843, 844]. Synonymous SNPs may therefore have direct implications on the pharmacokinetics and pharmacodynamics of drugs [845]. Furthermore, we noted using the online protein structure prediction tool *S*FOLD to examine the predicted 2D RNA structure for the regions containing the *ABCB1* SNPs, the rs1128503 G and A allele forms showed no differences in the secondary predicted mRNA structures. Instead, changes in the melting domains were noted as reflected in the energy reaction of the ΔG°_{37} scores, indicating that this polymorphism may still affect the transport through other processes, e.g., it may change interactions/ efficiencies of the transporter within the cellular environment [659, 846-848]. It may also hint that we need to investigate the protein dynamic folding which, however, was beyond the scope of this study. The importance of RNA is widely recognised in biological functions and that the RNA structures are essential to these processes[849]. Moreover, RNA structural modifications possibly caused by SNPs, may disrupt posttranslational control mechanism, potentially leading to the dysregulation of pain perception [850].

For *ABCB1* rs1045642 G>A, SNP effect analysis indicated the SNP to have a damaging effect by PolyPhen2. Following the 2D mRNA structure assessment, the predicted secondary structure for the A allele showed an increased multi-branch loop at that position (**Figure 4.1**) in comparison to the G allele form. Given this alteration, it can be hypothesised that this polymorphism could therefore potentially modulate the mechanism of the transporter through such structural changes. In addition, the A allele also showed an increase in the ΔG°_{37} score indicating a less stable mRNA structure compared to the G allele form, which supports the findings observed in the study conducted by Wang and Sadee (2006)[833].

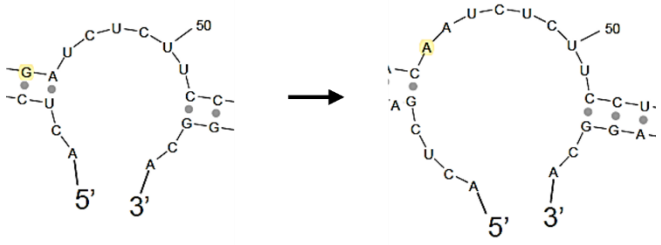


Figure 4.1: *ABCB1* rs1045642 G>A impact on RNA structure.

4.3.3 *OPRM1* rs1799971 A>G and rs540825 T>A SNP Associations

Analyses of the *OPRM1* genetic locus showed no independent associations for the individual polymorphisms investigated. The findings noted no significant differences in frequency distribution patterns of the minor alleles for rs1799971 A>G and rs540825 T>A. Several studies have however associated the rs1799971 G allele with higher pain [24, 561, 851, 852] in females [565, 853], and with increased pain sensitivity [564, 854]. Interestingly, the populations described are heterogenous at the population [USA (n=102); Czech republic (n=51/69); Taiwan (n=147); Norway (n=258/118); Singapore (n=270)] and clinical surgical setting (prostatectomy, hysterectomy, hernioplasty, lumbar disc herniations, and caesarean) . In contrast, some studies have noted no statistically significant links between the rs1799971 G allele and pain [471, 518, 567,

[855-857]. The lack of associations may be explained by several factors which include study design, surgical and clinical settings, confounders such as environmental factors, as well as ethnic background [28, 471, 740, 858-860]. Pain-related studies surrounding the *OPRM1* rs540825 T>A SNP are limited, which is a functional SNP that implicates a different isoform of the translated protein[566]. One study found *OPRM1* rs540825 T>A had a clinical effect on pain scores following major surgery, where the T allele compared to the A allele, was associated with increased analgesic requirements for postoperative pain [557]. Another study, publishing preliminary data reported that the rs540825 T>A was associated with reduced morphine dose, mediating opioid response [861]. In the present study, no significant association was noted between the genotype and allele frequency distribution of rs540825 T>A and pain, disability and combined (pain and disability) symptoms.

The inferred haplotypes for *OPRM1* however, implicated the genetic interval spanning these polymorphisms in modulating chronic shoulder pain and disability. The inferred *OPRM1* (rs1799971 A>G-rs540825 T>A) G-T haplotype was associated with a 67% reduced likelihood of reporting pain. Research data for the *OPRM1* rs1799971-rs540825 locus in postoperative pain is limited. However, a study conducted by De Gregori *et al.* (2016)[557] analysing seven *OPRM1* polymorphisms, including both rs1799971 A>G and rs540827 T>A, observed that haplotype carriers containing the G and T alleles required more postoperative analgesia (POA) than haplotype carriers containing the A and A alleles, respectively. This observation suggested that the rs1799971 G and rs540825 T allele, when in combination, required more POA may be in response to experiencing greater pain. It has been shown that the rs1799971 G allele increases the receptor binding affinity 3-fold for endogenous opioids, yet reduces the expression of the receptor and leads to high morphine requirements [862]. Also, *OPRM1* rs1799971 and rs540825 polymorphisms have both been investigated in pain and opioid studies

as both have been described to modify the mu-receptor properties, though inconsistencies remain [24, 469, 512, 555, 557, 561, 851, 863]. Although the functional SNP rs1799971 has been widely investigated, rs540825 remains understudied. Also of note, is that *OPRM1* subject to hypermethylation resulting from increased exposure to opioid use may also influence gene function and chronic musculoskeletal pain outcomes in addition to affecting addiction, a key side effect noted for opioid use [864, 865]. A systematic review focusing on pain highlighted that methylation and miRNA expression are the foremost epigenetic causes associated with alterations in gene function [866]. For instance, one study investigating BC patients treated with tramadol and paracetamol noted that at least two different miRNAs could influence the variability of acute and chronic pain [867]. The MAF frequency of rs1799971 fell within the range observed in the global population, while rs540825 was significantly low. In addition, compared to the global populations, these two *OPRM1* SNPs displayed the weakest LD in the mixed ancestry SA BCS. The only genotype effect observed in this cohort was for lymph node surgery, where A/A carriers were fewer than other genotypes. However, to the best of the author's knowledge, there has been no published research linking *OPRM1* genotypes to surgical outcomes. This area therefore remains in need of further scrutiny.

4.3.4 *In Silico* Exploration of *OPRM1* rs1799971 A>G and rs540825 T>A SNPs

Bioinformatic assessment of the selected SNPs, *OPRM1* rs1799971 indicated the A>G substitution to have a tolerated/benign effect on the resulting protein product. However, this nonsynonymous substitution results in an amino acid change within the receptors' extracellular n-terminal domain with the potential to influence binding affinity, agonist sensitivity as well as all downstream cascading effects [868-871]. Furthermore, for *OPRM1* rs1799971 A>G, the predicted secondary structure for the G allele noted the loss of one (out of three) internal loops at that position (**Figure 4.2**). Compared to the A allele, the G allele formed a hydrogen bond

with the opposite nucleotide at that position. Moreover, the predicted secondary structure for the G allele noted a decrease in ΔG°_{37} scores, which suggests a more stable protein compared to the A allele form. The hypothesis therefore is that this polymorphism could potentially modulate the mechanism of the μ-receptor binding through such structural changes [846, 848, 869].

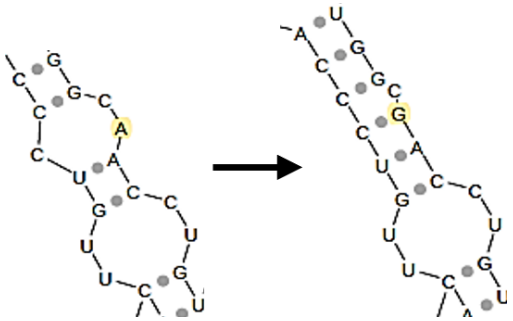


Figure 4.2: *OPRM1* rs1799971 A>G impact on RNA structure.

Similarly, *OPRM1* rs540825 T>A is predicted to have tolerated/benign effects on the results protein product with no structural differences between the T and A allele forms noted. Instead, the differences in ΔG°_{37} scores suggest that the nonsynonymous polymorphism may still affect the receptor's binding capacity through mRNA-structure-dependent processes, thereby potentially influencing drug response [551, 846, 848].

4.3.5 *COMT* rs6269 A>G, rs4633 C>T, rs4818 C>G, and rs4680 G>A SNP Associations

Genotype and allelic analysis of *COMT* showed the functional polymorphism rs4680 G>A A/A genotype to have a 3.23 and 3.81 increased odds of reporting moderate-high pain and combined (pain and disability), respectively. Similarly, the rs4680 A allele showed a 1.58 and 1.71 increased odds of reporting moderate-high pain and combined (pain and disability),

respectively. These findings agree with the published studies indicating that rs4680 A allele is associated with increased pain and that the A allele correlates with decreased *COMT* enzyme activity [25, 872, 873]. Furthermore, the levels of *COMT* activity have been linked to the regulation of neurotransmitters in the pain modulation pathway, including the opioid system [463, 736, 874]. However, some inconsistencies have been reported for this genetic locus [27, 787, 875, 876] _ENREF_25. For instance, in a study focusing on experimental and acute pain in postoperative breast cancer patients (Finland, n=1000), no significant associations were found for this genetic locus [787]. The authors speculate that this lack of association could stem from factors like the specific pain pathways activated, the timing and duration of reported pain, or the genomic environment, including specific haplotypes[787]. The role of potential epigenetic changes may also account for the disparity as discussed under *ABCB1* and *OPRM1* associations. Similarly, *COMT* expression could also be influenced by methylation as noted in a study examining the role of *COMT* in chronic fatigue syndrome and fibromyalgia [877].

Analyses of the central haploblock of *COMT* highlighted marked frequency differences between the SA BCS cohort and the reported global populations. It is hypothesized that this is reflective of the significant differences in the minor allele frequencies between the various populations for rs6269 A>G and rs4633 C>T. This highlights the importance of profiling the genetic structure of unique populations, as in the case of the SA mixed ancestry cohort. Evaluation of the LD structure between the SNP pairs further emphasizes the LD decay in the cohort investigated. This observation is not surprising, as it was previously described [878]. Specific haplotypes of the *COMT* haploblock were noted to be significantly associated with pain and combined (pain and disability), specifically the haplotype pairs rs6269A>G-rs4680 G>A. The observed G-G allele pair showed a 0.67 decreased odds of reporting moderate-high pain. Whereas the alternate A-A allele pair showed 2.09 and 2.18 increased odds for pain and

combined (pain and disability), respectively. These allele pairs reflect the high enzyme *COMT* activity associated with the G allele [765]**.** The study design was limited by sample size and therefore, did not allow for the evaluation of the full haploblock containing the four SNPs (rs6269 A>G, rs4633 C>T, rs4818 C>G, rs4680 G>A). In terms of the MAF distribution, it was observed that rs6269 and rs4633 were significantly more prevalent in the mixed ancestry SA BCS than in the global population. Whereas the SNPs, rs4818 and rs4680, noted similar MAF distributions. In addition, although an LD decay was observed for most of the SNP pairs, the SNP pair rs4818-rs4680, displayed a strong LD, like that of the global population. When comparing to the super populations, certain SNP pairs displayed weak and moderate LD patterns within the African population, resembling the LD patterns observed in the SA BCS cohort. This could be explained by the complex history and demographic dynamics unique to the SA mixed ancestry population [879]. The genetic effects of the *COMT* SNPs were observed to be linked to demographical/clinical variables, particularly the treatment modalities employed. For instance, fewer *COMT* rs6269 A/A genotype carriers received adjuvant Neo-CT and RT. As far as the relationship between *COMT* and breast cancer is concerned, conflicting reports are linking *COMT* activity to BC risk [880-883]. However, Neo-CT and RT are typically suggested to patients on a case-by-case basis, and the associations observed may be indicative of a sampling bias.

4.3.6 *In Silico* Exploration of *COMT* rs6269 A>G, rs4633 C>T, rs4818 C>G, and rs4680 G>A SNPs

Evaluation of the predicted consequential SNP effects by SIFT, PP2 and FATHMM noted *COMT* rs6269 A>G, rs4633 C>T, rs4818 C>G and rs4680 G>A, predicted all have tolerated/benign effects on the resulting protein product. The insignificant impact noted for the rs6269 A>G alleles was further supported by the 2D mRNA structure prediction, which could

be explained by the SNP's genomic location. *COMT* rs6269 A>G is within a promoter region of intron two, however, intronic SNPs have the potential to alter expression aspects such as splicing efficiency that can influence disease modification and susceptibility [659, 884]. For SNPs, rs4633 C>T, rs4818 C>G and rs4680 G>A, the 2D structures highlighted distinct structural mRNA changes contrary to the predicted consequences noted by SIFT, PP2 and FATHMM. The SNP's alternate alleles noted a loss of an internal loop (rs4633 T; **Figure 4.3**), a restructured hanging loop off the multi-branch (rs4818 G; **Figure 4.3**) and a complete 2D restructure with loss of hydrogen bonds and an increase of internal loops downstream (rs4680 A; **Figure 4.3**C), respectively. In addition, the predicted mRNA structures demonstrated significant changes within the energy reactions (ΔG°_{37}) highlighting the potential of the SNPs to alter protein availability and mechanisms through mRNA instability and translational processes, consistent with the literature [847, 848].

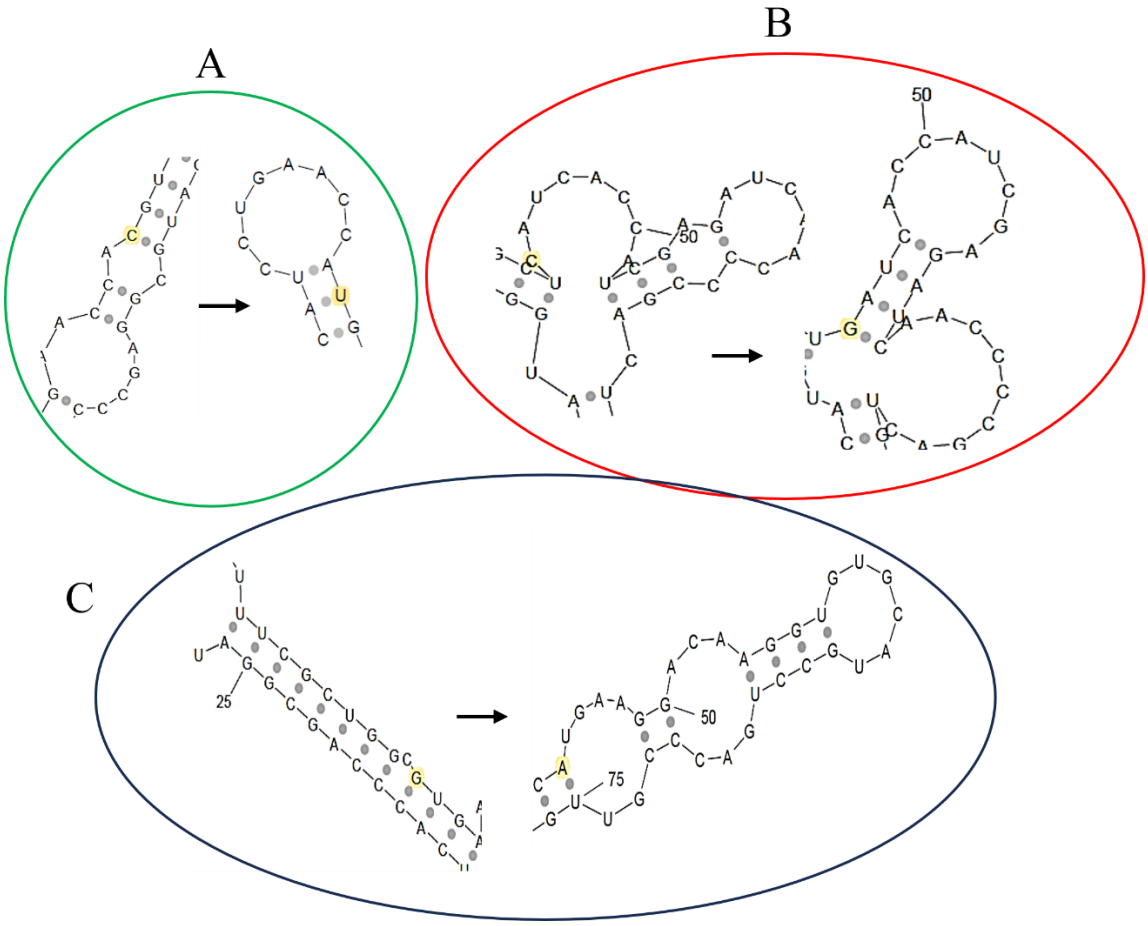


Figure 4.3: *COMT* rs4633 C>T, rs4818 C>G and rs4680 G>A impact on RNA structure.

BCS experience chronic pain on a variety of levels, and the accompanying genotype is likely to be just as complex [428, 554]. Given the multifaceted characteristic of pain, current research strongly suggests that an interaction between multiple genetic loci may well collectively contribute to modulating the pharmacological effects of prescribed medications [468, 554, 557, 680, 726, 885, 886]. Therefore, as a proxy for gene-gene interactions, specific allele-allele combinations were evaluated between the SNPs for *ABCB1*, *OPRM1* and *COMT* using the individual genotype data.

4.4 THE *ABCB1-OPRM1-COMT* GENE-GENE INTERACTION STUDY

4.4.1 Exploration of the *ABCB1* (rs1045642 G>A) – *OPRM1* (rs1799971 A>G- rs540825 T>A) Allele-Allele Interaction

Evaluation of the *ABCB1-OPRM1* allele combinations highlighted a combined genetic contribution to modulating disability and movement-related pain in this BCS cohort. Several associations were noted. The A-A-T allele-allele combination for the *ABCB1* (rs1045642 G>A) – *OPRM1* (rs1799971 A>G- rs540825 T>A) construct noted a 0.58 reduction in odds for pain and disability combined. Further examination of the two SNP allele-allele combination analysis for the *ABCB1* (rs1045642 G>A) – *OPRM1* (rs1799971 A>G- rs540825 T>A) construct revealed the inferred A-A (*ABCB1* rs1045642 G>A- *OPRM1* rs1799971 A>G) allele combination was associated with a 0.44 reduction in the likelihood of reporting combined (pain and disability). The likelihood of reporting pain and disability was equal when the *ABCB1* rs1045642 G and *OPRM1* rs1799971 A alleles were present. It is, however, important to approach this finding with caution considering the sample size and the number of SNPs under evaluation. Detecting multilocus genotype combinations through logistic regression can present challenges, particularly when there are too few or no data points for rare combinations, potentially hindering the outcomes [887].

Interestingly, in a previous study, haplotype carriers containing the *ABCB1* rs1045642 A in combination with the *OPRM1* rs1799971 A allele were observed to have lower methadone dosage and plasma concentrations than the alternate *ABCB1* allele [734]. The study concluded that *ABCB1* (lower) and *OPRM1* (higher) polymorphisms were associated with opposing dose requirements [734]. This supports the findings that the *ABCB1* rs1045642 A allele when inherited with the *OPRM1* rs1799971 A modulates pain scores, and in the previous study, in the form of opioid requirements. Moreover, the findings expanded on this known relationship by exploring

the complex interactions between *ABCB1* and *OPRM1* concerning chronic pain and disability within a genetically unique cohort.

Another noteworthy finding for *ABCB1* and *OPRM1* gene-gene interaction was for the *ABCB1* (rs1045642 G>A) - *OPRM1* (rs540825 T>A) allele-allele combination. Here the A-T allele combination was associated with 0.62 reduced risk of reporting moderate-high disability and combined (pain and disability). Coupled with the alternate combination, G-A, being associated with 1.57 and 1.50 increased risk of reporting moderate-high disability and combined (pain and disability). Research data for pain studies surrounding the gene-gene interactions between the *ABCB1* and *OPRM1* genes, and particularly these allele pairs, are limited. Based on these findings, the hypothesis here is that the *ABCB1* rs1045642 A allele may act as a potentially important regulator of opioid distribution. The *OPRM1* rs540825 T allele is reported to require more opioids and when inherited with the *ABCB1* rs1045642 A allele, resulted in reduced likelihood of reporting pain and disability [557]. This is further supported by the alternate allele combinations whereby the *OPRM1* rs540825 A allele which is reported to require fewer opioids inherited with the *ABCB1* rs1045642 G allele, was shown to have increased P-gp expression, an increased likelihood of reporting pain and disability was noted. This could be explained as nociceptive sensitization by exposure to opioids leading to opioid-induced hyperalgesia (OIH), the paradoxical effect that may intensify pre-existing pain [888]. Furthermore, it is reported that up-regulation of the *ABCB1 P*-gp membrane protein may contribute to opioid tolerance [889].

4.4.2 *In Silico* Exploration of *ABCB1 – OPRM1* Gene-Gene Interaction

Bioinformatic analyses noted a few shared functional pathways between the *ABCB1* and *OPRM1* genes using the GeneMANIA and EnrichR platforms. Noted in the GeneMANIA analysis, was the indirect interaction between *ABCB1* and *OPRM1* through secondary and tertiary gene-associated networks. The highlighted genes were observed to connect the *ABCB1* and *OPRM1* gene set through several networks such as co-expressed (*PLD2*, *ABCB4*, *SLC22A2*), physical interaction (*PLD2*, *SLC22A2*), predicted (*PLD2*, *ABCB4*), and pathway (*GNB1*) networks. Each of the genes identified is similar in function and has roles as either a substrate transporter such as *ABCB1* [795, 890, 891], form part of the signalling cascade associated with GPCRs as in the case of *OPRM1* [892, 893] or are involved in cellular homeostasis [894]. Interestingly, the *ABCB4* and *GNB1* genes were observed to share a genetic interaction with *ABCB1* and *OPRM1*. The role of investigating genetic or gene-gene interactions between independently acting genes is vital in the understanding of a complex phenotype such as chronic pain, especially if the effect of one gene is modest [895]. This shared gene-associated network, thus further supports the gene-gene interaction noted between *ABCB1* and *OPRM1* in the prevalence of chronic pain and disability.

Enrichment analysis further revealed both genes are targets for transcriptional regulation through microRNA binding, specifically the microRNA miR-16-15p. A recent study using carcinogenic tissue culture has shown that miR-16-5p activity has the potential to influence *ABCB1* expression and function [896]. Similarly, studies have predicted target sites for mir-16-5p in the three prime untranslated regions of *OPRM1* mRNA [897]. In addition, the analyses also noted both genes share binding domains for the *STAT4* transcriptional factor (TF) which encodes a DNA-binding protein that is critical in the transcription process following cytokine stimulation [898, 899]. The TF reportedly requires and will bind to the sequence

(T/A)TTCC(C/G)GGAA(T/A) present within the transcription region of a target gene [900]. *ABCB1* and *OPRM1* may have at least one or multiple sites containing this sequence according to the molecular signatures database (MSigDB) on Gene set enrichment analysis (GSEA) (STAT4_01 (gsea-msigdb.org). Using nuclear extracts from placental cells and promoter-binding TF profiling plate arrays, Speidel *et al.* (2018)[901] reported that the *ABCB1* promotor region has a strong binding affinity for the *STAT4* transcription factor indicating the presence of multiple binding sites. Other libraries screened during the enrichment analysis were pathway, gene ontology (GO), and diseases, where the GO library highlighted an association between the gene set and stress response (GO:0080134). Endogenous opioids are expressed throughout the brain in response to stressful events, which modulates the response through various mechanisms associated with each opioid receptor, including *OPRM1* [902]. More so, specifically, the *OPRM1* rs1799971 A>G SNP has been found to modify and regulate stress responsivity and the hypothalamic-pituitary-adrenal (HPA) axis in healthy volunteers [903, 904]. In addition, following the molecular profiling of three HPA axis components using mouse models and human samples, Lopez *et al.* (2021)[905] reported that *ABCB1* function is vital in the regulation of glucocorticoid uptake into the adrenal glands, thereby regulating stress adaptation. It is widely known that the HPA axis is an essential part of the brain that regulates the overall functionality of the system including stress response [906].

Combined, the role of *ABCB1* and *OPRM1* in chronic pain is further highlighted through their relationship to stress. Stress is described as a psychological state presenting with elevated levels of anxiety, and depression commonly observed concerning pain, which may lead to an allostatic imbalance [902]. The inability to manage pain and stress, especially if it is persistent, is thought to result the maintenance and aggravation, thereby keeping the cycle of pain and stress going [907]. The gene set *ABCB1* and *OPRM1*, were both associated with epilepsy (pathway and

rare diseases library), where *ABCB1* has been associated with drug resistance of several anti-epileptics in varying populations [493, 908-911]. Whereas increasing levels of endogenous opioids have been reported during epileptic incidents, although the exact role of *OPRM1* is unknown and thereby remains poorly understood [912, 913]. Also observed during the screening of the drug libraries were that both genes are relevant in the opioid pathway as the genes are consistently associated with morphine drug signatures [496, 512, 734, 737, 832, 914-916].

4.4.3 Exploration of the *OPRM1* (rs1799971 A>G- rs540825 T>A)- *COMT* (rs4680 G>A) Allele-Allele Interaction

The next interaction investigated the allele-allele combinations between the *OPRM1* and *COMT* SNPs. The gene-gene interactions between the *OPRM1* rs1799971 A>G, rs540825 T>A, and *COMT* (rs4680 G>A) SNPs noted a few specific allele-allele combinations associated with risks for reporting pain and combined symptoms. A significant interaction was observed for the *OPRM1* (rs1799971 A>G-rs540825 T>A)-*COMT* (rs4680 G>A) allele-allele combination in the pain category. The findings noted the A-T-A allele combination was associated with an increased risk for moderate-high pain. In addition, the G-T-G allele combination, with alternate alleles for rs1799971 A>G and rs4680 G>A, was associated with decreased risk for moderate-high pain. This finding underlines the significant interaction between functional *OPRM1* rs1799971 A>G and *COMT* rs4680 G>A SNPs that have been implicated in previous pain-related studies [554, 557, 680, 885, 886, 917]. Studies examining various pain settings (for example postoperative abdominal surgery and cancer pain), that were primarily focussed on cohorts that are of Caucasian origin [554, 557, 680, 885, 886, 917]. Thereby, warranting further analysis of the specific *OPRM1* rs1799971 A>G - *COMT* rs4680 G>A allele-allele combination. Analyses showed the A-A allele-allele pair had a 1.35 and 1.42 increased risk for reporting moderate-high pain and combined (pain and disability), respectively. Whereas the

alternate G-G allele-allele combination pair had a 0.23 decreased risk of reporting moderate-high pain. Although this study did not measure opioid requirements, it did measure reported pain scores. The findings contrasted with previous studies described in populations of European descent, which measured opioid requirements and rescue [554, 885, 917].

A novel finding in this study is the interaction between the *OPRM1* rs540825 T>A and *COMT* rs4680 G>A SNPs since no studies have yet explored the interaction between any *COMT* SNPs and the *OPRM1* rs540825 T>A polymorphism. The findings of this study showed the T-G allele combination was associated with an equal likelihood of reporting combined symptoms. In contrast, the alternate A-A combination was associated with an increased likelihood of reporting combined symptoms. The result supports the hypothesis that alternative exon splice variants are notable genetic sources with the potential to influence pain variability in response to opioid consumption [551]. The *OPRM1* rs540825 T>A polymorphism has been implicated in mood [558, 566] and fentanyl-induced emesis [551] studies, while pain-related studies are limited. This nonsynonymous polymorphism, rs540825 T>A (Glu>His), is found in the alternative exon of *OPRM1* that defines the MOR-1X protein isoform [566]. The biological significance attached to this polymorphism is attributed to its location in the C-termini of the isoform, crucial to receptor phosphorylation, internalization, and desensitization in response to μ-agonists [550]. In this interaction, assuming the functional *COMT* rs4680 polymorphism drives this interaction when breaking down genotype-phenotype data available for *COMT* rs4680 G>A, A/A carriers report higher pain but require fewer opioids [463, 918, 919]. This is hypothesized to be the result of diminished μ-opioid receptor response to pain in addition to an increase in MOR receptor availability [463, 736, 920]. Whereas carriers of the rs540825 A allele have previously been associated with requiring less opioids arguably in a response to less pain [557]. However, SNP studies for *OPRM1* rs540825 and *COMT* rs4680 are conflicting and limited, particularly

in the case of the rs540825 SNPs and therefore remain poorly understood. It could be that the requirement for fewer opioids may be due to agonists competing between an increase of MOR1 receptors, rather than that of a modified MOR-1X receptor. Regan *et al.* (2016)[553] hypothesized that opioids have a role in mediating cell-specific splicing and found morphine administration upregulated MOR-1X isoform expression. In this study, opioid requirements or receptor availability were not measured and therefore, these results warrant further scrutiny. When considering the assumption that the *COMT* rs4680 A allele drives this interaction, the study findings appear to be in line with the prior studies. The functional effect of *COMT* rs4680 is well described in various populations and associated with an increased risk of chronic pain after surgery [27, 557, 873, 921].

4.4.4 *In Silico* Exploration of the *OPRM1* - *COMT* Gene-Gene Interaction

Further examination of the interaction between *OPRM1* and *COMT* noted several secondary gene-associated networks using the bioinformatic approach. *OPRM1* and *COMT* were associated through physical and genetic interaction, co-expressed, shared protein domains, as well as pathway networks. The different associated networks noted here provide evidence and support the interactive relationship between *OPRM1* and *COMT*. For the reason that the secondary genes were either a subtype of the protein family, and therefore have functional similarity, or interact with the primary gene as in the case of *OPRD1*, *OPRL1* and *PENK* [631, 922, 923]. The enrichment analyses also highlighted interesting associations between the gene set, within each library screened. The gene set was associated with being found in specific cellular compartments that are specialized to the pain pathway and neuronal activity such as the axon, dendrites, and post-synaptic regions [924]. These nerve cell components work together allowing for the transmission of signals through chemical messengers (neurotransmitters) that will either induce or inhibit neuronal activity [924, 925]. The membrane-bound mu-opioid receptor can be

presynaptic which may reduce the inhibitory transmission, and postsynaptic whereby it directly inhibits the signal transmission [926]. Whereas the role of *COMT* is to regulate levels of types of neurotransmitters. The gene set was also associated with behavioral conditions such as substance abuse and addiction, as well as pain-orientated diseases as in the case of mononeuritis multiplex, basilar migraine [927, 928]. Pathway enrichment highlighted a link between this gene set and endometriosis, a chronic inflammatory condition that presents severe pain. Although the implication of the *OPRM1* and *COMT* genes are expected given their roles in the pain pathway, it is reported that the genes may also potentially impact risk for BC [929].

4.4.5 Exploration of the *ABCB1* (rs1128503 G>A-rs1045642 G>A)- *COMT* (rs4680 G>A) Allele-Allele Interaction

Reports of gene-gene interactions or synergistic effects between the *ABCB1* and *COMT* genes are limited. This study described the novel allele-allele combinations between *ABCB1* and *COMT* SNPs to be associated with pain and combined (pain and disability) symptoms. The *ABCB1* (rs1128503 G>A - rs1045642 G>A) – *COMT* (rs4680 G>A) A-A-G allele-allele combination was associated with a reduced likelihood of reporting combined pain and disability symptoms. This association was further supported with two SNP pair analyses as noted between the individual *ABCB1* rs1128503 G>A and *COMT* rs4680 G>A SNPs. The alternate alleles for each SNP, rs1128503 G and rs4680 A, were associated with an increased risk for pain and combined symptoms. The *ABCB1* (rs1045642 G>A) - *COMT* (rs4680 G>A) construct noted the complementary allele-allele pairs, G-A and A-G, were associated with increased and reduced likelihoods of reporting pain and combined symptoms, respectively. This study is the first to report an allele-specific interaction between these genes concerning the prevalence of chronic shoulder pain and disability in BCS. To date, numerous studies have been conducted in the clinical setting to elucidate the functional significance of *ABCB1* and

COMT SNPs, in pain- and opioid-related research with various outcomes [914, 916, 930-932]. However, the studies aimed to clarify the role of these SNPs either individually, or in haplotype form and to date no combinations of alleles have been reported as far as the authors are aware. Other than the functional role of drug distribution and through pain modulation of the dopaminergic pathways, the *ABCB1* and *COMT* genes are key players each within its independent pathway. As both genes are located on different chromosomes, the findings of this study showed the potential of allele-specific interaction across different chromosomes to influence the susceptibility of developing a particular phenotype.

4.4.6 *In Silico* Exploration of the *ABCB1- COMT* Gene-Gene Interaction

In the network analyses, it was observed that *ABCB1* and *COMT* share gene-associated networks with the *MAOA* and *COMTD1* genes, each having roles in the enzymatic activity of neurotransmitters like dopamine, but were more associated with the *COMT* gene (weight >40%); [933, 934]. The enrichment analyses also highlighted several associations for the gene set, most of which were for the disease and drug libraries. Screening of these libraries showed that both genes were associated with varying cancer types (e.g. thyroid cancer, renal cell carcinoma) and neurological (e.g. TAU syndrome, limb dystonia) disorders [935-939]. Upon the examination of screening the drugs library, a clear correlation emerges between diseases and drugs concerning each gene's function. The drugs library noted associations for specific drugs that are administered as treatment in cancer and neurological disorders [940-942]. These enrichment results displayed a clear demonstration of the genetic interaction that may exist between *ABCB1* and *COMT*.

4.4.7 Exploration of the *ABCB1* (rs1045642 G>A)- *OPRM1* (rs1799971 A>G)- *COMT* (rs4680 G>A) Allele-Allele Interaction

The collective gene-gene interaction between *ABCB1*, *OPRM1* and *COMT* has not been previously examined. Based on a literary report indicating that the three genes form part of the fundamental opioid signaling and pain pathway [464], this thesis demonstrates that SNPs within each *ABCB1*, *OPRM1* and *COMT* collectively, modulate chronic shoulder pain and disability in this SA BCS cohort. The results highlighted three specific allele-allele combinations formed by SNPs within each gene, which are associated with moderate-high pain, disability, and combined symptoms. These combinations include G-A-A, G-G-G and A-A-G. Notably, the former and latter allele-allele combinations containing the alternate forms of *ABCB1* rs1045642 G>A and *COMT* rs4680 G>A in combination with *OPRM1* rs1799971 A revealed contrasting associations. Furthermore, the associations of these allele-allele combination as presented in **Table 4.1**, were reflective of the independent gene associations noted in **Sections 4.3.1** and **4.3.5**.

Table 4.1: *ABCB1* (rs1045642)-*OPRM1* (rs1799971)-*COMT* (rs4680) gene-gene interaction associations.

ABCB1 **rs1045642 G>A**	***OPRM1*** **rs1799971 A>G**	***COMT*** **rs4680 G>A**	**Trend (odds)**	**Category**
MAJOR	MAJOR	**minor**	**Increased (1.93/1.63)**	Pain/Combined
Major	**minor**	MAJOR	Reduced (0.00)	Pain
minor	MAJOR	MAJOR	**Reduced (0.37/0.51)**	Disability/Combined

4.4.8 *In Silico* Exploration of the *ABCB1* - *OPRM1* - *COMT* Gene-Gene Interaction

In the bioinformatic analyses, the gene-associated networks for the gene set found an association with the *MAT1A* gene, a tumour suppression gene [943]. This correlation is, however, a tertiary link displaying weak associations for which data is limited, and therefore requires further in-depth exploration of the network. Enrichment analyses of *ABCB1*, *OPRM1*, and *COMT* revealed an association with the myocyte enhancer factor (*MEF2A).* A transcription factor that is vital for a wide range of cellular processes including neuronal differentiation, and skeletal muscle regeneration [944, 945]. Although significance was removed after adjusting, it could be hypothesized that expression of the gene set may potentially be influenced by the TF as it is widely distributed and vital in the brain [945]. The role of *MEF2A* may also be argued through its role in muscle regeneration as reports show it influences myoblast proliferation, differentiation and as well as myofibrogenesis [946-949]. Fibrosis is often associated with pain, which in theory is an associated outcome of dysregulated cellular proliferation [947, 949-951].

This potential interaction is however speculative since the relationship between the gene set, *MEF2A*, and chronic pain/disability is not well established, and therefore requires further scrutiny. Ontology terms highlighted an association of the gene set with the GCH1 Complex and plasma membrane regions. The GCH1 complex term describes the various steps in the production of Tetrahydrobiopterin (BH4), a crucial cofactor in the synthesis of neurotransmitters [952]. As previously highlighted, there is a significant relationship between *OPRM1*, *COMT* and neurotransmitters like dopamine; the enrichment findings support this relationship even further. Whereas the *ABCB1-GCH1* Complex association comes in the form of its role in drug delivery for crisis pain, specifically in sickle cell disease [953]. In the disease library, most of the enrichment correlation is observed for the gene set, with several pain and

movement and/or muscle-associated phenotypes. Emerging from the drugs library, the drugs associated, namely morphine, support the correlation between the gene set and pain phenotypes we have observed in the study.

5 CHAPTER FIVE: SUMMARY AND FUTURE PERSPECTIVES

5.1 SUMMARY

A growing challenge in **South African (SA**) healthcare is the frequency of chronic shoulder pain and disability among **breast cancer survivors (BCS)**. It is theorised that genetics may play a role in the development of these symptoms, and more so, a lack of adequate pain relief during the acute postoperative period regardless of opioid therapy. Evidence suggests that polymorphisms within genes functioning within the opioid signalling and pain pathways have the potential to influence the development of chronic musculoskeletal pain and disability conditions. As discussed in **Chapter 1, Sections 1.4.1, 1.4.2, and 1.4.3**. the widely studied ***ABCB1***, ***OPRM1***, and ***COMT*** genes have consistently been implicated in these conditions given the biological function that the translated proteins have in the opioid signalling and pain pathways. This thesis therefore sought to identify potential genetic contributors to variability in pain response and to understand the development of chronic pain and disability in SA BCS of **mixed ancestry** background. Particularly since the SA population has a diverse genetic background and an increasing BCS cohort that is of mixed ancestry.

The main aims of this thesis were, therefore, to evaluate the role of single nucleotide polymorphisms (SNPs) in candidate genes forming part of the pain-modulating pathway in a BCS cohort with symptoms of chronic pain and disability using the Shoulder Pain and Disability Index (SPADI), within a SA population of mixed ancestry. Additionally, the secondary aims were to explore an *in-silico* approach to evaluate the potential functional effects of the polymorphisms within the candidate genes on their encoding protein. In addition, highlighting potential biologically relevant pain-related pathways specific to this BCS cohort.

The specific objectives, therefore, were to describe the prevalence of BCS and frequencies of those reporting symptoms of chronic pain and disability using the Shoulder Pain and Disability Index (SPADI) in the SA cohort of mixed ancestry. Additionally, to conduct an exploratory sample analysis, evaluating the clinical profile of a subset of BCS during a 1-year treatment period relating to opioid administration and patient-reported outcome measures [(SPADI, Hospital Anxiety and Depression Scale (HADS) and Positive and Negative Affect (PANAS)].

The exploratory clinical sample analyses were a descriptive assessment that provided evidence of increasing levels of pain and disability during the 1-year course of BC treatment despite management with opioids and without clear evidence of relief. Specifically, pain, disability and combined symptoms scores were highest at 3 months postoperatively and noted a slight decline at 1 year, which are reported to be predictive of developing chronic pain. The clinical assessment also provided insight into the emotional and behavioural variables of participants during this period. Notably, anxiety, depression and negative affect increased over this period, while positive affect decreased. The patterns observed for these outcomes have been described in the literature in **Chapter 1**, **Sections 1.2.4** and **1.3.4**, and support the role of psychological exacerbations of chronic conditions. Although these findings were limited by various factors highlighted in **Chapter 4**, **Section 5.3.** The data aligned with the theory that polymorphisms within genes operating in the opioid signalling and pain pathway may contribute to inadequate relief and therefore impact the development of chronic symptoms.

The specific objectives for the genetic association studies (A: independent gene association and B: gene-gene interaction association) were to evaluate the genotype and allele frequencies of eight prioritised SNPs in three candidate genes: (i) *ABCB1* (rs1045642 G>A; rs1128503 G>A), (ii) *OPRM1* (rs1799971 A>G; rs540825 T>A) and (iii) *COMT* (rs6269 A>G;

rs4633 C>T; rs4818 C>G; rs4680 G>A). Construct inferred haplotypes as a proxy for gene associations, and allele-allele combinations as a proxy for gene-gene associations, with chronic pain and disability. Additionally, to conduct bioinformatic analyses to characterise the potential functional effects of the prioritised SNPs investigated, and to identify the functional associated networks related to the gene set explored and their potential protein network partners underlying pain-related associations.

In the independent genetic association analyses (**Table 5.1**) of the eight prioritised SNPs evaluated in the SA BCS, two independent gene associations were noted. The *ABCB1* rs1045642 G>A SNP was associated with reducing the likelihood of reporting disability and combined (pain and disability) symptoms. The *COMT* rs4680 G>A SNP was associated with an increasing likelihood of reporting pain and combined (pain and disability) symptoms. However, all three genes were implicated in the inferred haplotype analyses, whereby *ABCB1* (G-A/ A-A) inferred haplotypes were associated with reduced disability and combined (pain and disability) symptoms. The *OPRM1* (G-T) inferred haplotype was associated with reduced pain. Whereas the alternate haplotypes *COMT* (G-G and A-A) were associated with reduced and increased pain, respectively. This association was also reflected in the combined (pain and disability) group. Moreover, **evaluation of the gene-gene interaction association analyses** as summarised in **Table 5.1** using alleles as a proxy, noted the combined genetic intervals for *ABCB1-OPRM1-COMT* were associated with pain [G-A-A (increased); G-G-G (reduced)], disability [A-A-G (reduced)] and combined (pain and disability); [A-A-G (reduced); G-A-A (increased)] groups ($p<0.05$). Individual gene-gene interactions were also observed and are listed in **Table 5.1**.

Table 5.1: Summary of independent gene associations observed in the SA BCS cohort

Genotypes	Pain	Disability	Combined
***ABCB1* rs1128503 G>A**	–	–	–
***ABCB1* rs1045642 G>A**	–	**A/A**	**A/A**
***OPRM1* rs1799971 A>G**	–	–	–
***OPRM1* rs540825 T>A**	–	–	–
***COMT* rs6269 A>G**	–	–	–
***COMT* rs4633 C>T**	–	–	–
***COMT* rs4818 C>G**	–	–	–
***COMT* rs4680 G>A**	**A/A**	–	**A/A**
Inferred Haplotypes			
***ABCB1* rs1128503 G>A- rs1045642 G>A**	–	G-**A**	**A-A**
***OPRM1* rs1799971 A>G -rs540825 T>A**	**G**-T	–	–
***COMT* rs6269 A>G - rs4680 G>A**	**G**-G	–	–
	A-**A**	–	A-**A**
Inferred Allele-Allele Combinations			
***ABCB1* rs1045642 G>A-*OPRM1* rs1799971 A>G-rs540825 T>A**	–	–	**A**-A-T
***ABCB1* rs1045642 G>A-*OPRM1* rs1799971 A>G**			**A**-A
***ABCB1* rs1045642 G>A-*OPRM1* rs540825 T>A**	–	**A**-T	**A**-T
	–	G-**A**	G-**A**
***OPRM1* rs1799971 A>G -*COMT* rs4680 G>A**	A-**A**	–	A-**A**
	G-G	–	–
***OPRM1* rs540825 T>A-*COMT* rs4680 G>A**	**A-A**	–	–
***ABCB1* rs1128503 G>A- *COMT* rs4680 G>A**	G-**A**	–	G-**A**
***ABCB1* rs1045642 G>A- *COMT* rs4680 G>A**	G-**A**	G-**A**	G-**A**
	–	**A**-G	**A**-G
***ABCB1* rs1045642 G>A-*OPRM1* rs1799971 A>G- *COMT* rs4680 G>A**	G-A-**A**	A-A-G	G-A-**A**
	G-**G**-G	–	**A**-A-G

Summarized in this table are the associations observed in the SA BCS cohorts in the categories of pain, disability and combined (pain and disability). All gene-gene interaction associations observed are shown. Minor alleles are highlighted in **bold.** Reduced likelihood associations are indicated with the green blocks. Increased likelihood associations are indicated in blue blocks. No associations are denoted by a dash (–).

The *in-silico* exploration revealed that the SNPs for each gene, *ABCB1*, *OPRM1* and *COMT* were mostly benign by SIFT, PP2 and FATHMM standards apart from the *ABCB1* rs1045642 G>A SNP, predicted to be potentially damaging in effect. Furthermore, RNA structure analyses

revealed that the prioritised *ABCB1*, *OPRM1* and *COMT* SNPs have the potential to influence the structure or efficiency and impact the potential function of the translated proteins.

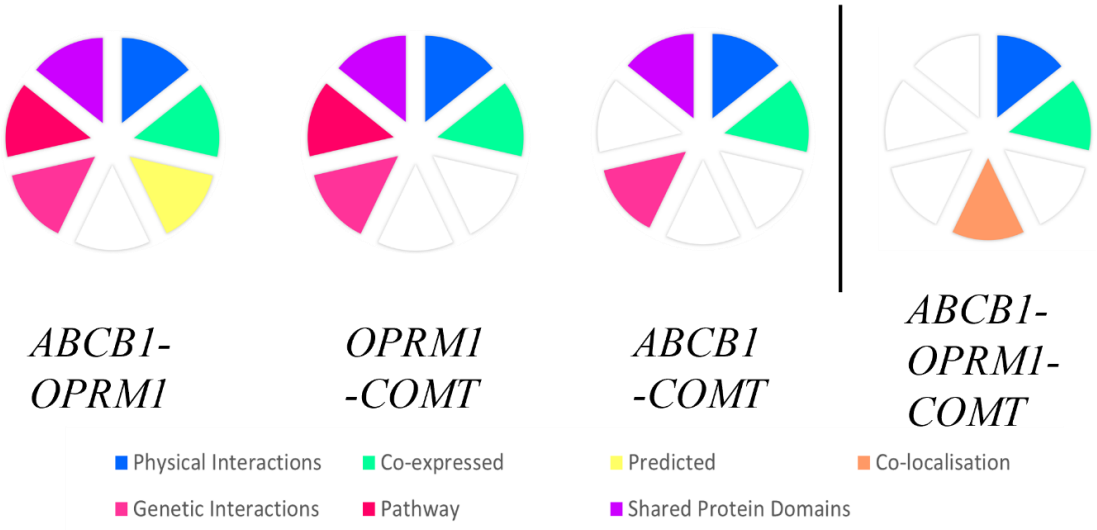


Figure 5.1: The corresponding gene-associated networks observed between the *ABCB1*, *OPRM1* and *COMT* genes.

The ***in-silico*** **investigation also supported** the data noted in the **gene-gene interaction analyses for the *ABCB1*, *OPRM1* and *COMT*** genes (**Figure 5.1**), highlighting that the gene set has shared networks that are co-expressed, co-localised and have physical interactions. In addition, enrichment exploration also revealed significant associations within the biological framework of different pathways. For example, in the disease library, several pain phenotypes that were not musculoskeletal-related were associated with the gene set. Other examples were that the gene set is found in the same biological compartments, and associated with pathways that involve neurobiological conditions, and more importantly, similar drug signatures (**Figure 5.2**).

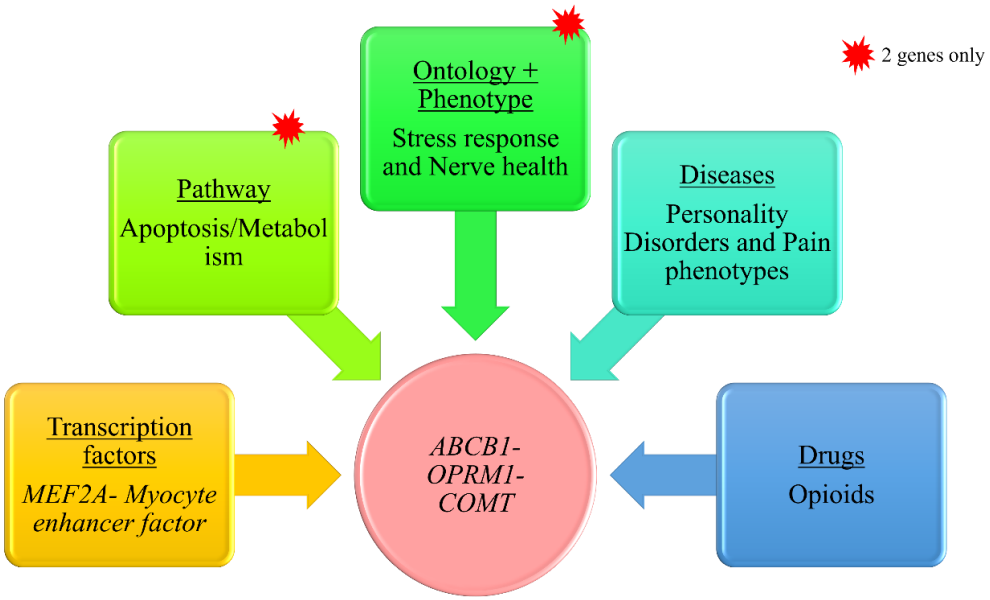


Figure 5.2: The corresponding libraries and associated enrichment data observed between the *ABCB1*, *OPRM1* and *COMT* genes.

5.2 CONCLUSION

The findings of this study support the hypothesis proposing that genetic variations within *ABCB1*, *OPRM1*, and *COMT* genes play an important role in shaping the emergence of chronic postoperative pain, particularly pain associated with movement. This thesis concluded with findings that demonstrated the correlation between the frequency of chronic shoulder pain and disability in SA BCS, and genetic variations with the *ABCB1*, *OPRM1*, and *COMT* genes.

The findings highlight a correlation between the *ABCB1* SNP, rs1045642 G>A, and the prevalence of disability, indicating movement-related pain. Although there is no independent association for the functional *OPRM1* SNPs, rs1799971 A>G, and rs540825 T>A, haplotype analyses showed an association which supports the contributory role of *OPRM1* in the opioid signaling and pain pathways. In addition to the independent association observed for *COMT* rs4680 G>A, gene interactions were observed highlighting the role of collective modulation in

pain and disability in the present cohort. Notably, the study suggests that genes influencing the persistence of pain and disability symptoms do not act independently; rather, they interact with each other. This underscores the complexity of genetic interactions in contributing to the development of chronic pain and related symptoms. Understanding the specific genetic factors that interact with and activate the development of chronic pain conditions is crucial for advancing therapeutic interventions. Additionally, in-silico analyses reveal strong relationships between the genes and important pathways and mechanisms, further strengthening and supporting the hypotheses presented in this study's aims. The clinical implications of this study aim to assist in the understanding of the pain mechanisms and opioid pathways towards the development of novel and innovative therapeutics for pain in personalized medicine.

5.3 LIMITATIONS

The ethnicity in this cohort was self-reported. We acknowledge that the BCS cohort was low, particularly in the subset of patients we analysed. Recruitment for the BCS cohort was conducted at a public hospital and one of the barriers included a high participant dropout rate and/ or several data points over the study period were not available for analyses. In addition, the observed patterns of prescribed opioids in the exploratory sample may not indicate the use of opioids. It is plausible that individuals may not adhere to their scripts or may even deviate to other forms of therapy drugs. As a result, the absence of these specific details may have impacted the analyses, potentially leading to a failure to adequately describe or account for confounding variables. Moreover, the exploratory sample set was severely underpowered and was used as a descriptive analysis of the SA BCS cohort describing the clinical profile related to trends of pain, disability, anxiety, depression, affect and pain management.

The main study exclusively used one instrument, the SPADI (Shoulder Pain and Disability Index) index, to measure these symptoms of pain and disability. We acknowledge that assessment tools such as the UCLA and SST, could have offered additional perspectives on symptoms, potentially impacting the classification and severity grading of the condition. For the genetic associations, the BCS cohort was powered at <80% to detect effects sizes of OR = 1.5 (Supplementary Table 2). The study followed a hypothesis approach in which both *ABCB1* *OPRM1* and *COMT* polymorphisms were previously implicated in the pain phenotype. More than two genetic polymorphisms were assessed (family-wise error rate) and, taken together with the small and underpowered (<80%) sample size, multiple testing was not included. Increasing the sample size may also increase the power to detect significant differences in (i) clinically relevant variables with genotype/allele frequencies, (ii) allow us to consider clinically relevant confounders and (iii) identify appropriate gene-gene locus associations in the regression analyses.

5.4 FUTURE PERSPECTIVES

The susceptibility of chronic shoulder pain and disability is collectively modulated by specific genes, namely *ABCB1*, *OPRM1*, and *COMT*. These genetic factors, therefore, need to be thoroughly examined in larger-scale studies to comprehend their impact on the chronicity of pain and disability symptoms. The exploratory sample analyses hint towards insights into acute pain patterns, inadequate pain relief, and psychological distress post-treatment. Studies, therefore, should examine and account for clinically relevant confounders. Moreover, comprehensive profiling of opioid consumption in combination with the genetic profile of these polymorphisms should be conducted to understand how SNPs could translate into variability in opioid response in this cohort. The collective genetic association data are supported by the in-silico findings; therefore, it is feasible that protein dynamic folding should be explored to

unravel a hypothesis for the mechanisms underpinning the findings. Future studies should, therefore, examine the role of genetic factors, identify clinically relevant confounders and opioid use, and understand how they all contribute to chronic pain and disability. Furthermore, incorporating functional genomics, proteomic, and epigenetic analyses of these variants, along with other clinically relevant genetic variations, may facilitate the exploration of the underlying mechanisms. This understanding will aid in paving the way for more effective interventions and treatments.

6 REFERENCES

1 Firfirey, F., September, A. V. and Shamley, D. (2022). "ABCB1 and OPRM1 single-nucleotide polymorphisms collectively modulate chronic shoulder pain and dysfunction in South African breast cancer survivors." Pharmacogenomics **23**(9): 513-530.

2 Firfirey, F., Shamley, D. and September, A. V. (2023). "Polymorphisms in COMT and OPRM1 Collectively Contribute to Chronic Shoulder Pain and Disability in South African Breast Cancer Survivor's." Genes **14**(1): 9.

3 Hidding, J. T., Beurskens, C. H., van der Wees, P. J.*, et al.* (2014). "Treatment related impairments in arm and shoulder in patients with breast cancer: a systematic review." Plos One **9**(5): e96748.

4 Boyages, J. (2017). "Radiation therapy and early breast cancer: current controversies." Med J Aust **207**(5): 216-222.5 Al-Gaithy, Z. K., Yaghmoor, B. E., Koumu, M. I.*, et al.* (2019). "Trends of mastectomy and breast-conserving surgery and related factors in female breast cancer patients treated at King Abdulaziz University Hospital, Jeddah, Saudi Arabia, 2009-2017: A retrospective cohort study." Ann Med Surg (Lond) **41**: 47-52.

6 Anyigba, C. A., Awandare, G. A. and Paemka, L. (2021). "Breast cancer in sub-Saharan Africa: The current state and uncertain future." Exp Biol Med (Maywood) **246**(12): 1377-1387.

7 Peuckmann, V., Ekholm, O., Rasmussen, N. K.*, et al.* (2009). "Chronic pain and other sequelae in long-term breast cancer survivors: nationwide survey in Denmark." Eur J Pain **13**(5): 478-485.

8 Hayes, S. C., Rye, S., Battistutta, D.*, et al.* (2010). "Upper-body morbidity following breast cancer treatment is common, may persist longer-term and adversely influences quality of life." Health Qual Life Outcomes **8**: 92.

9 Shamley, D., Lascurain-Aguirrebena, I., Oskrochi, R.*, et al.* (2012). "Shoulder morbidity after treatment for breast cancer is bilateral and greater after mastectomy." Acta Oncol **51**(8): 1045-1053.

10 Johansen, S., Fossa, K., Nesvold, I. L.*, et al.* (2014). "Arm and shoulder morbidity following surgery and radiotherapy for breast cancer." Acta Oncol **53**(4): 521-529.

11 Bodai, B. I. and Tuso, P. (2015). "Breast cancer survivorship: a comprehensive review of long-term medical issues and lifestyle recommendations." Perm J **19**(2): 48-79.

12 Shamley, D. (2015). "A Cross-Disciplinary Look at Shoulder Pain and Dysfunction after Treatment for Breast Cancer." International Journal of Cancer and Clinical Research **2**(1).

13 Calapai, M., Esposito, E., Puzzo, L.*, et al.* (2021). "Post-Mastectomy Pain: An Updated Overview on Risk Factors, Predictors, and Markers." Life (Basel) **11**(10): 1026.

14 Simon, L. S. (2012). "Relieving pain in America: A blueprint for transforming prevention, care, education, and research." Journal of Pain & Palliative Care Pharmacotherapy **26**(2): 197-198.

15 George, S. Z., Parr, J. J., Wallace, M. R.*, et al.* (2014). "Biopsychosocial influence on exercise-induced injury: genetic and psychological combinations are predictive of shoulder pain phenotypes." J Pain **15**(1): 68-80.

16 Meyr, A. J. and Saffran, B. (2008). "The pathophysiology of the chronic pain cycle." Clin Podiatr Med Surg **25**(3): 327-346; v.

17 Duenas, M., Ojeda, B., Salazar, A.*, et al.* (2016). "A review of chronic pain impact on patients, their social environment and the health care system." J Pain Res **9**: 457-467.

18 Lovelace, D. L., McDaniel, L. R. and Golden, D. (2019). "Long-Term Effects of Breast Cancer Surgery, Treatment, and Survivor Care." J Midwifery Womens Health **64**(6): 713-724.
19 Andersen, K. G., Duriaud, H. M., Jensen, H. E.*, et al.* (2015). "Predictive factors for the development of persistent pain after breast cancer surgery." Pain **156**(12): 2413-2422.
20 Wang, L., Guyatt, G. H., Kennedy, S. A.*, et al.* (2016). "Predictors of persistent pain after breast cancer surgery: a systematic review and meta-analysis of observational studies." CMAJ **188**(14): E352-E361.
21 Diatchenko, L., Slade, G. D., Nackley, A. G.*, et al.* (2005). "Genetic basis for individual variations in pain perception and the development of a chronic pain condition." Hum Mol Genet **14**(1): 135-143.
22 Sia, A. T., Sng, B. L., Lim, E. C.*, et al.* (2010). "The influence of ATP-binding cassette sub-family B member -1 (ABCB1) genetic polymorphisms on acute and chronic pain after intrathecal morphine for caesarean section: a prospective cohort study." Int J Obstet Anesth **19**(3): 254-260.
23 Lee, P. J., Delaney, P., Keogh, J.*, et al.* (2011). "Catecholamine-o-methyltransferase polymorphisms are associated with postoperative pain intensity." Clin J Pain **27**(2): 93-101.
24 Kolesnikov, Y., Gabovits, B., Levin, A.*, et al.* (2013). "Chronic pain after lower abdominal surgery: do catechol-O-methyl transferase/opioid receptor mu-1 polymorphisms contribute?" Mol Pain **9**: 19.
25 Martinez-Jauand, M., Sitges, C., Rodriguez, V.*, et al.* (2013). "Pain sensitivity in fibromyalgia is associated with catechol-O-methyltransferase (COMT) gene." Eur J Pain **17**(1): 16-27.
26 Hoofwijk, D. M. N., van Reij, R. R. I., Rutten, B. P. F.*, et al.* (2019). "Genetic polymorphisms and prediction of chronic post-surgical pain after hysterectomy-a subgroup analysis of a multicenter cohort study." Acta Anaesthesiol Scand **63**(8): 1063-1073.
27 Baumbauer, K. M., Ramesh, D., Perry, M.*, et al.* (2020). "Contribution of COMT and BDNF Genotype and Expression to the Risk of Transition From Acute to Chronic Low Back Pain." Clin J Pain **36**(6): 430-439.
28 Shi, C., Liu, J., Hu, J.*, et al.* (2022). "Genetic and Clinical Factors Associated with Opioid Response in Chinese Han Patients with Cancer Pain: An Exploratory Cross-Sectional Study." Pain Ther **11**(1): 269-288.
29 Harker, N., Lucas, W. C., Laubscher, R.*, et al.* (2020). "Is South Africa being spared the global opioid crisis? A review of trends in drug treatment demand for heroin, nyaope and codeine-related medicines in South Africa (2012–2017)." International Journal of Drug Policy **83**: 102839.
30 Gao, L., Mu, H., Lin, Y.*, et al.* (2023). "Review of the Current Situation of Postoperative Pain and Causes of Inadequate Pain Management in Africa." J Pain Res **16**: 1767-1778.
31 Fynn, A., Helberg, E., Godman, B.*, et al.* (2020). "Drug utilization review of tramadol hydrochloride in a regional hospital in South Africa; findings and implications." Hosp Pract (1995) **48**(2): 92-99.
32 Salz, T., Lavery, J. A., Lipitz-Snyderman, A. N.*, et al.* (2019). "Trends in Opioid Use Among Older Survivors of Colorectal, Lung, and Breast Cancers." J Clin Oncol **37**(12): 1001-1011.
33 Vieira, C. M. P., Fragoso, R. M., Pereira, D.*, et al.* (2019). "Pain polymorphisms and opioids: An evidence based review." Mol Med Rep **19**(3): 1423-1434.

34 Klepstad, P. (2007). "Genetic variability and opioid efficacy." Current Anaesthesia & Critical Care **18**(3): 149-156.
35 Kaye, A. D., Garcia, A. J., Hall, O. M.*, et al.* (2019). "Update on the pharmacogenomics of pain management." Pharmacogenomics and personalized medicine **12**: 125-143.
36 Zubair, M., Wang, S. and Ali, N. (2020). "Advanced Approaches to Breast Cancer Classification and Diagnosis." Front Pharmacol **11**: 632079.
37 Oyama, T., Iijima, K., Takei, H.*, et al.* (2000). "Atypical cystic lobule of the breast: an early stage of low-grade ductal carcinoma in-situ." Breast Cancer **7**(4): 326-331.
38 Garcia, M., Jemal, A., Ward, E.*, et al.* (2007). "Global cancer facts & figures 2007." Atlanta, GA: American cancer society **1**(3): 52.
39 Li, J., Chen, Z., Su, K.*, et al.* (2015). "Clinicopathological classification and traditional prognostic indicators of breast cancer." Int J Clin Exp Pathol **8**(7): 8500-8505.
40 Surakasula, A., Nagarjunapu, G. C. and Raghavaiah, K. V. (2014). "A comparative study of pre- and post-menopausal breast cancer: Risk factors, presentation, characteristics and management." Journal of research in pharmacy practice **3**(1): 12-18.
41 Momenimovahed, Z. and Salehiniya, H. (2019). "Epidemiological characteristics of and risk factors for breast cancer in the world." Breast cancer (Dove Medical Press) **11**: 151-164.
42 Giordano, S. H., Buzdar, A. U. and Hortobagyi, G. N. (2002). "Breast cancer in men." Ann Intern Med **137**(8): 678-687.
43 Abdelwahab Yousef, A. J. (2017). "Male Breast Cancer: Epidemiology and Risk Factors." Semin Oncol **44**(4): 267-272.
44 Sun, Y. S., Zhao, Z., Yang, Z. N.*, et al.* (2017). "Risk Factors and Preventions of Breast Cancer." International journal of biological sciences **13**(11): 1387-1397.
45 Siegel, R. L., Miller, K. D. and Jemal, A. (2017). "Cancer Statistics, 2017." CA Cancer J Clin **67**(1): 7-30.
46 Kim, Y., Yoo, K. Y. and Goodman, M. T. (2015). "Differences in incidence, mortality and survival of breast cancer by regions and countries in Asia and contributing factors." Asian Pac J Cancer Prev **16**(7): 2857-2870.
47 de Wit, E., Delport, W., Rugamika, C. E.*, et al.* (2010). "Genome-wide analysis of the structure of the South African Coloured Population in the Western Cape." Hum Genet **128**(2): 145-153.
48 Pfaff, C. L., Parra, E. J., Bonilla, C.*, et al.* (2001). "Population structure in admixed populations: effect of admixture dynamics on the pattern of linkage disequilibrium." Am J Hum Genet **68**(1): 198-207.
49 Ikediobi, O., Aouizerat, B., Xiao, Y.*, et al.* (2011). "Analysis of pharmacogenetic traits in two distinct South African populations." Hum Genomics **5**(4): 265-282.
50 Bhadoria, A. S., Kapil, U., Sareen, N.*, et al.* (2013). "Reproductive factors and breast cancer: a case-control study in tertiary care hospital of North India." Indian J Cancer **50**(4): 316-321.
51 Sisti, J. S., Bernstein, J. L., Lynch, C. F.*, et al.* (2015). "Reproductive factors, tumor estrogen receptor status and contralateral breast cancer risk: results from the WECARE study." Springerplus **4**(1): 825.
52 Dall, G. V. and Britt, K. L. (2017). "Estrogen Effects on the Mammary Gland in Early and Late Life and Breast Cancer Risk." Front Oncol **7**: 110.
53 Thakur, P., Seam, R. K., Gupta, M. K.*, et al.* (2017). "Breast cancer risk factor evaluation in a Western Himalayan state: A case-control study and comparison with the Western World." South Asian J Cancer **6**(3): 106-109.
54 Tamakoshi, K., Yatsuya, H., Wakai, K.*, et al.* (2005). "Impact of menstrual and reproductive factors on breast cancer risk in Japan: results of the JACC study." Cancer Sci **96**(1): 57-62.

55 Nguyen, J., Le, Q. H., Duong, B. H., *et al.* (2016). "A Matched Case-Control Study of Risk Factors for Breast Cancer Risk in Vietnam." Int J Breast Cancer **2016**: 7164623.
56 Kvale, G. (1992). "Reproductive factors in breast cancer epidemiology." Acta Oncol **31**(2): 187-194.
57 Albrektsen, G., Heuch, I., Tretli, S., *et al.* (1994). "Breast cancer incidence before age 55 in relation to parity and age at first and last births: a prospective study of one million Norwegian women." Epidemiology **5**(6): 604-611.
58 Lambe, M., Hsieh, C. C., Chan, H. W., *et al.* (1996). "Parity, age at first and last birth, and risk of breast cancer: a population-based study in Sweden." Breast Cancer Res Treat **38**(3): 305-311.
59 Lambe, M., Hsieh, C. C., Tsaih, S. W., *et al.* (1998). "Parity, age at first birth and the risk of carcinoma in situ of the breast." Int J Cancer **77**(3): 330-332.
60 MacMahon, B., Cole, P., Lin, T. M., *et al.* (1970). "Age at first birth and breast cancer risk." Bull World Health Organ **43**(2): 209-221.
61 Costantino, J. P., Gail, M. H., Pee, D., *et al.* (1999). "Validation studies for models projecting the risk of invasive and total breast cancer incidence." J Natl Cancer Inst **91**(18): 1541-1548.
62 Cnattingius, S., Torrang, A., Ekbom, A., *et al.* (2005). "Pregnancy characteristics and maternal risk of breast cancer." Jama **294**(19): 2474-2480.
63 Ma, H., Bernstein, L., Pike, M. C., *et al.* (2006). "Reproductive factors and breast cancer risk according to joint estrogen and progesterone receptor status: a meta-analysis of epidemiological studies." Breast cancer research : BCR **8**(4): R43.
64 Listyawardhani, Y., Mudigdo, A. and Adriani, R. B. (2018). Risk Factors of Breast Cancer in Women: A New Evidence from Surakarta, Central Java, Indonesia. Revitalizing Family Planning Program and Women's Empowerment for the Improvement of Population Well-being and Economic Development. Indonesia**:** 75-75.
65 Melbye, M., Wohlfahrt, J., Andersen, A. M., *et al.* (1999). "Preterm delivery and risk of breast cancer." Br J Cancer **80**(3-4): 609-613.
66 Cohn, B. A., Cirillo, P. M., Christianson, R. E., *et al.* (2001). "Placental characteristics and reduced risk of maternal breast cancer." J Natl Cancer Inst **93**(15): 1133-1140.
67 Hinkula, M., Pukkala, E., Kyyronen, P., *et al.* (2001). "Grand multiparity and the risk of breast cancer: population-based study in Finland." Cancer Causes Control **12**(6): 491-500.
68 Vatten, L. J., Romundstad, P. R., Trichopoulos, D., *et al.* (2002). "Pre-eclampsia in pregnancy and subsequent risk for breast cancer." Br J Cancer **87**(9): 971-973.
69 Fatima, N., Zaman, M. U. and Fatima, T. (2010). "Increased risk of breast cancer in multiparous and lactating women attending a breast care clinic in Pakistan: a paradigm shift?" Asian Pac J Cancer Prev **11**(5): 1219-1223.
70 Park, S. K., Kang, D., McGlynn, K. A., *et al.* (2008). "Intrauterine environments and breast cancer risk: meta-analysis and systematic review." Breast cancer research : BCR **10**(1): R8.
71 Anothaisintawee, T., Wiratkapun, C., Lerdsitthichai, P., *et al.* (2013). "Risk factors of breast cancer: a systematic review and meta-analysis." Asia Pac J Public Health **25**(5): 368-387.
72 Russo, J., Mailo, D., Hu, Y. F., *et al.* (2005). "Breast differentiation and its implication in cancer prevention." Clin Cancer Res **11**(2 Pt 2): 931s-936s.
73 Fortner, R. T., Sisti, J., Chai, B., *et al.* (2019). "Parity, breastfeeding, and breast cancer risk by hormone receptor status and molecular phenotype: results from the Nurses' Health Studies." Breast cancer research : BCR **21**(1): 40.
74 Plessis, L. D., Peer, N., English, R., *et al.* (2016). "Breastfeeding in South Africa : are we making progress?" South African Health Review **2016**(1): 109-123.

75 Auerbach, K. G. (1989). "Discrimination against breastfeeding: a racial/economic issue?" J Hum Lact **5**(1): 1-2.
76 Samuel, A. (2023). "Breastfeeding and breast cancer risk reduction in Sub-Saharan Africa: a look at the evidence." Tanzania Journal of Health Research **24**(2): 87-96.
77 Anstey, E. H., Shoemaker, M. L., Barrera, C. M.*, et al.* (2017). "Breastfeeding and Breast Cancer Risk Reduction: Implications for Black Mothers." American journal of preventive medicine **53**(3S1): S40-S46.
78 Roman, M., Quintana, M. J., Ferrer, J.*, et al.* (2017). "Cumulative risk of breast cancer screening outcomes according to the presence of previous benign breast disease and family history of breast cancer: supporting personalised screening." Br J Cancer **116**(11): 1480-1485.
79 Zendehdel, M., Niakan, B., Keshtkar, A.*, et al.* (2018). "Subtypes of Benign Breast Disease as a Risk Factor for Breast Cancer: A Systematic Review and Meta-Analysis Protocol." Iranian journal of medical sciences **43**(1): 1-8.
80 Kerlikowske, K., Gard, C. C., Tice, J. A.*, et al.* (2017). "Risk factors that increase risk of estrogen receptor–positive and–negative breast cancer." JNCI: Journal of the National Cancer Institute **109**(5).
81 Hartmann, L. C., Sellers, T. A., Frost, M. H.*, et al.* (2005). "Benign breast disease and the risk of breast cancer." N Engl J Med **353**(3): 229-237.
82 Arthur, R., Wang, Y., Ye, K.*, et al.* (2017). "Association between lifestyle, menstrual/reproductive history, and histological factors and risk of breast cancer in women biopsied for benign breast disease." Breast Cancer Res Treat **165**(3): 623-631.
83 Cancer, T. C. G. o. H. F. i. B. (1997). "Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52 705 women with breast cancer and 108 411 women without breast cancer." The lancet **350**(9084): 1047-1059.
84 Akoko, L. O., Rutashobya, A. K., Lutainulwa, E. W.*, et al.* (2022). "The effect of reproductive, hormonal, nutritional and lifestyle on breast cancer risk among black Tanzanian women: A case control study." Plos One **17**(2): e0263374.
85 Monninkhof, E. M., van der Schouw, Y. T. and Peeters, P. H. (1999). "Early age at menopause and breast cancer: are leaner women more protected? A prospective analysis of the Dutch DOM cohort." Breast Cancer Res Treat **55**(3): 285-291.
86 Beral, V. and Million Women Study, C. (2003). "Breast cancer and hormone-replacement therapy in the Million Women Study." Lancet **362**(9382): 419-427.
87 Dai, Q., Liu, B. and Du, Y. (2009). "Meta-analysis of the risk factors of breast cancer concerning reproductive factors and oral contraceptive use." Frontiers of Medicine in China **3**(4): 452-458.
88 White, N. D. (2018). "Hormonal Contraception and Breast Cancer Risk." Am J Lifestyle Med **12**(3): 224-226.
89 Kanadys, W., Baranska, A., Malm, M.*, et al.* (2021). "Use of Oral Contraceptives as a Potential Risk Factor for Breast Cancer: A Systematic Review and Meta-Analysis of Case-Control Studies Up to 2010." Int J Environ Res Public Health **18**(9).
90 Collaborative Group on Hormonal Factors in Breast, C. (1996). "Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies." Lancet **347**(9017): 1713-1727.
91 Morch, L. S., Skovlund, C. W., Hannaford, P. C.*, et al.* (2017). "Contemporary Hormonal Contraception and the Risk of Breast Cancer." N Engl J Med **377**(23): 2228-2239.

92 Nindrea, R. D., Aryandono, T. and Lazuardi, L. (2017). "Breast Cancer Risk From Modifiable and Non-Modifiable Risk Factors among Women in Southeast Asia: A Meta-Analysis." Asian Pac J Cancer Prev **18**(12): 3201-3206.
93 Ross, R. K., Paganini-Hill, A., Wan, P. C.*, et al.* (2000). "Effect of hormone replacement therapy on breast cancer risk: estrogen versus estrogen plus progestin." J Natl Cancer Inst **92**(4): 328-332.
94 Ye, J., Peng, H., Huang, X.*, et al.* (2022). "The association between endometriosis and risk of endometrial cancer and breast cancer: a meta-analysis." BMC Womens Health **22**(1): 455.
95 Marchbanks, P. A., McDonald, J. A., Wilson, H. G.*, et al.* (2002). "Oral contraceptives and the risk of breast cancer." N Engl J Med **346**(26): 2025-2032.
96 Iversen, L., Sivasubramaniam, S., Lee, A. J.*, et al.* (2017). "Lifetime cancer risk and combined oral contraceptives: the Royal College of General Practitioners' Oral Contraception Study." Am J Obstet Gynecol **216**(6): 580 e581-580 e589.
97 Beaber, E. F., Buist, D. S., Barlow, W. E.*, et al.* (2014). "Recent oral contraceptive use by formulation and breast cancer risk among women 20 to 49 years of age." Cancer research **74**(15): 4078-4089.
98 Kotsopoulos, J., Lubinski, J., Moller, P.*, et al.* (2014). "Timing of oral contraceptive use and the risk of breast cancer in BRCA1 mutation carriers." Breast Cancer Res Treat **143**(3): 579-586.
99 Lovett, J. L., Chima, M. A., Wexler, J. K.*, et al.* (2017). "Oral contraceptives cause evolutionarily novel increases in hormone exposureA risk factor for breast cancer." Evolution, medicine, and public health **2017**(1): 97-108.
100 Beral, V., Bull, D., Doll, R.*, et al.* (1997). "Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer." Lancet **350**(9084): 1047-1059.
101 Vinogradova, Y., Coupland, C. and Hippisley-Cox, J. (2020). "Use of hormone replacement therapy and risk of breast cancer: nested case-control studies using the QResearch and CPRD databases." BMJ **371**: m3873.
102 Zolfaroli, I., Tarin, J. J. and Cano, A. (2018). "Hormonal contraceptives and breast cancer: Clinical data." Eur J Obstet Gynecol Reprod Biol **230**: 212-216.
103 Brewer, H. R., Jones, M. E., Schoemaker, M. J.*, et al.* (2017). "Family history and risk of breast cancer: an analysis accounting for family structure." Breast Cancer Res Treat **165**(1): 193-200.
104 Liu, L., Hao, X., Song, Z.*, et al.* (2021). "Correlation between family history and characteristics of breast cancer." Sci Rep **11**(1): 6360.
105 Francies, F. Z., Hull, R., Khanyile, R.*, et al.* (2020). "Breast cancer in low-middle income countries: abnormality in splicing and lack of targeted treatment options." American journal of cancer research **10**(5): 1568-1591.
106 Greenup, R., Buchanan, A., Lorizio, W.*, et al.* (2013). "Prevalence of BRCA mutations among women with triple-negative breast cancer (TNBC) in a genetic counseling cohort." Annals of Surgical Oncology **20**(10): 3254-3258.
107 Tung, N., Battelli, C., Allen, B.*, et al.* (2015). "Frequency of mutations in individuals with breast cancer referred for BRCA 1 and BRCA 2 testing using next-generation sequencing with a 25-gene panel." Cancer **121**(1): 25-33.
108 Armstrong, N., Ryder, S., Forbes, C.*, et al.* (2019). "A systematic review of the international prevalence of BRCA mutation in breast cancer." Clin Epidemiol **11**: 543-561.

109 Wendt, C. and Margolin, S. (2019). "Identifying breast cancer susceptibility genes - a review of the genetic background in familial breast cancer." Acta Oncol **58**(2): 135-146.
110 Feng, Y., Spezia, M., Huang, S.*, et al.* (2018). "Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis." Genes Dis **5**(2): 77-106.
111 Ford, D., Easton, D. F., Bishop, D. T.*, et al.* (1994). "Risks of cancer in BRCA1-mutation carriers. Breast Cancer Linkage Consortium." Lancet **343**(8899): 692-695.
112 Brody, L. C. and Biesecker, B. B. (1998). "Breast cancer susceptibility genes. BRCA1 and BRCA2." Medicine **77**(3): 208-226.
113 Takaoka, M. and Miki, Y. (2018). "BRCA1 gene: function and deficiency." Int J Clin Oncol **23**(1): 36-44.
114 Schairer, C., Pfeiffer, R. M. and Gadalla, S. M. (2018). "Autoimmune diseases and breast cancer risk by tumor hormone-receptor status among elderly women." Int J Cancer **142**(6): 1202-1208.
115 Lai, Y. L., Chiang, C. J., Chen, Y. L.*, et al.* (2021). "Increased risk of second primary malignancies among endometrial cancer survivors receiving surgery alone: A population-based analysis." Cancer Med **10**(19): 6845-6854.
116 Fan, Y., Khan, N. H., Farhan Ali Khan, M.*, et al.* (2022). "Association of Hypertension and Breast Cancer: Antihypertensive Drugs as an Effective Adjunctive in Breast Cancer Therapy." Cancer Manag Res **14**: 1323-1329.
117 Huang, K., Xu, L., Jia, M.*, et al.* (2022). "Second primary malignancies in cervical cancer and endometrial cancer survivors: a population-based analysis." Aging (Albany NY) **14**(9): 3836-3855.
118 Boyle, P., Boniol, M., Koechlin, A.*, et al.* (2012). "Diabetes and breast cancer risk: a meta-analysis." Br J Cancer **107**(9): 1608-1617.
119 Eketunde, A. O. (2020). "Diabetes as a Risk Factor for Breast Cancer." Cureus **12**(5): e8010.
120 Lipscombe, L. L., Fischer, H. D., Austin, P. C.*, et al.* (2015). "The association between diabetes and breast cancer stage at diagnosis: a population-based study." Breast Cancer Res Treat **150**(3): 613-620.
121 Ayeni, O. A., Joffe, M., Cubasch, H.*, et al.* (2019). "Prevalence of comorbidities in women with and without breast cancer in Soweto, South Africa: Results from the SABC study." S Afr Med J **109**(4): 264-271.
122 Ayeni, O. A., Norris, S. A., Joffe, M.*, et al.* (2020). "The multimorbidity profile of South African women newly diagnosed with breast cancer." Int J Cancer **147**(2): 361-374.
123 Ayeni, O. A., Norris, S. A., Joffe, M.*, et al.* (2021). "Preexisting morbidity profile of women newly diagnosed with breast cancer in sub-Saharan Africa: African Breast Cancer-Disparities in Outcomes study." Int J Cancer **148**(9): 2158-2170.
124 Ayeni, O. A., Joffe, M., Mapanga, W.*, et al.* (2023). "Multimorbidity and overall survival among women with breast cancer: results from the South African Breast Cancer and HIV Outcomes Study." Breast cancer research : BCR **25**(1): 7.
125 Mohammed, A. M., Hamed, H. B., Noaman, M. K.*, et al.* (2023). "Metabolic syndrome and breast cancer risk." J Egypt Natl Canc Inst **35**(1): 42.
126 Lahmann, P. H., Hoffmann, K., Allen, N.*, et al.* (2004). "Body size and breast cancer risk: findings from the European Prospective Investigation into Cancer And Nutrition (EPIC)." Int J Cancer **111**(5): 762-771.
127 Bravi, F., Decarli, A. and Russo, A. G. (2018). "Risk factors for breast cancer in a cohort of mammographic screening program: a nested case-control study within the FRiCaM study." Cancer Med **7**(5): 2145-2152.

128 Miller, E. R., Wilson, C., Chapman, J.*, et al.* (2018). "Connecting the dots between breast cancer, obesity and alcohol consumption in middle-aged women: ecological and case control studies." BMC Public Health **18**(1): 460.
129 Wisse, A., Tryggvadottir, H., Simonsson, M.*, et al.* (2018). "Increasing preoperative body size in breast cancer patients between 2002 and 2016: implications for prognosis." Cancer Causes & Control **29**(7): 643-656.
130 Liu, K., Zhang, W., Dai, Z.*, et al.* (2018). "Association between body mass index and breast cancer risk: evidence based on a dose-response meta-analysis." Cancer Manag Res **10**: 143-151.
131 Ng, E. H., Gao, F., Ji, C. Y.*, et al.* (1997). "Risk factors for breast carcinoma in Singaporean Chinese women: the role of central obesity." Cancer **80**(4): 725-731.
132 Chen, X., Lu, W., Zheng, W.*, et al.* (2010). "Obesity and weight change in relation to breast cancer survival." Breast Cancer Res Treat **122**(3): 823-833.
133 Kawai, M., Kakugawa, Y., Nishino, Y.*, et al.* (2013). "Anthropometric factors, physical activity, and breast cancer risk in relation to hormone receptor and menopausal status in Japanese women: a case-control study." Cancer Causes Control **24**(5): 1033-1044.
134 Sinha, R. (2002). "An epidemiologic approach to studying heterocyclic amines." Mutat Res **506-507**: 197-204.
135 Taylor, E. F., Burley, V. J., Greenwood, D. C.*, et al.* (2007). "Meat consumption and risk of breast cancer in the UK Women's Cohort Study." Br J Cancer **96**(7): 1139-1146.
136 Sieri, S., Krogh, V., Ferrari, P.*, et al.* (2008). "Dietary fat and breast cancer risk in the European Prospective Investigation into Cancer and Nutrition." Am J Clin Nutr **88**(5): 1304-1312.
137 Jordan, I., Hebestreit, A., Swai, B.*, et al.* (2013). "Dietary patterns and breast cancer risk among women in northern Tanzania: a case-control study." Eur J Nutr **52**(3): 905-915.
138 Reynolds, P. (2013). "Smoking and breast cancer." J Mammary Gland Biol Neoplasia **18**(1): 15-23.
139 Rehman, S. and Husnain, S. M. (2014). "A probable risk factor of female breast cancer: study on benign and malignant breast tissue samples." Biol Trace Elem Res **157**(1): 24-29.
140 Health, U. D. o. and Services, H. (2014). The health consequences of smoking—50 years of progress: a report of the Surgeon General, Atlanta, GA: US Department of Health and Human Services, Centers for Disease ….
141 Jones, M. E., Schoemaker, M. J., Wright, L. B.*, et al.* (2017). "Smoking and risk of breast cancer in the Generations Study cohort." Breast cancer research : BCR **19**(1): 118.
142 Moussas, G. I. and Papadopoulou, A. G. (2017). "Substance abuse and cancer." Psychiatriki **28**(3): 234-241.
143 Jacobs, I., Taljaard-Krugell, C., Wicks, M.*, et al.* (2021). "Dietary Patterns and Breast Cancer Risk in Black Urban South African Women: The SABC Study." Nutrients **13**(11): 4106.
144 Bjerkaas, E., Parajuli, R., Weiderpass, E.*, et al.* (2013). "Smoking duration before first childbirth: an emerging risk factor for breast cancer? Results from 302,865 Norwegian women." Cancer Causes Control **24**(7): 1347-1356.
145 Catsburg, C., Miller, A. B. and Rohan, T. E. (2015). "Active cigarette smoking and risk of breast cancer." Int J Cancer **136**(9): 2204-2209.
146 Macacu, A., Autier, P., Boniol, M.*, et al.* (2015). "Active and passive smoking and risk of breast cancer: a meta-analysis." Breast Cancer Res Treat **154**(2): 213-224.
147 Dumitrescu, R. G. and Shields, P. G. (2005). "The etiology of alcohol-induced breast cancer." Alcohol **35**(3): 213-225.

148 Oyesanmi, O., Snyder, D., Sullivan, N.*, et al.* (2010). "Alcohol consumption and cancer risk: understanding possible causal mechanisms for breast and colorectal cancers." Evid Rep Technol Assess (Full Rep)(197): 1-151.
149 Fernandez, S. V. (2011). "Estrogen, alcohol consumption, and breast cancer." Alcoholism, clinical and experimental research **35**(3): 389-391.
150 Seitz, H. K., Pelucchi, C., Bagnardi, V.*, et al.* (2012). "Epidemiology and pathophysiology of alcohol and breast cancer: Update 2012." Alcohol Alcohol **47**(3): 204-212.
151 McDonald, J. A., Goyal, A. and Terry, M. B. (2013). "Alcohol Intake and Breast Cancer Risk: Weighing the Overall Evidence." Curr Breast Cancer Rep **5**(3): 208-221.
152 White, A. J., DeRoo, L. A., Weinberg, C. R.*, et al.* (2017). "Lifetime Alcohol Intake, Binge Drinking Behaviors, and Breast Cancer Risk." Am J Epidemiol **186**(5): 541-549.
153 Nwosu, E., Fismen, A. S., Helleve, A.*, et al.* (2022). "Trends in prevalence of overweight and obesity among South African and European adolescents: a comparative outlook." BMC Public Health **22**(1): 2287.
154 Fortner, R. T., Katzke, V., Kuhn, T.*, et al.* (2016). "Obesity and Breast Cancer." Recent Results Cancer Res **208**: 43-65.
155 Jacobs, I., Taljaard-Krugell, C., Ricci, C.*, et al.* (2019). "Dietary intake and breast cancer risk in black South African women: the South African Breast Cancer study." Br J Nutr **121**(5): 591-600.
156 Teras, L. R., Patel, A. V., Wang, M.*, et al.* (2020). "Sustained Weight Loss and Risk of Breast Cancer in Women 50 Years and Older: A Pooled Analysis of Prospective Data." J Natl Cancer Inst **112**(9): 929-937.
157 Friedenreich, C. M. and Cust, A. E. (2008). "Physical activity and breast cancer risk: impact of timing, type and dose of activity and population subgroup effects." Br J Sports Med **42**(8): 636-647.
158 Rockhill, B., Willett, W. C., Hunter, D. J.*, et al.* (1998). "Physical activity and breast cancer risk in a cohort of young women." J Natl Cancer Inst **90**(15): 1155-1160.
159 Dorgan, J. F. (1998). "Physical activity and breast cancer: is there a link?" J Natl Cancer Inst **90**(15): 1116-1117.
160 Xu, Y. and Rogers, C. J. (2020). "Physical Activity and Breast Cancer Prevention: Possible Role of Immune Mediators." Front Nutr **7**: 557997.
161 Warren, M. P. (1980). "The effects of exercise on pubertal progression and reproductive function in girls." J Clin Endocrinol Metab **51**(5): 1150-1157.
162 Merzenich, H., Boeing, H. and Wahrendorf, J. (1993). "Dietary fat and sports activity as determinants for age at menarche." Am J Epidemiol **138**(4): 217-224.
163 Harlow, S. D. and Ephross, S. A. (1995). "Epidemiology of menstruation and its relevance to women's health." Epidemiol Rev **17**(2): 265-286.
164 Kakizaki, M., Kuriyama, S., Sone, T.*, et al.* (2008). "Sleep duration and the risk of breast cancer: the Ohsaki Cohort Study." Br J Cancer **99**(9): 1502-1505.
165 White, A. J., Weinberg, C. R., Park, Y. M.*, et al.* (2017). "Sleep characteristics, light at night and breast cancer risk in a prospective cohort." Int J Cancer **141**(11): 2204-2214.
166 Cao, J., Eshak, E. S., Liu, K.*, et al.* (2019). "Sleep duration and risk of breast cancer: The JACC Study." Breast Cancer Res Treat **174**(1): 219-225.
167 Shigesato, M., Kawai, Y., Guillermo, C.*, et al.* (2020). "Association between sleep duration and breast cancer incidence: The multiethnic cohort." Int J Cancer **146**(3): 664-670.
168 Welp, E. A., Weiderpass, E., Boffetta, P.*, et al.* (1998). "Environmental risk factors of breast cancer." Scand J Work Environ Health **24**(1): 3-7.

169 Falck Jr, F., Ricci Jr, A., Wolff, M. S.*, et al.* (1992). "Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer." Archives of Environmental Health **47**(2): 143-146.
170 Wolff, M. S., Toniolo, P. G., Lee, E. W.*, et al.* (1993). "Blood levels of organochlorine residues and risk of breast cancer." J Natl Cancer Inst **85**(8): 648-652.
171 Verreault, R., Ayotte, P., Sauvi, L.*, et al.* (1994). "High organochlorine body burden in women with estrogen receptor-positive breast cancer." Journal of the National Cancer Institute **86**(3): 232-234.
172 Krieger, N., Wolff, M. S., Hiatt, R. A.*, et al.* (1994). "Breast cancer and serum organochlorines: a prospective study among white, black, and Asian women." JNCI: Journal of the National Cancer Institute **86**(8): 589-599.
173 DeBruin, L. S. and Josephy, P. D. (2002). "Perspectives on the chemical etiology of breast cancer." Environ Health Perspect **110 Suppl 1**(Suppl 1): 119-128.
174 Land, C. E. (1995). "Studies of cancer and radiation dose among atomic bomb survivors: the example of breast cancer." Jama **274**(5): 402-407.
175 Adami, H.-O., Signorello, L. B. and Trichopoulos, D. (1998). Towards an understanding of breast cancer etiology. Seminars in Cancer Biology, Elsevier.
176 Boice, J. D., Jr. (2001). "Radiation and breast carcinogenesis." Med Pediatr Oncol **36**(5): 508-513.
177 Coyle, Y. M. (2004). "The effect of environment on breast cancer risk." Breast Cancer Res Treat **84**(3): 273-288.
178 John, E. M., Phipps, A. I., Knight, J. A.*, et al.* (2007). "Medical radiation exposure and breast cancer risk: findings from the Breast Cancer Family Registry." Int J Cancer **121**(2): 386-394.
179 Moskowitz, C. S., Chou, J. F., Wolden, S. L.*, et al.* (2014). "Breast cancer after chest radiation therapy for childhood cancer." J Clin Oncol **32**(21): 2217-2223.
180 Henderson, T. O., Moskowitz, C. S., Chou, J. F.*, et al.* (2016). "Breast Cancer Risk in Childhood Cancer Survivors Without a History of Chest Radiotherapy: A Report From the Childhood Cancer Survivor Study." J Clin Oncol **34**(9): 910-918.
181 Council, N. R. (1990). "Health effects of exposure to low levels of ionizing radiation: BEIR V."
182 Robert, S. A., Strombom, I., Trentham-Dietz, A.*, et al.* (2004). "Socioeconomic risk factors for breast cancer: distinguishing individual- and community-level effects." Epidemiology **15**(4): 442-450.
183 Akinyemiju, T. F., Pisu, M., Waterbor, J. W.*, et al.* (2015). "Socioeconomic status and incidence of breast cancer by hormone receptor subtype." Springerplus **4**: 508.
184 Ewertz, M. and Duffy, S. W. (1988). "Risk of breast cancer in relation to reproductive factors in Denmark." Br J Cancer **58**(1): 99-104.
185 La Vecchia, C., Negri, E., Franceschi, S.*, et al.* (1993). "Long-term impact of reproductive factors on cancer risk." Int J Cancer **53**(2): 215-219.
186 Kelsey, J. L., Gammon, M. D. and John, E. M. (1993). "Reproductive factors and breast cancer." Epidemiol Rev **15**(1): 36-47.
187 Joffe, M., Ayeni, O., Norris, S. A.*, et al.* (2018). "Barriers to early presentation of breast cancer among women in Soweto, South Africa." Plos One **13**(2): e0192071.
188 McDonald, E. S., Clark, A. S., Tchou, J.*, et al.* (2016). "Clinical Diagnosis and Management of Breast Cancer." J Nucl Med **57 Suppl 1**: 9S-16S.
189 Mafu, T. S., September, A. V. and Shamley, D. (2018). "The potential role of angiogenesis in the development of shoulder pain, shoulder dysfunction, and lymphedema after breast cancer treatment." Cancer Manag Res **10**: 81-90.

190 Sharma, G. N., Dave, R., Sanadya, J.*, et al.* (2010). "Various types and management of breast cancer: an overview." Journal of advanced pharmaceutical technology & research **1**(2): 109-126.
191 Riis, M. (2020). "Modern surgical treatment of breast cancer." Ann Med Surg (Lond) **56**: 95-107.
192 Czajka, M. L. and Pfeifer, C. (2022). Breast cancer surgery. StatPearls [Internet], StatPearls Publishing.
193 Sabel, M. S. and Pierce, L. (2013). Breast-conserving therapy.
194 Zhang, B., Song, Q., Zhang, B.*, et al.* (2013). "A 10-year (1999 ~ 2008) retrospective multi-center study of breast cancer surgical management in various geographic areas of China." Breast **22**(5): 676-681.
195 McGuire, K. P., Santillan, A. A., Kaur, P.*, et al.* (2009). "Are mastectomies on the rise? A 13-year trend analysis of the selection of mastectomy versus breast conservation therapy in 5865 patients." Ann Surg Oncol **16**(10): 2682-2690.
196 Dragun, A. E., Huang, B., Tucker, T. C.*, et al.* (2012). "Increasing mastectomy rates among all age groups for early stage breast cancer: a 10-year study of surgical choice." Breast J **18**(4): 318-325.
197 Admoun, C. and Mayrovitz, H. (2021). "Choosing Mastectomy vs. Lumpectomy-With-Radiation: Experiences of Breast Cancer Survivors." Cureus **13**(10): e18433.
198 Panieri, E., Lazarus, D., Dent, D. M.*, et al.* (2003). "A study of the patient factors affecting reconstruction after mastectomy for breast carcinoma." Am Surg **69**(2): 95-97.
199 Bian, J., Krontiras, H. and Allison, J. (2008). "Outpatient mastectomy and breast reconstructive surgery." Ann Surg Oncol **15**(4): 1032-1039.
200 Brennan, M. E. and Spillane, A. J. (2013). "Uptake and predictors of post-mastectomy reconstruction in women with breast malignancy--systematic review." Eur J Surg Oncol **39**(6): 527-541.
201 Cubasch, H., Joffe, M., Ruff, P.*, et al.* (2017). "Breast conservation surgery versus total mastectomy among women with localized breast cancer in Soweto, South Africa." Plos One **12**(8): e0182125.
202 Lambert, M., Mendenhall, E., Kim, A. W.*, et al.* (2020). "Health system experiences of breast cancer survivors in urban South Africa." Womens Health (Lond) **16**: 1745506520949419.
203 Research, E. O. F., Cancer, T. O. and Liver, E. A. F. T. S. O. T. (2012). "EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma." Journal of hepatology **56**(4): 908-943.
204 Hamza, A. and Elrefaey, S. (2014). "Non-surgical treatment of early breast cancer: techniques on the way." Gland Surg **3**(3): 149-150.
205 Izzo, F., Granata, V., Grassi, R.*, et al.* (2019). "Radiofrequency Ablation and Microwave Ablation in Liver Tumors: An Update." Oncologist **24**(10): e990-e1005.
206 Dupuy, D. E., Zagoria, R. J., Akerley, W.*, et al.* (2000). "Percutaneous radiofrequency ablation of malignancies in the lung." AJR Am J Roentgenol **174**(1): 57-59.
207 Iannuccilli, J. D. and Dupuy, D. E. (2013). "How to set up a successful tumor ablation practice." Techniques in Vascular and Interventional Radiology **16**(4): 201-208.
208 Knavel, E. M. and Brace, C. L. (2013). "Tumor ablation: common modalities and general practices." Tech Vasc Interv Radiol **16**(4): 192-200.
209 Akhan, O., Guler, E., Akinci, D.*, et al.* (2016). "Radiofrequency ablation for lung tumors: outcomes, effects on survival, and prognostic factors." Diagn Interv Radiol **22**(1): 65-71.
210 Jeffrey, S. S., Birdwell, R. L., Ikeda, D. M.*, et al.* (1999). "Radiofrequency ablation of breast cancer: first report of an emerging technology." Arch Surg **134**(10): 1064-1068.

211 Mayo-Smith, W. W. and Dupuy, D. E. (2004). "Adrenal neoplasms: CT-guided radiofrequency ablation--preliminary results." Radiology **231**(1): 225-230.
212 Tateishi, R., Shiina, S., Teratani, T.*, et al.* (2005). "Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases." Cancer **103**(6): 1201-1209.
213 Hiraki, T., Sakurai, J., Tsuda, T.*, et al.* (2006). "Risk factors for local progression after percutaneous radiofrequency ablation of lung tumors: evaluation based on a preliminary review of 342 tumors." Cancer **107**(12): 2873-2880.
214 Peek, M. C. L., Ahmed, M., Napoli, A.*, et al.* (2017). "Minimally invasive ablative techniques in the treatment of breast cancer: a systematic review and meta-analysis." Int J Hyperthermia **33**(2): 191-202.
215 Ito, T., Oura, S., Nagamine, S.*, et al.* (2018). "Radiofrequency Ablation of Breast Cancer: A Retrospective Study." Clin Breast Cancer **18**(4): e495-e500.
216 Zhou, Y. F. (2011). "High intensity focused ultrasound in clinical tumor ablation." World J Clin Oncol **2**(1): 8-27.
217 Izadifar, Z., Izadifar, Z., Chapman, D.*, et al.* (2020). "An Introduction to High Intensity Focused Ultrasound: Systematic Review on Principles, Devices, and Clinical Applications." J Clin Med **9**(2): 460.
218 Sabel, M. S., Nehs, M. A., Su, G.*, et al.* (2005). "Immunologic response to cryoablation of breast cancer." Breast Cancer Res Treat **90**(1): 97-104.
219 Sabel, M. S., Su, G., Griffith, K. A.*, et al.* (2010). "Rate of freeze alters the immunologic response after cryoablation of breast cancer." Ann Surg Oncol **17**(4): 1187-1193.
220 Littrup, P. J., Bang, H. J., Currier, B. P.*, et al.* (2013). "Soft-tissue cryoablation in diffuse locations: feasibility and intermediate term outcomes." J Vasc Interv Radiol **24**(12): 1817-1825.
221 Simmons, R. M., Ballman, K. V., Cox, C.*, et al.* (2016). "A Phase II Trial Exploring the Success of Cryoablation Therapy in the Treatment of Invasive Breast Carcinoma: Results from ACOSOG (Alliance) Z1072." Ann Surg Oncol **23**(8): 2438-2445.
222 Pusceddu, C., Paliogiannis, P., Nigri, G.*, et al.* (2019). "Cryoablation In The Management Of Breast Cancer: Evidence To Date." Breast cancer (Dove Medical Press) **11**: 283-292.
223 Regen-Tuero, H. C., Ward, R. C., Sikov, W. M.*, et al.* (2021). "Cryoablation and Immunotherapy for Breast Cancer: Overview and Rationale for Combined Therapy." Radiol Imaging Cancer **3**(2): e200134.
224 Chew, H. K. (2001). "Adjuvant therapy for breast cancer: who should get what?" The Western journal of medicine **174**(4): 284-287.
225 Maier, P., Hartmann, L., Wenz, F.*, et al.* (2016). "Cellular Pathways in Response to Ionizing Radiation and Their Targetability for Tumor Radiosensitization." Int J Mol Sci **17**(1).
226 Maani, E. V. and Maani, C. V. (2021). "Radiation therapy." StatPearls [Internet].
227 Shirato, H., Le, Q. T., Kobashi, K.*, et al.* (2018). "Selection of external beam radiotherapy approaches for precise and accurate cancer treatment." Journal of radiation research **59**(suppl_1): i2-i10.
228 Ogawa, Y. (2016). "Paradigm Shift in Radiation Biology/Radiation Oncology—Exploitation of the "H2O2 Effect" for Radiotherapy Using Low-LET (Linear Energy Transfer) Radiation such as X-rays and High-Energy Electrons." Cancers **8**(3): 28.
229 Prise, K. M. (2006). "New advances in radiation biology." Occup Med (Lond) **56**(3): 156-161.
230 Durante, M. and Loeffler, J. S. (2010). "Charged particles in radiation oncology." Nat Rev Clin Oncol **7**(1): 37-43.

231 Guadagnolo, B. A., Liao, K. P., Elting, L.*, et al.* (2013). "Use of radiation therapy in the last 30 days of life among a large population-based cohort of elderly patients in the United States." J Clin Oncol **31**(1): 80-87.
232 Liauw, S. L., Connell, P. P. and Weichselbaum, R. R. (2013). "New paradigms and future challenges in radiation oncology: an update of biological targets and technology." Sci Transl Med **5**(173): 173sr172.
233 Baskar, R., Dai, J., Wenlong, N.*, et al.* (2014). "Biological response of cancer cells to radiation treatment." Frontiers in molecular biosciences **1**: 24.
234 Yang, T. J. and Ho, A. Y. (2013). "Radiation therapy in the management of breast cancer." Surg Clin North Am **93**(2): 455-471.
235 Group, E. B. C. T. C. (2005). "Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: an overview of the randomised trials." The lancet **366**(9503): 2087-2106.
236 Veronesi, U., Cascinelli, N., Mariani, L.*, et al.* (2002). "Twenty-year follow-up of a randomized study comparing breast-conserving surgery with radical mastectomy for early breast cancer." N Engl J Med **347**(16): 1227-1232.
237 Litiere, S., Werutsky, G., Fentiman, I. S.*, et al.* (2012). "Breast conserving therapy versus mastectomy for stage I-II breast cancer: 20 year follow-up of the EORTC 10801 phase 3 randomised trial." Lancet Oncol **13**(4): 412-419.
238 Kayali, M., Jaoude, J. A., Ramia, P.*, et al.* (2021). "Post-lumpectomy radiation therapy boost in breast cancer patients: evidence revisited." ecancermedicalscience **15**: 1194.
239 Kim, C. S. and Algan, O. (2021). "Radiation therapy for early stage breast cancer." StatPearls [Internet].
240 Palumbo, M. O., Kavan, P., Miller, W. H., Jr.*, et al.* (2013). "Systemic cancer therapy: achievements and challenges that lie ahead." Front Pharmacol **4**: 57.
241 Dewhirst, M. W. and Secomb, T. W. (2017). "Transport of drugs from blood vessels to tumour tissue." Nature reviews. Cancer **17**(12): 738-750.
242 Amjad, M. T., Chidharla, A. and Kasi, A. (2021). "Cancer Chemotherapy." StatPearls [Internet].
243 Hortobagyi, G. N. (1998). "Treatment of breast cancer." N Engl J Med **339**(14): 974-984.
244 Fisher, B., Bryant, J., Wolmark, N.*, et al.* (1998). "Effect of preoperative chemotherapy on the outcome of women with operable breast cancer." J Clin Oncol **16**(8): 2672-2685.
245 Singletary, S. E. (2001). "Neoadjuvant chemotherapy in the treatment of stage II and III breast cancer." Am J Surg **182**(4): 341-346.
246 Fujii, T., Le Du, F., Xiao, L.*, et al.* (2015). "Effectiveness of an Adjuvant Chemotherapy Regimen for Early-Stage Breast Cancer: A Systematic Review and Network Meta-analysis." JAMA oncology **1**(9): 1311-1318.
247 Fisusi, F. A. and Akala, E. O. (2019). "Drug Combinations in Breast Cancer Therapy." Pharmaceutical nanotechnology **7**(1): 3-23.
248 Early Breast Cancer Trialists' Collaborative, G., Peto, R., Davies, C.*, et al.* (2012). "Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials." Lancet **379**(9814): 432-444.
249 Rossi, L., Stevens, D., Pierga, J. Y.*, et al.* (2015). "Impact of Adjuvant Chemotherapy on Breast Cancer Survival: A Real-World Population." Plos One **10**(7): e0132853.
250 Scagliotti, G. V. and Selvaggi, G. (2006). "Antimetabolites and cancer: emerging data with a focus on antifolates." Expert Opin Ther Pat **16**(2): 189-200.
251 Garon, E. B. and Dubinett, S. M. (2011). "Mitotic inhibitors." Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer **6**(11 Suppl 4): S1791-1792.

252 Diseases, N. I. o. D. a. D. a. K. (2012). Alkylating Agents. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury. Bethesda (MD), National Institute of Diabetes and Digestive and Kidney Diseases.
253 Dou, Q. P. and Zonder, J. A. (2014). "Overview of proteasome inhibitor-based anti-cancer therapies: perspective on bortezomib and second generation proteasome inhibitors versus future generation inhibitors of ubiquitin-proteasome system." Current cancer drug targets **14**(6): 517-536.
254 Sreerama, L. (2014). Alkylating Agents. Encyclopedia of Cancer**:** 1-6.
255 Egler, R. A., Ahuja, S. P. and Matloub, Y. (2016). "L-asparaginase in the treatment of patients with acute lymphoblastic leukemia." Journal of pharmacology & pharmacotherapeutics **7**(2): 62-71.
256 Rahman, M. (2016). Metabolic Pathways and Chemotherapy Drugs. Frontiers in Drug Design & Discovery**:** 3-35.
257 Huang, C. Y., Ju, D. T., Chang, C. F.*, et al.* (2017). "A review on the effects of current chemotherapy drugs and natural agents in treating non-small cell lung cancer." BioMedicine **7**(4): 23.
258 Schneble, E., Jinga, D. C. and Peoples, G. (2015). "Breast Cancer Immunotherapy." Maedica (Bucur) **10**(2): 185-191.
259 Henriques, B., Mendes, F. and Martins, D. (2021). "Immunotherapy in Breast Cancer: When, How, and What Challenges?" Biomedicines **9**(11).
260 Debien, V., De Caluwé, A., Wang, X.*, et al.* (2023). "Immunotherapy in breast cancer: an overview of current strategies and perspectives." npj Breast Cancer **9**(1): 7.
261 Abdou, Y., Goudarzi, A., Yu, J. X.*, et al.* (2022). "Immunotherapy in triple negative breast cancer: beyond checkpoint inhibitors." npj Breast Cancer **8**(1): 121.
262 Emens, L. A. (2018). "Breast Cancer Immunotherapy: Facts and Hopes." Clinical Cancer Research **24**(3): 511-520.
263 Schröder, R., Illert, A. L., Erbes, T.*, et al.* (2022). "The epigenetics of breast cancer - Opportunities for diagnostics, risk stratification and therapy." Epigenetics **17**(6): 612-624.
264 Draganescu, M. and Carmocan, C. (2017). "Hormone Therapy in Breast Cancer." Chirurgia (Bucur) **112**(4): 413-417.
265 Bui, K. T., Willson, M. L., Goel, S.*, et al.* (2020). "Ovarian suppression for adjuvant treatment of hormone receptor-positive early breast cancer." The Cochrane database of systematic reviews **3**(3): CD013538.
266 Flaum, L. E. and Gradishar, W. J. (2018). Advances in Endocrine Therapy for Postmenopausal Metastatic Breast Cancer. Optimizing Breast Cancer Management. W. J. Gradishar. Cham, Springer International Publishing**:** 141-154.
267 Awan, A. and Esfahani, K. (2018). "Endocrine therapy for breast cancer in the primary care setting." Current oncology (Toronto, Ont.) **25**(4): 285-291.
268 Shah, R. and O'Regan, R. M. (2018). "Adjuvant Endocrine Therapy." Cancer Treat Res **173**: 15-29.
269 Tremont, A., Lu, J. and Cole, J. T. (2017). "Endocrine Therapy for Early Breast Cancer: Updated Review." The Ochsner journal **17**(4): 405-411.
270 Patel, H. K. and Bihani, T. (2018). "Selective estrogen receptor modulators (SERMs) and selective estrogen receptor degraders (SERDs) in cancer treatment." Pharmacol Ther **186**: 1-24.
271 Hernando, C., Ortega-Morillo, B., Tapia, M.*, et al.* (2021). "Oral Selective Estrogen Receptor Degraders (SERDs) as a Novel Breast Cancer Therapy: Present and Future from a Clinical Perspective." Int J Mol Sci **22**(15): 7812.

272 Lin, Y. Z., Lee, C. C., Cho, D. Y.*, et al.* (2021). "Suppression of breast cancer cells resistant to a pure anti-estrogen with CAR-transduced natural killer cells." American journal of cancer research **11**(9): 4455-4469.
273 Liu, S., Han, S. J. and Smith, C. L. (2013). "Cooperative activation of gene expression by agonists and antagonists mediated by estrogen receptor heteroligand dimer complexes." Mol Pharmacol **83**(5): 1066-1077.
274 Miller, K. D., Nogueira, L., Devasia, T.*, et al.* (2022). "Cancer treatment and survivorship statistics, 2022." CA Cancer J Clin **72**(5): 409-436.
275 Richards, M. A., Westcombe, A. M., Love, S. B.*, et al.* (1999). "Influence of delay on survival in patients with breast cancer: a systematic review." Lancet **353**(9159): 1119-1126.
276 Unger-Saldana, K., Miranda, A., Zarco-Espinosa, G.*, et al.* (2015). "Health system delay and its effect on clinical stage of breast cancer: Multicenter study." Cancer **121**(13): 2198-2206.
277 Ginsburg, O., Yip, C. H., Brooks, A.*, et al.* (2020). "Breast cancer early detection: A phased approach to implementation." Cancer **126 Suppl 10**(Suppl 10): 2379-2393.
278 Marzorati, C., Riva, S. and Pravettoni, G. (2017). "Who Is a Cancer Survivor? A Systematic Review of Published Definitions." J Cancer Educ **32**(2): 228-237.
279 Maajani, K., Jalali, A., Alipour, S.*, et al.* (2019). "The Global and Regional Survival Rate of Women With Breast Cancer: A Systematic Review and Meta-analysis." Clin Breast Cancer **19**(3): 165-177.
280 Coleman, M. P., Quaresma, M., Berrino, F.*, et al.* (2008). "Cancer survival in five continents: a worldwide population-based study (CONCORD)." Lancet Oncol **9**(8): 730-756.
281 Holli, K. and Isola, J. (1997). "Effect of age on the survival of breast cancer patients." European journal of cancer (Oxford, England : 1990) **33**(3): 425-428.
282 Allemani, C., Weir, H. K., Carreira, H.*, et al.* (2015). "Global surveillance of cancer survival 1995-2009: analysis of individual data for 25,676,887 patients from 279 population-based registries in 67 countries (CONCORD-2)." Lancet **385**(9972): 977-1010.
283 Allemani, C., Matsuda, T., Di Carlo, V.*, et al.* (2018). "Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries." Lancet **391**(10125): 1023-1075.
284 Yfantis, A., Sarafis, P., Moisoglou, I.*, et al.* (2020). "How breast cancer treatments affect the quality of life of women with non-metastatic breast cancer one year after surgical treatment: a cross-sectional study in Greece." BMC Surg **20**(1): 210.
285 Knox, J. B. L. (2020). "The Vitality of Mortality: Being-Toward-Death and Long-Term Cancer Survivorship." J Med Philos **45**(6): 703-724.
286 Council, N. R. (2005). From cancer patient to cancer survivor: lost in transition, National Academies Press.
287 Mogal, H. D., Howard-McNatt, M., Dodson, R.*, et al.* (2017). "Quality of life of older African American breast cancer survivors: a population-based study." Support Care Cancer **25**(5): 1431-1438.
288 Dinapoli, L., Colloca, G., Di Capua, B.*, et al.* (2021). "Psychological Aspects to Consider in Breast Cancer Diagnosis and Treatment." Curr Oncol Rep **23**(3): 38.
289 Barlow, D. H. (2000). "Unraveling the mysteries of anxiety and its disorders from the perspective of emotion theory." Am Psychol **55**(11): 1247-1263.
290 Ng, C. G., Mohamed, S., Kaur, K.*, et al.* (2017). "Perceived distress and its association with depression and anxiety in breast cancer patients." Plos One **12**(3): e0172975.

291 Harris, J., Cornelius, V., Ream, E.*, et al.* (2017). "Anxiety after completion of treatment for early-stage breast cancer: a systematic review to identify candidate predictors and evaluate multivariable model development." Support Care Cancer **25**(7): 2321-2333.
292 Krasne, M., Ruddy, K. J., Poorvu, P. D.*, et al.* (2022). "Coping strategies and anxiety in young breast cancer survivors." Support Care Cancer **30**(11): 9109-9116.
293 Maass, S. W., Roorda, C., Berendsen, A. J.*, et al.* (2015). "The prevalence of long-term symptoms of depression and anxiety after breast cancer treatment: A systematic review." Maturitas **82**(1): 100-108.
294 Stark, D. P. and House, A. (2000). "Anxiety in cancer patients." Br J Cancer **83**(10): 1261-1267.
295 Cui, R. (2015). "Editorial: A Systematic Review of Depression." Curr Neuropharmacol **13**(4): 480.
296 Seav, S. M., Dominick, S. A., Stepanyuk, B.*, et al.* (2015). "Management of sexual dysfunction in breast cancer survivors: a systematic review." Womens Midlife Health **1**(1): 9.
297 Fobair, P., Stewart, S. L., Chang, S.*, et al.* (2006). "Body image and sexual problems in young women with breast cancer." Psychooncology **15**(7): 579-594.
298 Haque, R., Hsu, J. W., Avila, C.*, et al.* (2021). "Insomnia and Susceptibility to Depressive Symptoms and Fatigue in Diverse Breast Cancer Survivors." J Womens Health (Larchmt) **30**(11): 1604-1615.
299 Palesh, O. G., Roscoe, J. A., Mustian, K. M.*, et al.* (2010). "Prevalence, demographics, and psychological associations of sleep disruption in patients with cancer: University of Rochester Cancer Center-Community Clinical Oncology Program." J Clin Oncol **28**(2): 292-298.
300 Walker, J., Holm Hansen, C., Martin, P.*, et al.* (2013). "Prevalence of depression in adults with cancer: a systematic review." Ann Oncol **24**(4): 895-900.
301 Amiel, C. R., Fisher, H. M. and Antoni, M. H. (2016). Concerns about breast cancer, pain, and fatigue in non-metastatic breast cancer patients undergoing primary treatment. Healthcare, MDPI.
302 Dunne, M. and Keenan, K. (2016). "CE: Late and Long-Term Sequelae of Breast Cancer Treatment." Am J Nurs **116**(6): 36-45.
303 Overgaard, M., Bentzen, S., Christensen, J. J.*, et al.* (1987). "The value of the NSD formula in equation of acute and late radiation complications in normal tissue following 2 and 5 fractions per week in breast cancer patients treated with postmastectomy irradiation." Radiotherapy and Oncology **9**(1): 1-11.
304 Lyngholm, C. D., Christiansen, P. M., Damsgaard, T. E.*, et al.* (2013). "Long-term follow-up of late morbidity, cosmetic outcome and body image after breast conserving therapy. A study from the Danish Breast Cancer Cooperative Group (DBCG)." Acta Oncologica **52**(2): 259-269.
305 Williams, N. R., Williams, S., Kanapathy, M.*, et al.* (2019). "Radiation-induced fibrosis in breast cancer: A protocol for an observational cross-sectional pilot study for personalised risk estimation and objective assessment." Int J Surg Protoc **14**: 9-13.
306 Wynn, T. A. (2008). "Cellular and molecular mechanisms of fibrosis." J Pathol **214**(2): 199-210.
307 Antar, S. A., Ashour, N. A., Marawan, M. E.*, et al.* (2023). "Fibrosis: Types, Effects, Markers, Mechanisms for Disease Progression, and Its Relation with Oxidative Stress, Immunity, and Inflammation." Int J Mol Sci **24**(4).
308 Wuraola, F., Olasehinde, O., Di Bernardo, M.*, et al.* (2023). "Prevalence and determinants of lymphedema in newly diagnosed Nigerian breast cancer patients using bioimpedance estimations." ecancermedicalscience **17**: 1506.

309 Ren, Y., Kebede, M. A., Ogunleye, A. A.*, et al.* (2022). "Burden of lymphedema in long-term breast cancer survivors by race and age." Cancer **128**(23): 4119-4128.
310 Armer, J. M., Ballman, K. V., McCall, L.*, et al.* (2019). "Lymphedema symptoms and limb measurement changes in breast cancer survivors treated with neoadjuvant chemotherapy and axillary dissection: results of American College of Surgeons Oncology Group (ACOSOG) Z1071 (Alliance) substudy." Supportive Care in Cancer **27**: 495-503.
311 He, L., Qu, H., Wu, Q.*, et al.* (2020). "Lymphedema in survivors of breast cancer." Oncol Lett **19**(3): 2085-2096.
312 Rockson, S. G. (2001). "Lymphedema." The American Journal of Medicine **110**(4): 288-295.
313 Gillespie, T. C., Sayegh, H. E., Brunelle, C. L.*, et al.* (2018). "Breast cancer-related lymphedema: risk factors, precautionary measures, and treatments." Gland Surg **7**(4): 379-403.
314 McEvoy, M. P., Gomberawalla, A., Smith, M.*, et al.* (2022). "The prevention and treatment of breast cancer- related lymphedema: A review." Frontiers in Oncology **12**.
315 Fu, M. R. (2014). "Breast cancer-related lymphedema: Symptoms, diagnosis, risk reduction, and management." World J Clin Oncol **5**(3): 241-247.
316 Yeung, W., McPhail, S. M. and Kuys, S. S. (2015). "A systematic review of axillary web syndrome (AWS)." Journal of Cancer Survivorship **9**: 576-598.
317 Koehler, L. A., Haddad, T. C., Hunter, D. W.*, et al.* (2019). "Axillary web syndrome following breast cancer surgery: symptoms, complications, and management strategies." Breast cancer (Dove Medical Press) **11**: 13-19.
318 Jeong, S., Song, B. J., Rhu, J.*, et al.* (2021). "A Risk Factor Analysis of Axillary Web Syndrome in Patients After Breast Cancer Surgery: A Single Center Study in Korea." Ann Rehabil Med **45**(5): 401-409.
319 Lippi, L., de Sire, A., Losco, L.*, et al.* (2022). "Axillary Web Syndrome in Breast Cancer Women: What Is the Optimal Rehabilitation Strategy after Surgery? A Systematic Review." J Clin Med **11**(13).
320 Alharazy, S. M. (2023). "Occurrence of axillary web syndrome without surgical intervention: a case report." J Int Med Res **51**(1): 3000605231152384.
321 Palta, S., Saroa, R. and Palta, A. (2014). "Overview of the coagulation system." Indian journal of anaesthesia **58**(5): 515.
322 Lee, T. S., Kilbreath, S. L., Refshauge, K. M.*, et al.* (2008). "Prognosis of the upper limb following surgery and radiation for breast cancer." Breast Cancer Res Treat **110**(1): 19-37.
323 Chrischilles, E. A., Riley, D., Letuchy, E.*, et al.* (2019). "Upper extremity disability and quality of life after breast cancer treatment in the Greater Plains Collaborative clinical research network." Breast Cancer Res Treat **175**(3): 675-689.
324 Feuk, G. I. B. (2000). "Morbidity from Axillary Treatment in Breast Cancer: A Follow-up Study in a District Hospital." Acta Oncologica **39**(3): 335-336.
325 Kärki, A., Simonen, R., Mälkiä, E.*, et al.* (2005). "Impairments, activity limitations and participation restrictions 6 and 12 months after breast cancer operation." J Rehabil Med **37**(3): 180-188.
326 Shamley, D., Srinaganathan, R., Oskrochi, R.*, et al.* (2009). "Three-dimensional scapulothoracic motion following treatment for breast cancer." Breast Cancer Res Treat **118**(2): 315-322.
327 Hamood, R., Hamood, H., Merhasin, I.*, et al.* (2018). "Chronic pain and other symptoms among breast cancer survivors: prevalence, predictors, and effects on quality of life." Breast Cancer Res Treat **167**(1): 157-169.

328 Raja, S. N., Carr, D. B., Cohen, M.*, et al.* (2020). "The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises." Pain **161**(9): 1976-1982.
329 Merskey, H. (1986). "Pain terms: a current list with definitions and notes on usage." Pain **3**: 217-221.
330 Mills, S. E. E., Nicolson, K. P. and Smith, B. H. (2019). "Chronic pain: a review of its epidemiology and associated factors in population-based studies." Br J Anaesth **123**(2): e273-e283.
331 Lucas, J., van Doorn, P., Hegedus, E.*, et al.* (2022). "A systematic review of the global prevalence and incidence of shoulder pain." BMC Musculoskelet Disord **23**(1): 1073.
332 Giacalone, A., Alessandria, P. and Ruberti, E. (2019). "The Physiotherapy Intervention for Shoulder Pain in Patients Treated for Breast Cancer: Systematic Review." Cureus **11**(12): e6416.
333 Kamerman, P. R., Bradshaw, D., Laubscher, R.*, et al.* (2020). "Almost 1 in 5 South African adults have chronic pain: a prevalence study conducted in a large nationally representative sample." Pain **161**(7): 1629-1635.
334 Boucheron, P., Anele, A., Zietsman, A.*, et al.* (2021). "Self-reported arm and shoulder problems in breast cancer survivors in Sub-Saharan Africa: the African Breast Cancer-Disparities in Outcomes cohort study." Breast cancer research : BCR **23**(1): 109.
335 Urwin, M., Symmons, D., Allison, T.*, et al.* (1998). "Estimating the burden of musculoskeletal disorders in the community: the comparative prevalence of symptoms at different anatomical sites, and the relation to social deprivation." Ann Rheum Dis **57**(11): 649-655.
336 MacDermid, J. C., Ramos, J., Drosdowech, D.*, et al.* (2004). "The impact of rotator cuff pathology on isometric and isokinetic strength, function, and quality of life." J Shoulder Elbow Surg **13**(6): 593-598.
337 Burbank, K. M., Stevenson, J. H., Czarnecki, G. R.*, et al.* (2008). "Chronic shoulder pain: part I. Evaluation and diagnosis." Am Fam Physician **77**(4): 453-460.
338 McCausland, C., Sawyer, E., Eovaldi, B. J.*, et al.* (2018). "Anatomy, shoulder and upper limb, shoulder muscles."
339 Barnechea Rey, A. R. (2021). Anatomy and Kinematics of the Shoulder Joint. Orthopaedic Biomechanics in Sports Medicine. J. Koh, S. Zaffagnini, R. Kuroda, U. G. Longo and F. Amirouche. Cham, Springer International Publishing**:** 111-133.
340 Bakhsh, W. and Nicandri, G. (2018). "Anatomy and Physical Examination of the Shoulder." Sports Med Arthrosc Rev **26**(3): e10-e22.
341 Javed, O., Maldonado, K. A. and Ashmyan, R. (2024). Anatomy, Shoulder and Upper Limb, Muscles. StatPearls. Treasure Island (FL), StatPearls Publishing

342 Boykin, R. E., Heuer, H. J. D., Vaishnav, S.*, et al.* (2010). "Rotator cuff disease – basics of diagnosis and treatment." Rheumatology Reports **2**(1).
343 Donnelly, T. D., Ashwin, S., Macfarlane, R. J.*, et al.* (2013). "Clinical assessment of the shoulder." Open Orthop J **7**: 310-315.
344 Poppen, N. K. and Walker, P. S. (1976). "Normal and abnormal motion of the shoulder." J Bone Joint Surg Am **58**(2): 195-201.
345 Barnes, C. J., Van Steyn, S. J. and Fischer, R. A. (2001). "The effects of age, sex, and shoulder dominance on range of motion of the shoulder." J Shoulder Elbow Surg **10**(3): 242-246.
346 Gismervik, S. O., Drogset, J. O., Granviken, F.*, et al.* (2017). "Physical examination tests of the shoulder: a systematic review and meta-analysis of diagnostic test performance." BMC Musculoskelet Disord **18**(1): 41.

347 Self, E. (2002). "Clinical guidelines for shoulder pain." Orthopaedic Knowledge Update: Shoulder and Elbow **2**: 443-467.
348 Cakir, M., Samanci, N., Balci, N.*, et al.* (2003). "Musculoskeletal manifestations in patients with thyroid disease." Clin Endocrinol (Oxf) **59**(2): 162-167.
349 Iannotti, J. P. and Kwon, Y. W. (2005). "Management of persistent shoulder pain: a treatment algorithm." Am J Orthop (Belle Mead NJ) **34**(12 Suppl): 16-23.
350 Younger, J., McCue, R. and Mackey, S. (2009). "Pain outcomes: a brief review of instruments and techniques." Curr Pain Headache Rep **13**(1): 39-43.
351 Mellor, F. and Knapp, K. (2020). Research Outcome Measures. Medical Imaging and Radiotherapy Research: Skills and Strategies. A. Ramlaul. Cham, Springer International Publishing**:** 167-183.
352 Schofield, S., McCormick, J. and Roscoe-Cutting, C. (2021). "Measuring mild-to-moderate acute pain–a regulatory perspective."
353 Pantaleon, L. (2019). "Why measuring outcomes is important in health care." J Vet Intern Med **33**(2): 356-362.
354 Fink, R. (2000). "Pain assessment: the cornerstone to optimal pain management." Proc (Bayl Univ Med Cent) **13**(3): 236-239.
355 Aldon-Villegas, R., Ridao-Fernández, C., Torres-Enamorado, D.*, et al.* (2021). "How to assess shoulder functionality: a systematic review of existing validated outcome measures." Diagnostics **11**(5): 845.
356 Roy, J.-S., Braën, C., Leblond, J.*, et al.* (2015). "Diagnostic accuracy of ultrasonography, MRI and MR arthrography in the characterisation of rotator cuff disorders: a systematic review and meta-analysis." British Journal of Sports Medicine **49**(20): 1316-1328.
357 Allen, G. M. (2018). "The diagnosis and management of shoulder pain." J Ultrason **18**(74): 234-239.
358 Singh, J. P. (2012). "Shoulder ultrasound: What you need to know." Indian J Radiol Imaging **22**(4): 284-292.
359 Jiang, L., He, J., Chen, C. P. C.*, et al.* (2020). "The Ultrasonographic Features of Shoulder Pain Patients in a Tertiary Hospital in South China." Biomed Res Int **2020**: 3024793.
360 Tran, G., Cowling, P., Smith, T.*, et al.* (2018). "What Imaging-Detected Pathologies Are Associated With Shoulder Symptoms and Their Persistence? A Systematic Literature Review." Arthritis Care Res (Hoboken) **70**(8): 1169-1184.
361 Nijs, J., Torres-Cueco, R., Van Wilgen, P.*, et al.* (2014). "Applying modern pain neuroscience in clinical practice: criteria for the classification of central sensitization pain." Pain Physician **17**(5): 447-457.
362 Nijs, J., Leysen, L., Pas, R.*, et al.* (2018). "Treatment of pain following cancer: applying neuro-immunology in rehabilitation practice." Disability and Rehabilitation **40**(6): 714-721.
363 Leysen, L., Adriaenssens, N., Nijs, J.*, et al.* (2019). "Chronic Pain in Breast Cancer Survivors: Nociceptive, Neuropathic, or Central Sensitization Pain?" Pain Pract **19**(2): 183-195.
364 Nicholson, B. (2006). "Differential diagnosis: nociceptive and neuropathic pain." Am J Manag Care **12**(9 Suppl): S256-262.
365 Riedel, W. and Neeck, G. (2001). "Nociception, pain, and antinociception: current concepts." Z Rheumatol **60**(6): 404-415.
366 Kost, R. G. and Straus, S. E. (1996). "Postherpetic neuralgia--pathogenesis, treatment, and prevention." N Engl J Med **335**(1): 32-42.
367 Sun, Z. C., Ma, S. B., Chu, W. G.*, et al.* (2020). "Canonical Transient Receptor Potential (TRPC) Channels in Nociception and Pathological Pain." Neural Plast **2020**: 3764193.

368 Swieboda, P., Filip, R., Prystupa, A.*, et al.* (2013). "Assessment of pain: types, mechanism and treatment." Ann Agric Environ Med **Spec no. 1**: 2-7.
369 Cohen, S. P. and Mao, J. (2014). "Neuropathic pain: mechanisms and their clinical implications." BMJ **348**: f7656.
370 Doody, O. and Bailey, M. E. (2019). "Understanding pain physiology and its application to person with intellectual disability." J Intellect Disabil **23**(1): 5-18.
371 Osuch, E. and Marais, A. (2018). "An update on available pain medications." South African Family Practice **60**(3): 14-20.
372 Hodges, P. W. and Tucker, K. (2011). "Moving differently in pain: a new theory to explain the adaptation to pain." Pain **152**(3 Suppl): S90-S98.
373 Van der Gucht, E., Dams, L., Meeus, M.*, et al.* (2020). "Kinesiophobia contributes to pain-related disability in breast cancer survivors: a cross-sectional study." Support Care Cancer **28**(9): 4501-4508.
374 Rasmussen, G. H. F., Madeleine, P., Arroyo-Morales, M.*, et al.* (2023). "Pain sensitivity and shoulder function among breast cancer survivors compared to matched controls: a case-control study." J Cancer Surviv **17**(1): 150-159.
375 Taxonomy, I. T. F. i. (1994). Pain terms: a current list with definitions and notes on usage, IASP Press Seattle, Washington**:** 206-213.
376 Belfer, I. and Dai, F. (2010). "Phenotyping and genotyping neuropathic pain." Curr Pain Headache Rep **14**(3): 203-212.
377 Woolf, C. J. and Mannion, R. J. (1999). "Neuropathic pain: aetiology, symptoms, mechanisms, and management." Lancet **353**(9168): 1959-1964.
378 Woolf, C. J. (2004). "Dissecting out mechanisms responsible for peripheral neuropathic pain: implications for diagnosis and therapy." Life Sci **74**(21): 2605-2610.
379 Latremoliere, A. and Woolf, C. J. (2009). "Central sensitization: a generator of pain hypersensitivity by central neural plasticity." J Pain **10**(9): 895-926.
380 Campbell, J. N. and Meyer, R. A. (2006). "Mechanisms of neuropathic pain." Neuron **52**(1): 77-92.
381 McMahon, S. B., Lewin, G. R. and Wall, P. D. (1993). "Central hyperexcitability triggered by noxious inputs." Curr Opin Neurobiol **3**(4): 602-610.
382 Meller, S. T. and Gebhart, G. F. (1994). "Spinal mediators of hyperalgesia." Drugs **47 Suppl 5**: 10-20; discussion 46-17.
383 Greco, R., Tassorelli, C., Sandrini, G.*, et al.* (2008). "Role of calcitonin gene-related peptide and substance P in different models of pain." Cephalalgia **28**(2): 114-126.
384 Kumar, A., Pottabathini, R., Bhatnagar, A.*, et al.* (2016). "Pharmacological Management of Neuropathic Pain: Current Trends and Possible Approaches." Archives of Neuroscience **4**(1).
385 Klit, H., Finnerup, N. B. and Jensen, T. S. (2009). "Central post-stroke pain: clinical characteristics, pathophysiology, and management." Lancet Neurol **8**(9): 857-868.
386 Mallick-Searle, T., Snodgrass, B. and Brant, J. M. (2016). "Postherpetic neuralgia: epidemiology, pathophysiology, and pain management pharmacology." J Multidiscip Healthc **9**: 447-454.
387 Kaeley, N., Ahmad, S., Pathania, M.*, et al.* (2019). "Prevalence and patterns of peripheral neuropathy in patients of rheumatoid arthritis." J Family Med Prim Care **8**(1): 22-26.
388 Baron, R., Binder, A., Attal, N.*, et al.* (2016). "Neuropathic low back pain in clinical practice." Eur J Pain **20**(6): 861-873.
389 Elsakka, K. M., J, M. D. and Allam, A. E. (2024). Ilioinguinal Neuralgia. StatPearls. Treasure Island (FL), StatPearls Publishing.

390 Cuesta-Vargas, A. I., Roldan-Jimenez, C., Pajares, B.*, et al.* (2018). "Central sensitization in breast cancer survivors." Journal of Applied Biobehavioral Research **23**(2): e12120.
391 De Groef, A., Meeus, M., De Vrieze, T.*, et al.* (2018). "Unraveling Self-Reported Signs of Central Sensitization in Breast Cancer Survivors with Upper Limb Pain: Prevalence Rate and Contributing Factors." Pain Physician **21**(3): E247-E256.
392 Nijs, J., George, S. Z., Clauw, D. J.*, et al.* (2021). "Central sensitisation in chronic pain conditions: latest discoveries and their potential for precision medicine." The Lancet Rheumatology **3**(5): e383-e392.
393 Hurth, A., Nijzink-Ter Steege, J., Scheepbouwer, P.*, et al.* (2021). "Assessment of Central Sensitization in Breast Cancer Survivors: Convergent Validity and Use of the Central Sensitization Inventory (CSI) and Its Short-Form as a Clustering Tool." Clin Pract **11**(3): 607-618.
394 Eller-Smith, O. C., Nicol, A. L. and Christianson, J. A. (2018). "Potential Mechanisms Underlying Centralized Pain and Emerging Therapeutic Interventions." Front Cell Neurosci **12**: 35.
395 Woolf, C. J. (2011). "Central sensitization: implications for the diagnosis and treatment of pain." Pain **152**(3 Suppl): S2-S15.
396 Bair, E., Brownstein, N. C., Ohrbach, R.*, et al.* (2013). "Study protocol, sample characteristics, and loss to follow-up: the OPPERA prospective cohort study." J Pain **14**(12 Suppl): T2-19.
397 Bair, E., Ohrbach, R., Fillingim, R. B.*, et al.* (2013). "Multivariable modeling of phenotypic risk factors for first-onset TMD: the OPPERA prospective cohort study." J Pain **14**(12 Suppl): T102-115.
398 Fillingim, R. B., Slade, G. D., Diatchenko, L.*, et al.* (2011). "Summary of findings from the OPPERA baseline case-control study: implications and future directions." J Pain **12**(11 Suppl): T102-107.
399 Greenspan, J. D., Slade, G. D., Bair, E.*, et al.* (2013). "Pain sensitivity and autonomic factors associated with development of TMD: the OPPERA prospective cohort study." J Pain **14**(12 Suppl): T63-74 e61-66.
400 Harte, S. E., Harris, R. E. and Clauw, D. J. (2018). "The neurobiology of central sensitization." Journal of Applied Biobehavioral Research **23**(2): e12137.
401 Somogyi, A. A., Barratt, D. T. and Coller, J. K. (2007). "Pharmacogenetics of opioids." Clin Pharmacol Ther **81**(3): 429-444.
402 Gach, K., Wyrebska, A., Fichna, J.*, et al.* (2011). "The role of morphine in regulation of cancer cell growth." Naunyn Schmiedebergs Arch Pharmacol **384**(3): 221-230.
403 Aubrun, F., Mazoit, J. X. and Riou, B. (2012). "Postoperative intravenous morphine titration." Br J Anaesth **108**(2): 193-201.
404 Goodsell, D. S. (2005). "The molecular perspective: morphine." Stem cells **23**(1): 144-145.
405 Houmes, R. J., Voets, M. A., Verkaaik, A.*, et al.* (1992). "Efficacy and safety of tramadol versus morphine for moderate and severe postoperative pain with special regard to respiratory depression." Anesth Analg **74**(4): 510-514.
406 Lewis, K. S. and Han, N. H. (1997). "Tramadol: a new centrally acting analgesic." Am J Health Syst Pharm **54**(6): 643-652.
407 Dean, L. and Kane, M. (2021). "Tramadol therapy and CYP2D6 genotype."
408 Fry, M., Ryan, J. and Alexander, N. (2004). "A prospective study of nurse initiated panadeine forte: expanding pain management in the ED." Accid Emerg Nurs **12**(3): 136-140.
409 Botting, R. M. (2000). "Mechanism of action of acetaminophen: is there a cyclooxygenase 3?" Clinical Infectious Diseases **31**(Supplement_5): S202-S210.

410 Kirchheiner, J., Schmidt, H., Tzvetkov, M.*, et al.* (2007). "Pharmacokinetics of codeine and its metabolite morphine in ultra-rapid metabolizers due to CYP2D6 duplication." The Pharmacogenomics Journal **7**(4): 257-265.
411 Mazaleuskaya, L. L., Sangkuhl, K., Thorn, C. F.*, et al.* (2015). "PharmGKB summary: pathways of acetaminophen metabolism at the therapeutic versus toxic doses." Pharmacogenetics and genomics **25**(8): 416.
412 Ademikanra, A., Olayiwola, A. and Oyewole, O. (2023). "Introduction to Opioids: A Review." Biomedicine and Chemical Sciences **2**(1): 01-06.
413 Zin, C. S., Nazar, N. I., Rahman, N. S.*, et al.* (2018). "Trends and patterns of analgesic prescribing in Malaysian public hospitals from 2010 to 2016: tramadol predominately used." J Pain Res **11**: 1959-1966.
414 Unlugenc, H., Ozalevli, M., Gunes, Y.*, et al.* (2003). "Pre-emptive analgesic efficacy of tramadol compared with morphine after major abdominal surgery." Br J Anaesth **91**(2): 209-213.
415 Saritha, M. and Mohan, P. R. (2019). "Prescribing patterns of tramadol in hemodialysis patients." International Journal of Basic & Clinical Pharmacology **8**(10): 4.
416 Scott, L. J. and Perry, C. M. (2000). "Tramadol: a review of its use in perioperative pain." Drugs **60**(1): 139-176.
417 Siu, A. Y. C. and Chung, C. H. (2017). "A Pilot Study on the use of Tramadol Hydrochloride for Pain Control in an Emergency Department." Hong Kong Journal of Emergency Medicine **11**(1): 16-20.
418 Subedi, M., Bajaj, S., Kumar, M. S.*, et al.* (2019). "An overview of tramadol and its usage in pain management and future perspective." Biomed Pharmacother **111**: 443-451.
419 Duan, G., Bao, X., Yang, G.*, et al.* (2019). "Patient-controlled intravenous tramadol versus patient-controlled intravenous hydromorphone for analgesia after secondary cesarean delivery: a randomized controlled trial to compare analgesic, anti-anxiety and anti-depression effects." J Pain Res **12**: 49-59.
420 Chi, X., Li, M., Mei, W.*, et al.* (2017). "Comparison of patient-controlled intravenous analgesia with sufentanil versus tramadol in post-cesarean section pain management and lactation after general anesthesia - a prospective, randomized, double-blind, controlled study." J Pain Res **10**: 1521-1527.
421 Moron Merchante, I., Pergolizzi, J. V., Jr., van de Laar, M.*, et al.* (2013). "Tramadol/Paracetamol fixed-dose combination for chronic pain management in family practice: a clinical review." ISRN Family Med **2013**: 638469.
422 Kostnapfel, T., Korosec, A. and Kastelic, A. (2020). "Tramadol as the most prescribed opioid analgesic medication in Slovenia in recent years." Heroin Addiction and Related Clinical Problems **22**(5): 13-18.
423 Aweke, Z., Seyoum, F., Shitemaw, T.*, et al.* (2020). "Comparison of preemptive paracetamol, paracetamol-diclofenac & paracetamol-tramadol combination on postoperative pain after elective abdominal surgery under general anesthesia, Ethiopia: a randomized control trial study, 2018." BMC Anesthesiol **20**(1): 191.
424 Martinez, V., Guichard, L. and Fletcher, D. (2015). "Effect of combining tramadol and morphine in adult surgical patients: a systematic review and meta-analysis of randomized trials." Br J Anaesth **114**(3): 384-395.
425 Hwang, Y. and Oh, J. (2022). "The relationship between shoulder pain and shoulder disability in women: The mediating role of sleep quality and psychological disorders." Medicine **101**(41): e31118.
426 Leysen, L., Beckwee, D., Nijs, J.*, et al.* (2017). "Risk factors of pain in breast cancer survivors: a systematic review and meta-analysis." Support Care Cancer **25**(12): 3607-3643.

427 Schou Bredal, I., Smeby, N. A., Ottesen, S.*, et al.* (2014). "Chronic pain in breast cancer survivors: comparison of psychosocial, surgical, and medical characteristics between survivors with and without pain." Journal of pain and symptom management **48**(5): 852-862.
428 Bao, T., Seidman, A., Li, Q.*, et al.* (2018). "Living with chronic pain: perceptions of breast cancer survivors." Breast Cancer Res Treat **169**(1): 133-140.
429 Divella, M., Vetrugno, L., Bertozzi, S.*, et al.* (2020). "Patient-reported pain and other symptoms among breast cancer survivors: prevalence and risk factors." Tumori Journal **106**(6): 480-490.
430 De Groef, A., Meeus, M., Heathcote, L. C.*, et al.* (2023). "Treating persistent pain after breast cancer: practice gaps and future directions." J Cancer Surviv **17**(6): 1698-1707.
431 Katz, J., Jackson, M., Kavanagh, B. P.*, et al.* (1996). "Acute pain after thoracic surgery predicts long-term post-thoracotomy pain." Clin J Pain **12**(1): 50-55.
432 Ramsay, M. A. (2000). Acute postoperative pain management. Baylor University medical center proceedings, Taylor & Francis.
433 Edwards, R. R., Haythornthwaite, J. A., Smith, M. T.*, et al.* (2009). "Catastrophizing and depressive symptoms as prospective predictors of outcomes following total knee replacement." Pain Res Manag **14**(4): 307-311.
434 Frumkin, M. R. and Rodebaugh, T. L. (2021). "The role of affect in chronic pain: A systematic review of within-person symptom dynamics." J Psychosom Res **147**: 110527.
435 Ene, K. W., Nordberg, G., Johansson, F. G.*, et al.* (2006). "Pain, psychological distress and health-related quality of life at baseline and 3 months after radical prostatectomy." BMC Nursing **5**(1): 8.
436 Chaplin, J. M. and Morton, R. P. (1999). "A prospective, longitudinal study of pain in head and neck cancer patients." Head Neck **21**(6): 531-537.
437 Gunnarsson, H., Safipour, J., Elmqvist, C.*, et al.* (2021). "Different pain variables could independently predict anxiety and depression in subjects with chronic musculoskeletal pain." Scandinavian Journal of Pain **21**(2): 274-282.
438 Edwards, S. L., Beesley, J., French, J. D.*, et al.* (2013). "Beyond GWASs: illuminating the dark road from association to function." Am J Hum Genet **93**(5): 779-797.
439 Smith, B. H., Elliott, A. M., Chambers, W. A.*, et al.* (2001). "The impact of chronic pain in the community." Fam Pract **18**(3): 292-299.
440 Li, S., Brimmers, A., van Boekel, R. L. M.*, et al.* (2023). "A systematic review of genome-wide association studies for pain, nociception, neuropathy, and pain treatment responses." Pain **164**(9): 1891-1911.
441 Martin, C., De Baerdemaeker, A., Poelaert, J.*, et al.* (2016). "Controlled-release of opioids for improved pain management." Materials Today **19**(9): 491-502.
442 Derendorf, H., Lesko, L. J., Chaikin, P.*, et al.* (2000). "Pharmacokinetic/pharmacodynamic modeling in drug research and development." J Clin Pharmacol **40**(12 Pt 2): 1399-1418.
443 Romberg, R., Olofsen, E., Sarton, E.*, et al.* (2004). "Pharmacokinetic-pharmacodynamic modeling of morphine-6-glucuronide-induced analgesia in healthy volunteers: absence of sex differences." Anesthesiology **100**(1): 120-133.
444 Fatima, T. S., Fathima, S. T., Kandadai, R. M.*, et al.* (2023). "Association of Catechol-O-Methyltransferase Gene Polymorphisms and Haplotypes in the Levodopa-Induced Adverse Events in Subjects with Parkinson's Disease." Indian J Clin Biochem **38**(2): 262-274.
445 Benet, L. Z. and Zia-Amirhosseini, P. (1995). "Basic principles of pharmacokinetics." Toxicol Pathol **23**(2): 115-123.
446 Smith, H. S. (2009). "Opioid metabolism." Mayo Clin Proc **84**(7): 613-624.

447 Scott, S. A. (2011). "Personalizing medicine with clinical pharmacogenetics." Genet Med **13**(12): 987-995.
448 Kapur, B. M., Lala, P. K. and Shaw, J. L. (2014). "Pharmacogenetics of chronic pain management." Clin Biochem **47**(13-14): 1169-1187.
449 Cox, J. J., Reimann, F., Nicholas, A. K.*, et al.* (2006). "An SCN9A channelopathy causes congenital inability to experience pain." Nature **444**(7121): 894-898.
450 Zanger, U. M. and Schwab, M. (2013). "Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation." Pharmacol Ther **138**(1): 103-141.
451 Somogyi, A. A., Coller, J. K. and Barratt, D. T. (2015). "Pharmacogenetics of opioid response." Clin Pharmacol Ther **97**(2): 125-127.
452 Sadhasivam, S., Chidambaran, V., Olbrecht, V. A.*, et al.* (2014). "Genetics of pain perception, COMT and postoperative pain management in children." Pharmacogenomics **15**(3): 277-284.
453 Campbell, C. M. and Edwards, R. R. (2012). "Ethnic differences in pain and pain management." Pain management **2**(3): 219-230.
454 De Gregori, S., De Gregori, M., Ranzani, G. N.*, et al.* (2012). "Morphine metabolism, transport and brain disposition." Metab Brain Dis **27**(1): 1-5.
455 Konig, J., Muller, F. and Fromm, M. F. (2013). "Transporters and drug-drug interactions: important determinants of drug disposition and effects." Pharmacol Rev **65**(3): 944-966.
456 Coles, L. D., Lee, I. J., Voulalas, P. J.*, et al.* (2009). "Estradiol and progesterone-mediated regulation of P-gp in P-gp overexpressing cells (NCI-ADR-RES) and placental cells (JAR)." Mol Pharm **6**(6): 1816-1825.
457 Wandel, C., Kim, R., Wood, M.*, et al.* (2002). "Interaction of morphine, fentanyl, sufentanil, alfentanil, and loperamide with the efflux drug transporter P-glycoprotein." Anesthesiology **96**(4): 913-920.
458 Geier, A., Wagner, M., Dietrich, C. G.*, et al.* (2007). "Principles of hepatic organic anion transporter regulation during cholestasis, inflammation and liver regeneration." Biochim Biophys Acta **1773**(3): 283-308.
459 Skarke, C., Langer, M., Jarrar, M.*, et al.* (2004). "Probenecid interacts with the pharmacokinetics of morphine-6-glucuronide in humans." Anesthesiology **101**(6): 1394-1399.
460 Al-Hasani, R. and Bruchas, M. R. (2011). "Molecular mechanisms of opioid receptor-dependent signaling and behavior." Anesthesiology **115**(6): 1363-1381.
461 Cuitavi, J., Hipolito, L. and Canals, M. (2021). "The Life Cycle of the Mu-Opioid Receptor." Trends Biochem Sci **46**(4): 315-328.
462 Dhaliwal, A. and Gupta, M. (2020). Physiology, Opioid Receptor, StatPearls Publishing, Treasure Island (FL).
463 Zubieta, J. K., Heitzeg, M. M., Smith, Y. R.*, et al.* (2003). "COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor." Science **299**(5610): 1240-1243.
464 Laugsand, E. A., Fladvad, T., Skorpen, F.*, et al.* (2011). "Clinical and genetic factors associated with nausea and vomiting in cancer patients receiving opioids." European journal of cancer (Oxford, England : 1990) **47**(11): 1682-1691.
465 Pacheco Dda, F., Klein, A., Perez, A. C.*, et al.* (2009). "Central antinociception induced by mu-opioid receptor agonist morphine, but not delta- or kappa-, is mediated by cannabinoid CB1 receptor." Br J Pharmacol **158**(1): 225-231.
466 Sprouse-Blum, A. S., Smith, G., Sugai, D.*, et al.* (2010). "Understanding endorphins and their importance in pain management." Hawaii Med J **69**(3): 70-71.

467 Przewlocki, R. (2004). "Opioid abuse and brain gene expression." Eur J Pharmacol **500**(1-3): 331-349.
468 Matic, M., de Wildt, S. N., Tibboel, D.*, et al.* (2017). "Analgesia and Opioids: A Pharmacogenetics Shortlist for Implementation in Clinical Practice." Clin Chem **63**(7): 1204-1213.
469 Li, J., Wei, Z., Zhang, J.*, et al.* (2019). "Candidate gene analyses for acute pain and morphine analgesia after pediatric day surgery: African American versus European Caucasian ancestry and dose prediction limits." Pharmacogenomics J **19**(6): 570-581.
470 Margarit, C., Roca, R., Inda, M. D.*, et al.* (2019). "Genetic Contribution in Low Back Pain: A Prospective Genetic Association Study." Pain Pract **19**(8): 836-847.
471 Wang, X. S., Song, H. B., Chen, S.*, et al.* (2015). "Association of single nucleotide polymorphisms of ABCB1, OPRM1 and COMT with pain perception in cancer patients." J Huazhong Univ Sci Technolog Med Sci **35**(5): 752-758.
472 Dimeloe, S., Frick, C., Fischer, M.*, et al.* (2014). "Human regulatory T cells lack the cyclophosphamide-extruding transporter ABCB1 and are more susceptible to cyclophosphamide-induced apoptosis." Eur J Immunol **44**(12): 3614-3620.
473 Mora Lagares, L., Minovski, N., Caballero Alfonso, A. Y.*, et al.* (2020). "Homology Modeling of the Human P-glycoprotein (ABCB1) and Insights into Ligand Binding through Molecular Docking Studies." Int J Mol Sci **21**(11): 4058.
474 Fung, K. L. and Gottesman, M. M. (2009). "A synonymous polymorphism in a common MDR1 (ABCB1) haplotype shapes protein function." Biochim Biophys Acta **1794**(5): 860-871.
475 Zawadzka, I., Jelen, A., Pietrzak, J.*, et al.* (2020). "The impact of ABCB1 gene polymorphism and its expression on non-small-cell lung cancer development, progression and therapy - preliminary report." Sci Rep **10**(1): 6188.
476 Linton, K. J. (2007). "Structure and function of ABC transporters." Physiology (Bethesda) **22**(2): 122-130.
477 Dean, M. and Allikmets, R. (2001). "Complete characterization of the human ABC gene family." J Bioenerg Biomembr **33**(6): 475-479.
478 Hodges, L. M., Markova, S. M., Chinn, L. W.*, et al.* (2011). "Very important pharmacogene summary: ABCB1 (MDR1, P-glycoprotein)." Pharmacogenetics and genomics **21**(3): 152-161.
479 Chen, C. J., Chin, J. E., Ueda, K.*, et al.* (1986). "Internal duplication and homology with bacterial transport proteins in the mdr1 (P-glycoprotein) gene from multidrug-resistant human cells." Cell **47**(3): 381-389.
480 Gottesman, M. M. and Ling, V. (2006). "The molecular basis of multidrug resistance in cancer: the early years of P-glycoprotein research." FEBS Lett **580**(4): 998-1009.
481 Wolking, S., Schaeffeler, E., Lerche, H.*, et al.* (2015). "Impact of Genetic Polymorphisms of ABCB1 (MDR1, P-Glycoprotein) on Drug Disposition and Potential Clinical Implications: Update of the Literature." Clinical Pharmacokinetics **54**(7): 709-735.
482 Beuselinck, B., Karadimou, A., Lambrechts, D.*, et al.* (2013). "Single-nucleotide polymorphisms associated with outcome in metastatic renal cell carcinoma treated with sunitinib." Br J Cancer **108**(4): 887-900.
483 Beuselinck, B., Lambrechts, D., Van Brussel, T.*, et al.* (2014). "Efflux pump ABCB1 single nucleotide polymorphisms and dose reductions in patients with metastatic renal cell carcinoma treated with sunitinib." Acta Oncol **53**(10): 1413-1422.
484 Jiang, B., Yan, L. J. and Wu, Q. (2019). "ABCB1 (C1236T) Polymorphism Affects P-Glycoprotein-Mediated Transport of Methotrexate, Doxorubicin, Actinomycin D, and Etoposide." DNA Cell Biol **38**(5): 485-490.

485 Brazeau, D. A., Attwood, K., Meaney, C. J., *et al.* (2020). "Beyond Single Nucleotide Polymorphisms: CYP3A5(*)3(*)6(*)7 Composite and ABCB1 Haplotype Associations to Tacrolimus Pharmacokinetics in Black and White Renal Transplant Recipients." Front Genet **11**: 889.

486 Rychlik-Sych, M., Baranska, M., Dudarewicz, M., *et al.* (2018). "Haplotypes of ABCB1 1236C >T (rs1128503), 2677G >T/A (rs2032582), and 3435C >T (rs1045642) in patients with bullous pemphigoid." Arch Dermatol Res **310**(6): 515-522.

487 Xie, W. W., Zhang, L., Wu, R. R., *et al.* (2015). "Case-control association study of ABCB1 gene and major depressive disorder in a local Chinese Han population." Neuropsychiatr Dis Treat **11**: 1967-1971.

488 Zhong, X., Liu, M. Y., Sun, X. H., *et al.* (2016). "Association between ABCB1 polymorphisms and haplotypes and Alzheimer's disease: a meta-analysis." Sci Rep **6**: 32708.

489 Hattori, S., Suda, A., Kishida, I., *et al.* (2018). "Effects of ABCB1 gene polymorphisms on autonomic nervous system activity during atypical antipsychotic treatment in schizophrenia." Bmc Psychiatry **18**(1): 231.

490 Levran, O., O'Hara, K., Peles, E., *et al.* (2008). "ABCB1 (MDR1) genetic variants are associated with methadone doses required for effective treatment of heroin dependence." Hum Mol Genet **17**(14): 2219-2227.

491 Hamidovic, A., Hahn, K. and Kolesar, J. (2010). "Clinical significance of ABCB1 genotyping in oncology." J Oncol Pharm Pract **16**(1): 39-44.

492 Wolf, S. J., Bachtiar, M., Wang, J., *et al.* (2011). "An update on ABCB1 pharmacogenetics: insights from a 3D model into the location and evolutionary conservation of residues corresponding to SNPs associated with drug pharmacokinetics." Pharmacogenomics J **11**(5): 315-325.

493 Wolking, S., Schaeffeler, E., Lerche, H., *et al.* (2015). "Impact of Genetic Polymorphisms of ABCB1 (MDR1, P-Glycoprotein) on Drug Disposition and Potential Clinical Implications: Update of the Literature." Clin Pharmacokinet **54**(7): 709-735.

494 Candiotti, K., Yang, Z., Xue, L., *et al.* (2013). "Single-nucleotide polymorphism C3435T in the ABCB1 gene is associated with opioid consumption in postoperative pain." Pain Med **14**(12): 1977-1984.

495 Rhodin, A., Gronbladh, A., Ginya, H., *et al.* (2013). "Combined analysis of circulating beta-endorphin with gene polymorphisms in OPRM1, CACNAD2 and ABCB1 reveals correlation with pain, opioid sensitivity and opioid-related side effects." Mol Brain **6**: 8.

496 Bastami, S., Gupta, A., Zackrisson, A. L., *et al.* (2014). "Influence of UGT2B7, OPRM1 and ABCB1 gene polymorphisms on postoperative morphine consumption." Basic Clin Pharmacol Toxicol **115**(5): 423-431.

497 Hoffmeyer, S., Burk, O., von Richter, O., *et al.* (2000). "Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo." Proc Natl Acad Sci U S A **97**(7): 3473-3478.

498 Hsin, C. H., Stoffel, M. S., Gazzaz, M., *et al.* (2020). "Combinations of common SNPs of the transporter gene ABCB1 influence apparent bioavailability, but not renal elimination of oral digoxin." Sci Rep **10**(1): 12457.

499 Parchure, A. S. and Peng, Y. B. (2020). "The impact of opioid analgesics and the pharmacogenomics of abcb1 in opioid dependence and pharmacotherapies: A short review." The Open Pain Journal **13**(1).

500 Ambudkar, S. V., Dey, S., Hrycyna, C. A., *et al.* (1999). "Biochemical, cellular, and pharmacological aspects of the multidrug transporter." Annu Rev Pharmacol Toxicol **39**(1): 361-398.

501 Zahari, Z., Lee, C. S., Ibrahim, M. A., *et al.* (2017). "Relationship Between ABCB1 Polymorphisms and Cold Pain Sensitivity Among Healthy Opioid-naive Malay Males." Pain Pract **17**(7): 930-940.
502 Tang, K., Ngoi, S.-M., Gwee, P.-C., *et al.* (2002). "Distinct haplotype profiles and strong linkage disequilibrium at the MDR1 multidrug transporter gene locus in three ethnic Asian populations." Pharmacogenetics and genomics **12**(6): 437-450.
503 Kim, R. B., Leake, B. F., Choo, E. F., *et al.* (2001). "Identification of functionally variant MDR1 alleles among European Americans and African Americans." Clin Pharmacol Ther **70**(2): 189-199.
504 Singh, A. B., Bousman, C. A., Ng, C. H., *et al.* (2012). "ABCB1 polymorphism predicts escitalopram dose needed for remission in major depression." Transl Psychiatry **2**(11): e198.
505 Panczyk, M., Balcerczak, E., Piaskowski, S., *et al.* (2009). "ABCB1 gene polymorphisms and haplotype analysis in colorectal cancer." Int J Colorectal Dis **24**(8): 895-905.
506 Weissfeld, J. L., Diergaarde, B., Nukui, T., *et al.* (2014). "Inherited variation in the ATP-binding cassette transporter ABCB1 and survival after chemotherapy for stage III-IV lung cancer." Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer **9**(9): 1264-1271.
507 Parvin, M. N., Aziz, M. A., Rabbi, S. N. I., *et al.* (2021). "Assessment of the Link of ABCB1 and NR3C1 gene polymorphisms with the prednisolone resistance in pediatric nephrotic syndrome patients of Bangladesh: A genotype and haplotype approach." J Adv Res **33**: 141-151.
508 Coller, J. K., Barratt, D. T., Dahlen, K., *et al.* (2006). "ABCB1 genetic variability and methadone dosage requirements in opioid-dependent individuals." Clin Pharmacol Ther **80**(6): 682-690.
509 Beer, B., Erb, R., Pavlic, M., *et al.* (2013) Association of polymorphisms in pharmacogenetic candidate genes (OPRD1, GAL, ABCB1, OPRM1) with opioid dependence in European population: a case-control study. Plos One **8**, e75359 DOI: 10.1371/journal.pone.0075359
510 Christoffersen, D. J., Damkier, P., Feddersen, S., *et al.* (2016). "The ABCB1, rs9282564, AG and TT Genotypes and the COMT, rs4680, AA Genotype are Less Frequent in Deceased Patients with Opioid Addiction than in Living Patients with Opioid Addiction." Basic Clin Pharmacol Toxicol **119**(4): 381-388.
511 Fonseca, F., de la Torre, R., Diaz, L., *et al.* (2011). "Contribution of cytochrome P450 and ABCB1 genetic variability on methadone pharmacokinetics, dose requirements, and response." Plos One **6**(5): e19527.
512 Gong, X. D., Wang, J. Y., Liu, F., *et al.* (2013). "Gene polymorphisms of OPRM1 A118G and ABCB1 C3435T may influence opioid requirements in Chinese patients with cancer pain." Asian Pac J Cancer Prev **14**(5): 2937-2943.
513 Takashina, Y., Naito, T., Mino, Y., *et al.* (2012). "Impact of CYP3A5 and ABCB1 gene polymorphisms on fentanyl pharmacokinetics and clinical responses in cancer patients undergoing conversion to a transdermal system." Drug Metab Pharmacokinet **27**(4): 414-421.
514 Dzambazovska-Trajkovska, V., Nojkov, J., Kartalov, A., *et al.* (2016). "Association of Single-Nucleotide Polymorhism C3435T in the ABCB1 Gene with Opioid Sensitivity in Treatment of Postoperative Pain." Pril (Makedon Akad Nauk Umet Odd Med Nauki) **37**(2-3): 73-80.
515 Horvat, C. M., Au, A. K., Conley, Y. P., *et al.* (2017). "ABCB1 genotype is associated with fentanyl requirements in critically ill children." Pediatric research **82**(1): 29-35.

516 Mamie, C., Rebsamen, M. C., Morris, M. A., *et al.* (2013). "First evidence of a polygenic susceptibility to pain in a pediatric cohort." Anesth Analg **116**(1): 170-177.
517 Soultati, I., Ntenti, C., Tsaousi, G., *et al.* (2023). "Effect of common OPRM1, COMT, SLC6A4, ABCB1, and CYP2B6 polymorphisms on perioperative analgesic and propofol demands on patients subjected to thyroidectomy surgery." Pharmacol Rep **75**(2): 386-396.
518 Aubrun, F., Zahr, N., Langeron, O., *et al.* (2018). "Opioid-related genetic polymorphisms do not influence postoperative opioid requirement: A prospective observational study." Eur J Anaesthesiol **35**(7): 496-504.
519 Fojo, A., Lebo, R., Shimizu, N., *et al.* (1986). "Localization of multidrug resistance-associated DNA sequences to human chromosome 7." Somat Cell Mol Genet **12**(4): 415-420.
520 Choudhuri, S. and Klaassen, C. D. (2006). "Structure, function, expression, genomic organization, and single nucleotide polymorphisms of human ABCB1 (MDR1), ABCC (MRP), and ABCG2 (BCRP) efflux transporters." Int J Toxicol **25**(4): 231-259.
521 Ishikawa, T., Hirano, H., Onishi, Y., *et al.* (2004). "Functional evaluation of ABCB1 (P-glycoprotein) polymorphisms: high-speed screening and structure-activity relationship analyses." Drug Metab Pharmacokinet **19**(1): 1-14.
522 Chen, C. J., Clark, D., Ueda, K., *et al.* (1990). "Genomic organization of the human multidrug resistance (MDR1) gene and origin of P-glycoproteins." J Biol Chem **265**(1): 506-514.
523 Tulsyan, S., Mittal, R. D. and Mittal, B. (2016). "The effect of ABCB1 polymorphisms on the outcome of breast cancer treatment." Pharmacogenomics and personalized medicine **9**: 47-58.
524 Klaassen, C. D. and Aleksunes, L. M. (2010). "Xenobiotic, bile acid, and cholesterol transporters: function and regulation." Pharmacol Rev **62**(1): 1-96.
525 Schinkel, A. H., Kemp, S., Dolle, M., *et al.* (1993). "N-glycosylation and deletion mutants of the human MDR1 P-glycoprotein." J Biol Chem **268**(10): 7474-7481.
526 Goodfellow, H. R., Sardini, A., Ruetz, S., *et al.* (1996). "Protein kinase C-mediated phosphorylation does not regulate drug transport by the human multidrug resistance P-glycoprotein." J Biol Chem **271**(23): 13668-13674.
527 Idriss, H. T., Hannun, Y. A., Boulpaep, E., *et al.* (2000). "Regulation of volume-activated chloride channels by P-glycoprotein: phosphorylation has the final say!" J Physiol **524 Pt 3**(Pt 3): 629-636.
528 Zhang, Z., Wu, J. Y., Hait, W. N., *et al.* (2004). "Regulation of the stability of P-glycoprotein by ubiquitination." Mol Pharmacol **66**(3): 395-403.
529 Borst, P. and Elferink, R. O. (2002). "Mammalian ABC transporters in health and disease." Annu Rev Biochem **71**(1): 537-592.
530 de Boer, A. G., van der Sandt, I. C. and Gaillard, P. J. (2003). "The role of drug transporters at the blood-brain barrier." Annu Rev Pharmacol Toxicol **43**(1): 629-656.
531 Thiebaut, F., Tsuruo, T., Hamada, H., *et al.* (1987). "Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues." Proc Natl Acad Sci U S A **84**(21): 7735-7738.
532 Staud, F., Ceckova, M., Micuda, S., *et al.* (2010). Expression and Function of P-Glycoprotein in Normal Tissues: Effect on Pharmacokinetics. Multi-Drug Resistance in Cancer. J. Zhou. Totowa, NJ, Humana Press**:** 199-222.
533 Schinkel, A. H. and Jonker, J. W. (2012). "Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview." Advanced drug delivery reviews **64**: 138-153.
534 Schinkel, A. H. (1997). "The physiological function of drug-transporting P-glycoproteins." Semin Cancer Biol **8**(3): 161-170.

535 Schumacher, T., Krohn, M., Hofrichter, J.*, et al.* (2012). "ABC transporters B1, C1 and G2 differentially regulate neuroregeneration in mice." Plos One **7**(4): e35613.
536 Karthika, C. and Sureshkumar, R. (2020). "P-Glycoprotein Efflux Transporters and Its Resistance Its Inhibitors and Therapeutic Aspects."
537 Wessler, J. D., Grip, L. T., Mendell, J.*, et al.* (2013). "The P-glycoprotein transport system and cardiovascular drugs." J Am Coll Cardiol **61**(25): 2495-2502.
538 Ueda, K., Okamura, N., Hirai, M.*, et al.* (1992). "Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone." J Biol Chem **267**(34): 24248-24252.
539 Meijer, O. C., Karssen, A. M. and de Kloet, E. R. (2003). "Cell- and tIssue-specific effects of corticosteroids in relation to glucocorticoid resistance: examples from the brain." The Journal of endocrinology **178**(1): 13-18.
540 Callaghan, R., Luk, F. and Bebawy, M. (2014). "Inhibition of the multidrug resistance P-glycoprotein: time for a change of strategy?" Drug metabolism and disposition: the biological fate of chemicals **42**(4): 623-631.
541 Waldhoer, M., Bartlett, S. E. and Whistler, J. L. (2004). "Opioid receptors." Annu Rev Biochem **73**(1): 953-990.
542 Rosenbaum, D. M., Rasmussen, S. G. and Kobilka, B. K. (2009). "The structure and function of G-protein-coupled receptors." Nature **459**(7245): 356-363.
543 Snyder, S. H. and Pasternak, G. W. (2003). "Historical review: Opioid receptors." Trends Pharmacol Sci **24**(4): 198-205.
544 Samoshkin, A., Convertino, M., Viet, C. T.*, et al.* (2015). "Structural and functional interactions between six-transmembrane mu-opioid receptors and beta2-adrenoreceptors modulate opioid signaling." Sci Rep **5**(1): 18198.
545 Diatchenko, L., Robinson, J. E. and Maixner, W. (2011). "Elucidation of mu-opioid gene structure: how genetics can help predict therapeutic response to opioids." European Journal of Pain Supplements **5**(2): 433-438.
546 Klepstad, P., Rakvåg, T., Kaasa, S.*, et al.* (2004). "The 118 A> G polymorphism in the human μ-opioid receptor gene may increase morphine requirements in patients with pain caused by malignant disease." Acta Anaesthesiologica Scandinavica **48**(10): 1232-1239.
547 Shabalina, S. A., Zaykin, D. V., Gris, P.*, et al.* (2009). "Expansion of the human mu-opioid receptor gene architecture: novel functional variants." Hum Mol Genet **18**(6): 1037-1051.
548 Pan, Y. X. (2002). "Identification and characterization of a novel promoter of the mouse mu opioid receptor gene (Oprm) that generates eight splice variants." Gene **295**(1): 97-108.
549 Pan, Y. X., Xu, J., Bolan, E.*, et al.* (2005). "Identification of four novel exon 5 splice variants of the mouse mu-opioid receptor gene: functional consequences of C-terminal splicing." Mol Pharmacol **68**(3): 866-875.
550 Pan, Y. X., Xu, J., Mahurter, L.*, et al.* (2003). "Identification and characterization of two new human mu opioid receptor splice variants, hMOR-1O and hMOR-1X." Biochem Biophys Res Commun **301**(4): 1057-1061.
551 Pang, G. S., Ithnin, F., Wong, Y. Y.*, et al.* (2012). "A non-synonymous single nucleotide polymorphism in an OPRM1 splice variant is associated with fentanyl-induced emesis in women undergoing minor gynaecological surgery." Plos One **7**(11): e48416.
552 Regan, P. M. (2015). Regulation and Functional Impact of Opioid Receptor Splicing in Response to Morphine, Temple University. Libraries.

553 Regan, P. M., Sariyer, I. K., Langford, T. D.*, et al.* (2016). "Morphine-induced MOR-1X and ASF/SF2 Expressions Are Independent of Transcriptional Regulation: Implications for MOR-1X Signaling." Journal of cellular physiology **231**(7): 1542-1553.

554 Reyes-Gibby, C. C., Shete, S., Rakvag, T.*, et al.* (2007). "Exploring joint effects of genes and the clinical efficacy of morphine for cancer pain: OPRM1 and COMT gene." Pain **130**(1-2): 25-30.

555 Zhao, Z., Lv, B., Zhao, X.*, et al.* (2019). "Effects of OPRM1 and ABCB1 gene polymorphisms on the analgesic effect and dose of sufentanil after thoracoscopic-assisted radical resection of lung cancer." Biosci Rep **39**(1).

556 Chidambaran, V., Gang, Y., Pilipenko, V.*, et al.* (2020). "Systematic Review and Meta-Analysis of Genetic Risk of Developing Chronic Postsurgical Pain." J Pain **21**(1-2): 2-24.

557 De Gregori, M., Diatchenko, L., Ingelmo, P. M.*, et al.* (2016). "Human Genetic Variability Contributes to Postoperative Morphine Consumption." J Pain **17**(5): 628-636.

558 Masih, J. and Verbeke, W. (2019). Exploring Association of Opioid Receptor Genes Polymorphism with Positive and Negative Moods using Positive and Negative Affective States Scale (PANAS).

559 Schwantes-An, T. H., Zhang, J., Chen, L. S.*, et al.* (2016). "Association of the OPRM1 Variant rs1799971 (A118G) with Non-Specific Liability to Substance Dependence in a Collaborative de novo Meta-Analysis of European-Ancestry Cohorts." Behavior genetics **46**(2): 151-169.

560 Wonkam, A., Mnika, K., Ngo Bitoungui, V. J.*, et al.* (2018). "Clinical and genetic factors are associated with pain and hospitalisation rates in sickle cell anaemia in Cameroon." Br J Haematol **180**(1): 134-146.

561 Bartosova, O., Polanecky, O., Perlik, F.*, et al.* (2015). "OPRM1 and ABCB1 polymorphisms and their effect on postoperative pain relief with piritramide." Physiol Res **64**(Suppl 4): S521-527.

562 Bjorland, S., Moen, A., Schistad, E.*, et al.* (2016). "Genes associated with persistent lumbar radicular pain; a systematic review." BMC Musculoskelet Disord **17**(1): 500.

563 Erbi, I., Ciantelli, M., Farinella, R.*, et al.* (2020). "Role of OPRM1, clinical and anthropometric variants in neonatal pain reduction." Sci Rep **10**(1): 7091.

564 Sia, A. T., Lim, Y., Lim, E. C.*, et al.* (2008). "A118G single nucleotide polymorphism of human mu-opioid receptor gene influences pain perception and patient-controlled intravenous morphine consumption after intrathecal morphine for postcesarean analgesia." Anesthesiology **109**(3): 520-526.

565 Hasvik, E., Iordanova Schistad, E., Grovle, L.*, et al.* (2014). "Subjective health complaints in patients with lumbar radicular pain and disc herniation are associated with a sex - OPRM1 A118G polymorphism interaction: a prospective 1-year observational study." BMC Musculoskelet Disord **15**: 161.

566 Garriock, H. A., Tanowitz, M., Kraft, J. B.*, et al.* (2010). "Association of mu-opioid receptor variants and response to citalopram treatment in major depressive disorder." Am J Psychiatry **167**(5): 565-573.

567 Jurewicz, A., Bohatyrewicz, A., Pawlak, M.*, et al.* (2022). "No Association between Genetic Variants of the COMT and OPRM1 Genes and Pain Perception among Patients Undergoing Total Hip or Knee Arthroplasty for Primary Osteoarthritis." Genes (Basel) **13**(10): 1775.

568 Wang, J. B., Johnson, P. S., Persico, A. M.*, et al.* (1994). "Human mu opiate receptor. cDNA and genomic clones, pharmacologic characterization and chromosomal assignment." FEBS Lett **338**(2): 217-222.

569 Pasternak, G. W. and Pan, Y. X. (2013). "Mu opioids and their receptors: evolution of a concept." Pharmacol Rev **65**(4): 1257-1317.
570 Wei, L. N. and Loh, H. H. (2011). Transcriptional and Epigenetic Regulation of Opioid Receptor Genes: Present and Future. Annual Review of Pharmacology and Toxicology, Vol 51, 2011. A. K. Cho. **51:** 75-97.
571 Wendel, B. and Hoehe, M. R. (1998). "The human μ opioid receptor gene: 5 regulatory and intronic sequences." Journal of molecular medicine **76**(7): 525-532.
572 Kraus, J. r., Börner, C., Giannini, E.*, et al.* (2001). "Regulation of μ-opioid receptor gene transcription by interleukin-4 and influence of an allelic variation within a STAT6 transcription factor binding site." Journal of Biological Chemistry **276**(47): 43901-43908.
573 Borner, C., Hollt, V. and Kraus, J. (2002). "Involvement of activator protein-1 in transcriptional regulation of the human mu-opioid receptor gene." Mol Pharmacol **61**(4): 800-805.
574 Börner, C., Wöltje, M., Höllt, V.*, et al.* (2004). "STAT6 transcription factor binding sites with mismatches within the canonical 5′-TTC… GAA-3′ motif involved in regulation of δ-and μ-opioid receptors." Journal of neurochemistry **91**(6): 1493-1500.
575 Kraus, J., Borner, C., Giannini, E.*, et al.* (2003). "The role of nuclear factor kappaB in tumor necrosis factor-regulated transcription of the human mu-opioid receptor gene." Mol Pharmacol **64**(4): 876-884.
576 Borner, C., Hollt, V. and Kraus, J. (2006). "Cannabinoid receptor type 2 agonists induce transcription of the mu-opioid receptor gene in Jurkat T cells." Mol Pharmacol **69**(4): 1486-1491.
577 Bedini, A., Baiula, M. and Spampinato, S. (2008). "Transcriptional activation of human mu-opioid receptor gene by insulin-like growth factor-I in neuronal cells is modulated by the transcription factor REST." Journal of neurochemistry **105**(6): 2166-2178.
578 Ono, T., Kaneda, T., Muto, A.*, et al.* (2009). "Positive transcriptional regulation of the human micro opioid receptor gene by poly(ADP-ribose) polymerase-1 and increase of its DNA binding affinity based on polymorphism of G-172 -> T." J Biol Chem **284**(30): 20175-20183.
579 Liu, H., Li, H., Guo, L.*, et al.* (2010). "Mechanisms involved in phosphatidylinositol 3-kinase pathway mediated up-regulation of the mu opioid receptor in lymphocytes." Biochem Pharmacol **79**(3): 516-523.
580 Andria, M. L. and Simon, E. J. (2001). "Identification of a neurorestrictive suppressor element (NRSE) in the human mu-opioid receptor gene." Brain Res Mol Brain Res **91**(1-2): 73-80.
581 Xu, Y. and Carr, L. G. (2001). "Transcriptional regulation of the human mu opioid receptor (hMOR) gene: evidence of positive and negative cis-acting elements in the proximal promoter and presence of a distal promoter." DNA Cell Biol **20**(7): 391-402.
582 Hwang, C. K., Song, K. Y., Kim, C. S.*, et al.* (2007). "Evidence of endogenous mu opioid receptor regulation by epigenetic control of the promoters." Mol Cell Biol **27**(13): 4720-4736.
583 Moore, L. D., Le, T. and Fan, G. (2013). "DNA methylation and its basic function." Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology **38**(1): 23-38.
584 Oertel, B. G., Doehring, A., Roskam, B.*, et al.* (2012). "Genetic-epigenetic interaction modulates mu-opioid receptor regulation." Hum Mol Genet **21**(21): 4751-4760.
585 Chidambaran, V., Zhang, X., Martin, L. J.*, et al.* (2017). "DNA methylation at the mu-1 opioid receptor gene (OPRM1) promoter predicts preoperative, acute, and chronic postsurgical pain after spine fusion." Pharmacogenomics and personalized medicine **10**: 157.

586 Bannister, A. J. and Kouzarides, T. (2011). "Regulation of chromatin by histone modifications." Cell Res **21**(3): 381-395.
587 HealyShannon, KhanProtiti, HeShihua*, et al.* (2012). "Histone H3 phosphorylation, immediate-early gene expression, and the nucleosomal response: a historical perspective1This article is part of Special Issue entitled Asilomar Chromatin and has undergone the Journal's usual peer review process." Biochemistry and Cell Biology **90**(1): 39-54.
588 Rossetto, D., Avvakumov, N. and Cote, J. (2012). "Histone phosphorylation: a chromatin modification involved in diverse nuclear events." Epigenetics **7**(10): 1098-1108.
589 Sawicka, A. and Seiser, C. (2012). "Histone H3 phosphorylation - a versatile chromatin modification for different occasions." Biochimie **94**(11): 2193-2201.
590 Swygert, S. G. and Peterson, C. L. (2014). "Chromatin dynamics: interplay between remodeling enzymes and histone modifications." Biochim Biophys Acta **1839**(8): 728-736.
591 Alaskhar Alhamwe, B., Khalaila, R., Wolf, J.*, et al.* (2018). "Histone modifications and their role in epigenetics of atopy and allergic diseases." Allergy, asthma, and clinical immunology : official journal of the Canadian Society of Allergy and Clinical Immunology **14**: 39.
592 Sun, N., Yu, L., Gao, Y.*, et al.* (2021). "MeCP2 Epigenetic Silencing of Oprm1 Gene in Primary Sensory Neurons Under Neuropathic Pain Conditions." Front Neurosci **15**: 743207.
593 Formisano, L., Noh, K. M., Miyawaki, T.*, et al.* (2007). "Ischemic insults promote epigenetic reprogramming of mu opioid receptor expression in hippocampal neurons." Proc Natl Acad Sci U S A **104**(10): 4170-4175.
594 Nilsen, T. W. and Graveley, B. R. (2010). "Expansion of the eukaryotic proteome by alternative splicing." Nature **463**(7280): 457-463.
595 Wang, Y., Liu, J., Huang, B. O.*, et al.* (2015). "Mechanism of alternative splicing and its regulation." Biomedical reports **3**(2): 152-158.
596 Black, D. L. (1992). "Activation of c-src neuron-specific splicing by an unusual RNA element in vivo and in vitro." Cell **69**(5): 795-807.
597 Chan, R. C. and Black, D. L. (1997). "Conserved intron elements repress splicing of a neuron-specific c-src exon in vitro." Mol Cell Biol **17**(5): 2970.
598 Chan, R. C. and Black, D. L. (1997). "The polypyrimidine tract binding protein binds upstream of neural cell-specific c-src exon N1 to repress the splicing of the intron downstream." Mol Cell Biol **17**(8): 4667-4676.
599 Carstens, R. P., Eaton, J. V., Krigman, H. R.*, et al.* (1997). "Alternative splicing of fibroblast growth factor receptor 2 (FGF-R2) in human prostate cancer." Oncogene **15**(25): 3059-3065.
600 Cooper, T. A. and Mattox, W. (1997). "The regulation of splice-site selection, and its role in human disease." Am J Hum Genet **61**(2): 259-266.
601 Carstens, R. P., McKeehan, W. L. and Garcia-Blanco, M. A. (1998). "An intronic sequence element mediates both activation and repression of rat fibroblast growth factor receptor 2 pre-mRNA splicing." Mol Cell Biol **18**(4): 2205-2217.
602 Carstens, R. P., Wagner, E. J. and Garcia-Blanco, M. A. (2000). "An intronic splicing silencer causes skipping of the IIIb exon of fibroblast growth factor receptor 2 through involvement of polypyrimidine tract binding protein." Mol Cell Biol **20**(19): 7388-7400.
603 Spraggon, L. and Cartegni, L. (2013). "Antisense Modulation of RNA Processing as a Therapeutic Approach in Cancer Therapy." Drug Discov Today Ther Strateg **10**(3): e139-e148.

604 Xiong, H. Y., Alipanahi, B., Lee, L. J.*, et al.* (2015). "The human splicing code reveals new insights into the genetic determinants of disease." Science **347**(6218).
605 Liu, S., Kang, W.-J., Abrimian, A.*, et al.* (2021). "Alternative Pre-mRNA Splicing of the Mu Opioid Receptor Gene, OPRM1: Insight into Complex Mu Opioid Actions." Biomolecules **11**(10): 1525.
606 Oladosu, F. A., Maixner, W. and Nackley, A. G. (2015). "Alternative Splicing of G Protein-Coupled Receptors: Relevance to Pain Management." Mayo Clin Proc **90**(8): 1135-1151.
607 Keren, H., Lev-Maor, G. and Ast, G. (2010). "Alternative splicing and evolution: diversification, exon definition and function." Nat Rev Genet **11**(5): 345-355.
608 Markovic, D. and Challiss, R. A. (2009). "Alternative splicing of G protein-coupled receptors: physiology and pathophysiology." Cellular and molecular life sciences : CMLS **66**(20): 3337-3352.
609 Wise, H. (2012). "The roles played by highly truncated splice variants of G protein-coupled receptors." J Mol Signal **7**(1): 13.
610 Xu, J., Lu, Z., Narayan, A.*, et al.* (2017). "Alternatively spliced mu opioid receptor C termini impact the diverse actions of morphine." J Clin Invest **127**(4): 1561-1573.
611 Pasternak, G. W. (2018). Chapter Twelve - Mu Opioid Pharmacology: 40 Years to the Promised Land. Advances in Pharmacology. G. W. Pasternak and J. T. Coyle, Academic Press. **82:** 261-291.
612 Pan, L., Xu, J., Yu, R.*, et al.* (2005). "Identification and characterization of six new alternatively spliced variants of the human mu opioid receptor gene, Oprm." Neuroscience **133**(1): 209-220.
613 Xu, J., Xu, M., Hurd, Y. L.*, et al.* (2009). "Isolation and characterization of new exon 11-associated N-terminal splice variants of the human mu opioid receptor gene." J Neurochem **108**(4): 962-972.
614 Xu, J., Xu, M., Brown, T.*, et al.* (2013). "Stabilization of the mu-opioid receptor by truncated single transmembrane splice variants through a chaperone-like action." J Biol Chem **288**(29): 21211-21227.
615 Narayan, A., Hunkele, A., Xu, J.*, et al.* (2021). "Mu Opioids Induce Biased Signaling at the Full-Length Seven Transmembrane C-Terminal Splice Variants of the mu Opioid Receptor Gene, Oprm1." Cell Mol Neurobiol **41**(5): 1059-1074.
616 Stein, C. (1993). "Peripheral mechanisms of opioid analgesia." Anesth Analg **76**(1): 182-191.
617 Merrer, J. L., Becker, J. A. J., Befort, K.*, et al.* (2009). "Reward Processing by the Opioid System in the Brain." Physiological Reviews **89**(4): 1379-1412.
618 Zhao, X. F. (2020). "G protein-coupled receptors function as cell membrane receptors for the steroid hormone 20-hydroxyecdysone." Cell Commun Signal **18**(1): 146.
619 Nalini Sehgal, M., Howard Smith, M. and Laxmaiah Manchikanti, M. (2011). "Peripherally acting opioids and clinical implications for pain control." Pain Physician **14**: 249-258.
620 Sobczak, M., Salaga, M., Storr, M. A.*, et al.* (2014). "Physiology, signaling, and pharmacology of opioid receptors and their ligands in the gastrointestinal tract: current concepts and future perspectives." J Gastroenterol **49**(1): 24-45.
621 Coggeshall, R. E., Zhou, S. and Carlton, S. M. (1997). "Opioid receptors on peripheral sensory axons." Brain Res **764**(1-2): 126-132.
622 Jaber, L., Swaim, W. D. and Dionne, R. A. (2003). "Immunohistochemical localization of mu-opioid receptors in human dental pulp." J Endod **29**(2): 108-110.
623 Khodorova, A., Navarro, B., Jouaville, L. S.*, et al.* (2003). "Endothelin-B receptor activation triggers an endogenous analgesic cascade at sites of peripheral injury." Nat Med **9**(8): 1055-1061.

624 Ibrahim, M. M., Porreca, F., Lai, J.*, et al.* (2005). "CB2 cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids." Proc Natl Acad Sci U S A **102**(8): 3093-3098.
625 Wenk, H. N., Brederson, J. D. and Honda, C. N. (2006). "Morphine directly inhibits nociceptors in inflamed skin." J Neurophysiol **95**(4): 2083-2097.
626 Bergstrom, J., Ahmed, M., Li, J.*, et al.* (2006). "Opioid peptides and receptors in joint tissues: study in the rat." J Orthop Res **24**(6): 1193-1199.
627 Philippe, D., Chakass, D., Thuru, X.*, et al.* (2006). "Mu opioid receptor expression is increased in inflammatory bowel diseases: implications for homeostatic intestinal inflammation." Gut **55**(6): 815-823.
628 Zollner, C. and Stein, C. (2007). "Opioids." Handb Exp Pharmacol(177): 31-63.
629 Abbadie, C., Pan, Y., Drake, C. T.*, et al.* (2000). "Comparative immunohistochemical distributions of carboxy terminus epitopes from the mu-opioid receptor splice variants MOR-1D, MOR-1 and MOR-1C in the mouse and rat CNS." Neuroscience **100**(1): 141-153.
630 Xu, J., Lu, Z., Xu, M.*, et al.* (2014). "Differential expressions of the alternatively spliced variant mRNAs of the micro opioid receptor gene, OPRM1, in brain regions of four inbred mouse strains." Plos One **9**(10): e111267.
631 Feng, Y., He, X., Yang, Y.*, et al.* (2012). "Current research on opioid receptor function." Curr Drug Targets **13**(2): 230-246.
632 Pathan, H. and Williams, J. (2012). "Basic opioid pharmacology: an update." Br J Pain **6**(1): 11-16.
633 Bunchorntavakul, C. and Reddy, K. R. (2012). "Pruritus in chronic cholestatic liver disease." Clinics in liver disease **16**(2): 331-346.
634 P Headrick, J., Pepe, S. and N Peart, J. (2012). "Non-analgesic effects of opioids: cardiovascular effects of opioids and their receptor systems." Current Pharmaceutical Design **18**(37): 6090-6100.
635 Husain, S., Abdul, Y. and E Potter, D. (2012). "Non-analgesic effects of opioids: neuroprotection in the retina." Current Pharmaceutical Design **18**(37): 6101-6108.
636 Azhagiya Singam, E. R., Tachachartvanich, P., La Merrill, M. A.*, et al.* (2019). "Structural Dynamics of Agonist and Antagonist Binding to the Androgen Receptor." J Phys Chem B **123**(36): 7657-7666.
637 Corder, G., Castro, D. C., Bruchas, M. R.*, et al.* (2018). "Endogenous and Exogenous Opioids in Pain." Annu Rev Neurosci **41**: 453-473.
638 Faria, J., Barbosa, J., Moreira, R.*, et al.* (2018). "Comparative pharmacology and toxicology of tramadol and tapentadol." Eur J Pain **22**(5): 827-844.
639 Manandhar, P., Connor, M. and Santiago, M. (2022). "Tapentadol shows lower intrinsic efficacy at μ receptor than morphine and oxycodone." Pharmacology Research & Perspectives **10**(1): e00921.
640 Edinoff, A. N., Kaplan, L. A., Khan, S.*, et al.* (2021). "Full Opioid Agonists and Tramadol: Pharmacological and Clinical Considerations." Anesthesiology and pain medicine **11**(4): e119156.
641 Brownstein, M. J. (1993). "A brief history of opiates, opioid peptides, and opioid receptors." Proceedings of the National Academy of Sciences **90**(12): 5391-5393.
642 Kritikos, P. G. and Papadaki, S. (1967). The history of the poppy and of opium and their expansion in antiquity in the eastern Mediterranean area, UN New York.
643 Higginbotham, J. A., Markovic, T., Massaly, N.*, et al.* (2022). "Endogenous opioid systems alterations in pain and opioid use disorder." Frontiers in Systems Neuroscience **16**.
644 Theriot, J., Sabir, S. and Azadfard, M. (2019). "Opioid antagonists."

645 van Dorp, E., Yassen, A. and Dahan, A. (2007). "Naloxone treatment in opioid addiction: the risks and benefits." Expert Opin Drug Saf **6**(2): 125-132.
646 Zhang, X., Sun, M. Y., Zhang, X.*, et al.* (2022). "Dynamic recognition of naloxone, morphine and endomorphin1 in the same pocket of micro-opioid receptors." Frontiers in molecular biosciences **9**: 925404.
647 Aronson, J. K. E. (2016). Opioid receptor antagonists. Meyler's Side Effects of Drugs. J. K. Aronson. Oxford, Elsevier**:** 381.
648 Desai, C. (2016). "Meyler's side effects of drugs: The international encyclopedia of adverse drug reactions and interactions." Indian Journal of Pharmacology **48**(2): 224.
649 Abdelraheem, E., Thair, B., Varela, R. F.*, et al.* (2022). "Methyltransferases: Functions and Applications." ChemBioChem **23**(18): e202200212.
650 Richter, M. (2013). "Functional diversity of organic molecule enzyme cofactors." Nat Prod Rep **30**(10): 1324-1345.
651 Zubieta, C., Ross, J. R., Koscheski, P.*, et al.* (2003). "Structural basis for substrate recognition in the salicylic acid carboxyl methyltransferase family." Plant Cell **15**(8): 1704-1716.
652 Qazi, T. J., Quan, Z., Mir, A.*, et al.* (2018). "Epigenetics in Alzheimer's Disease: Perspective of DNA Methylation." Mol Neurobiol **55**(2): 1026-1044.
653 Wang, F. Y., Wang, P., Zhao, D. F.*, et al.* (2021). "Analytical methodologies for sensing catechol-O-methyltransferase activity and their applications." J Pharm Anal **11**(1): 15-27.
654 Reenilä, I. (1999). "Catechol-O-methyltransferase activity: Assay, distribution and pharmacological modification."
655 Bai, H. W., Shim, J. Y., Yu, J.*, et al.* (2007). "Biochemical and molecular modeling studies of the O-methylation of various endogenous and exogenous catechol substrates catalyzed by recombinant human soluble and membrane-bound catechol-O-methyltransferases." Chem Res Toxicol **20**(10): 1409-1425.
656 Guldberg, H. C. and Marsden, C. A. (1975). "Catechol-O-methyl transferase: pharmacological aspects and physiological role." Pharmacol Rev **27**(2): 135-206.
657 Knisely, M. R., Conley, Y. P., Smoot, B.*, et al.* (2019). "Associations Between Catecholaminergic and Serotonergic Genes and Persistent Arm Pain Severity Following Breast Cancer Surgery." J Pain **20**(9): 1100-1111.
658 Hoofwijk, D. M., van Reij, R. R., Rutten, B. P.*, et al.* (2016). "Genetic polymorphisms and their association with the prevalence and severity of chronic postsurgical pain: a systematic review." Br J Anaesth **117**(6): 708-719.
659 Nackley, A. G., Shabalina, S. A., Tchivileva, I. E.*, et al.* (2006). "Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure." Science **314**(5807): 1930-1933.
660 Liao, S. Y., Lin, S. H., Liu, C. M.*, et al.* (2009). "Genetic variants in COMT and neurocognitive impairment in families of patients with schizophrenia." Genes Brain Behav **8**(2): 228-237.
661 Männistö, P. T. and Kaakkola, S. (1999). "Catechol-O-methyltransferase (COMT): Biochemistry, Molecular Biology, Pharmacology, and Clinical Efficacy of the New Selective COMT Inhibitors." Pharmacological reviews **51**(4): 593-628.
662 Liu, H. L. and Wang, W. C. (2003). "Protein engineering to improve the thermostability of glucoamylase from Aspergillus awamori based on molecular dynamics simulations." Protein Eng **16**(1): 19-25.
663 Machius, M., Declerck, N., Huber, R.*, et al.* (2003). "Kinetic stabilization of Bacillus licheniformis alpha-amylase through introduction of hydrophobic residues at the surface." J Biol Chem **278**(13): 11546-11553.

664 Schmack, K., Rossler, H., Sekutowicz, M.*, et al.* (2015). "Linking unfounded beliefs to genetic dopamine availability." Front Hum Neurosci **9**: 521.
665 Stein, M. B., Fallin, M. D., Schork, N. J.*, et al.* (2005). "COMT polymorphisms and anxiety-related personality traits." Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology **30**(11): 2092-2102.
666 Sweet, R. A., Devlin, B., Pollock, B. G.*, et al.* (2005). "Catechol-O-methyltransferase haplotypes are associated with psychosis in Alzheimer disease." Mol Psychiatry **10**(11): 1026-1036.
667 Molero, P., Ortuno, F., Zalacain, M.*, et al.* (2007). "Clinical involvement of catechol-O-methyltransferase polymorphisms in schizophrenia spectrum disorders: influence on the severity of psychotic symptoms and on the response to neuroleptic treatment." Pharmacogenomics J **7**(6): 418-426.
668 Michaelovsky, E., Gothelf, D., Korostishevsky, M.*, et al.* (2008). "Association between a common haplotype in the COMT gene region and psychiatric disorders in individuals with 22q11.2DS." Int J Neuropsychopharmacol **11**(3): 351-363.
669 Jacobsen, L. M., Schistad, E. I., Storesund, A.*, et al.* (2012). "The COMT rs4680 Met allele contributes to long-lasting low back pain, sciatica and disability after lumbar disc herniation." Eur J Pain **16**(7): 1064-1069.
670 Lucenteforte, E., Vannacci, A., Crescioli, G.*, et al.* (2019). "Opioid response in paediatric cancer patients and the Val158Met polymorphism of the human catechol-O-methyltransferase (COMT) gene: an Italian study on 87 cancer children and a systematic review." Bmc Cancer **19**(1): 113.
671 Kambur, O. and Männistö, P. T. (2010). Catechol-O-Methyltransferase and Pain. International Review of Neurobiology. E. Nissinen, Academic Press. **95:** 227-279.
672 Barbosa, F. R., Matsuda, J. B., Mazucato, M.*, et al.* (2012). "Influence of catechol-O-methyltransferase (COMT) gene polymorphisms in pain sensibility of Brazilian fibromialgia patients." Rheumatol Int **32**(2): 427-430.
673 Tammimaki, A. and Mannisto, P. T. (2012). "Catechol-O-methyltransferase gene polymorphism and chronic human pain: a systematic review and meta-analysis." Pharmacogenetics and genomics **22**(9): 673-691.
674 Yang, M., Baser, R. E., Khanin, R.*, et al.* (2023). "COMT Val158Met Affects the Analgesic Response to Acupuncture Among Cancer Survivors with Chronic Pain." The Journal of pain.
675 Korczeniewska, O. A., Kuo, F., Huang, C. Y.*, et al.* (2021). "Genetic variation in catechol-O-methyltransferase is associated with individual differences in conditioned pain modulation in healthy subjects." J Gene Med **23**(11): e3374.
676 Omair, A., Mannion, A. F., Holden, M.*, et al.* (2015). "Catechol-O-methyltransferase (COMT) gene polymorphisms are associated with baseline disability but not long-term treatment outcome in patients with chronic low back pain." Eur Spine J **24**(11): 2425-2431.
677 Chiang, D. L. C., Rice, D. A., Helsby, N. A.*, et al.* (2023). "The incidence, impact, and risk factors for moderate to severe persistent pain after breast cancer surgery: a prospective cohort study." Pain Med **24**(9): 1023-1034.
678 Tan, E. C., Lim, E. C., Ocampo, C. E.*, et al.* (2016). "Common variants of catechol-O-methyltransferase influence patient-controlled analgesia usage and postoperative pain in patients undergoing total hysterectomy." Pharmacogenomics J **16**(2): 186-192.
679 Diatchenko, L., Nackley, A. G., Slade, G. D.*, et al.* (2006). "Catechol-O-methyltransferase gene polymorphisms are associated with multiple pain-evoking stimuli." Pain **125**(3): 216-224.

680 Khalil, H., Sereika, S. M., Dai, F.*, et al.* (2017). "OPRM1 and COMT Gene-Gene Interaction Is Associated With Postoperative Pain and Opioid Consumption After Orthopedic Trauma." Biol Res Nurs **19**(2): 170-179.
681 Nackley, A. G. and Diatchenko, L. (2010). Assessing Potential Functionality of Catechol-O-methyltransferase (COMT) Polymorphisms Associated with Pain Sensitivity and Temporomandibular Joint Disorders. Analgesia: Methods and Protocols. A. Szallasi. **617:** 375-393.
682 Segall, S. K., Nackley, A. G., Diatchenko, L.*, et al.* (2010). "Comt1 genotype and expression predicts anxiety and nociceptive sensitivity in inbred strains of mice." Genes Brain Behav **9**(8): 933-946.
683 McLean, S. A., Diatchenko, L., Lee, Y. M.*, et al.* (2011). "Catechol O-methyltransferase haplotype predicts immediate musculoskeletal neck pain and psychological symptoms after motor vehicle collision." J Pain **12**(1): 101-107.
684 Smith, S. B., Maixner, D. W., Greenspan, J. D.*, et al.* (2011). "Potential genetic risk factors for chronic TMD: genetic associations from the OPPERA case control study." J Pain **12**(11 Suppl): T92-101.
685 Segall, S. K., Maixner, W., Belfer, I.*, et al.* (2012). "Janus molecule I: dichotomous effects of COMT in neuropathic vs nociceptive pain modalities." CNS Neurol Disord Drug Targets **11**(3): 222-235.
686 Belfer, I., Segall, S. K., Lariviere, W. R.*, et al.* (2013). "Pain modality- and sex-specific effects of COMT genetic functional variants." Pain **154**(8): 1368-1376.
687 Vargas-Alarcon, G., Fragoso, J. M., Cruz-Robles, D.*, et al.* (2007). "Catechol-O-methyltransferase gene haplotypes in Mexican and Spanish patients with fibromyalgia." Arthritis Res Ther **9**(5): R110.
688 Park, D. J., Kim, S. H., Nah, S. S.*, et al.* (2016). Association between catechol-O-methyl transferase gene polymorphisms and fibromyalgia in a Korean population: A case-control study. European journal of pain (London, England). **20:** 1131.
689 Zhang, F., Tong, J., Hu, J.*, et al.* (2015). "COMT gene haplotypes are closely associated with postoperative fentanyl dose in patients." Anesth Analg **120**(4): 933-940.
690 Tenhunen, J., Salminen, M., Lundstrom, K.*, et al.* (1994). "Genomic organization of the human catechol O-methyltransferase gene and its expression from two distinct promoters." Eur J Biochem **223**(3): 1049-1059.
691 Lundstr, K., Salminen, M., Jalanko, A.*, et al.* (1991). "Cloning and Characterization of Human Placental Catechol--Methyltransferase cDNA." DNA and Cell Biology **10**(3): 181-189.
692 Salminen, M., Lundstrom, K., Tilgmann, C.*, et al.* (1990). "Molecular cloning and characterization of rat liver catechol-O-methyltransferase." Gene **93**(2): 241-247.
693 Qayyum, A., Zai, C. C., Hirata, Y.*, et al.* (2015). "The Role of the Catechol-o-Methyltransferase (COMT) GeneVal158Met in Aggressive Behavior, a Review of Genetic Studies." Curr Neuropharmacol **13**(6): 802-814.
694 Grossman, M. H., Emanuel, B. S. and Budarf, M. L. (1992). "Chromosomal mapping of the human catechol-O-methyltransferase gene to 22q11.1----q11.2." Genomics **12**(4): 822-825.
695 Faisst, S. and Meyer, S. (1992). "Compilation of vertebrate-encoded transcription factors." Nucleic Acids Res **20**(1): 3-26.
696 Imagawa, M., Chiu, R. and Karin, M. (1987). "Transcription factor AP-2 mediates induction by two different signal-transduction pathways: protein kinase C and cAMP." Cell **51**(2): 251-260.
697 Caruso, M., Iacobini, C., Passananti, C.*, et al.* (1990). "Protein recognition sites in polyomavirus enhancer: formation of a novel site for NF-1 factor in an enhancer mutant and characterization of a site in the enhancer D domain." Embo j **9**(3): 947-955.

698 Chen, J., Song, J., Yuan, P.*, et al.* (2011). "Orientation and cellular distribution of membrane-bound catechol-O-methyltransferase in cortical neurons: implications for drug development." J Biol Chem **286**(40): 34752-34760.
699 Xu, Q., Ma, J. Z., Payne, T. J.*, et al.* (2010). "Determination of Methylated CpG Sites in the Promoter Region of Catechol-O-Methyltransferase (COMT) and their Involvement in the Etiology of Tobacco Smoking." Front Psychiatry **1**: 16.
700 Murphy, B. C., O'Reilly, R. L. and Singh, S. M. (2005). "Site-specific cytosine methylation in S-COMT promoter in 31 brain regions with implications for studies involving schizophrenia." Am J Med Genet B Neuropsychiatr Genet **133B**(1): 37-42.
701 Sasaki, M., Kaneuchi, M., Sakuragi, N.*, et al.* (2003). "Multiple promoters of catechol-O-methyltransferase gene are selectively inactivated by CpG hypermethylation in endometrial cancer." Cancer Res **63**(12): 3101-3106.
702 Wu, Q., Odwin-Dacosta, S., Cao, S.*, et al.* (2019). "Estrogen down regulates COMT transcription via promoter DNA methylation in human breast cancer cells." Toxicol Appl Pharmacol **367**: 12-22.
703 Krahmer, N., Najafi, B., Schueder, F.*, et al.* (2018). "Organellar Proteomics and Phospho-Proteomics Reveal Subcellular Reorganization in Diet-Induced Hepatic Steatosis." Dev Cell **47**(2): 205-221 e207.
704 Fornes, R., Manti, M., Qi, X.*, et al.* (2019). "Mice exposed to maternal androgen excess and diet-induced obesity have altered phosphorylation of catechol-O-methyltransferase in the placenta and fetal liver." Int J Obes (Lond) **43**(11): 2176-2188.
705 Overbye, A. and Seglen, P. O. (2009). "Phosphorylated and non-phosphorylated forms of catechol O-methyltransferase in rat liver, brain and other tissues." The Biochemical journal **417**(2): 535-545.
706 Schendzielorz, N., Oinas, J. P., Myohanen, T. T.*, et al.* (2013). "Catechol-O-methyltransferase (COMT) protein expression and activity after dopaminergic and noradrenergic lesions of the rat brain." Plos One **8**(4): e61392.
707 Eisenhofer, G., Keiser, H., Friberg, P.*, et al.* (1998). "Plasma metanephrines are markers of pheochromocytoma produced by catechol-O-methyltransferase within tumors." The Journal of Clinical Endocrinology & Metabolism **83**(6): 2175-2185.
708 Nomura, T., Inoue, K., Creveling, C. R.*, et al.* (1996). "Immunocytochemical localization of aromaticl-amino acid decarboxylase and catechol-O-methyltransferase in blood vessel wall of the human dental pulp." Brain Research **735**(2): 314-316.
709 John, K., Ragavan, N., Pratt, M. M.*, et al.* (2009). "Quantification of phase I/II metabolizing enzyme gene expression and polycyclic aromatic hydrocarbon-DNA adduct levels in human prostate." Prostate **69**(5): 505-519.
710 Tenhunen, J., Heikkila, P., Alanko, A.*, et al.* (1999). "Soluble and membrane-bound catechol-O-methyltransferase in normal and malignant mammary gland." Cancer Lett **144**(1): 75-84.
711 Reenila, I. and Mannisto, P. T. (2001). "Catecholamine metabolism in the brain by membrane-bound and soluble catechol-o-methyltransferase (COMT) estimated by enzyme kinetic values." Med Hypotheses **57**(5): 628-632.
712 Myohanen, T. T., Schendzielorz, N. and Mannisto, P. T. (2010). "Distribution of catechol-O-methyltransferase (COMT) proteins and enzymatic activities in wild-type and soluble COMT deficient mice." J Neurochem **113**(6): 1632-1643.
713 Matsumoto, M., Weickert, C. S., Akil, M.*, et al.* (2003). "Catechol O-methyltransferase mRNA expression in human and rat brain: evidence for a role in cortical neuronal function." Neuroscience **116**(1): 127-137.
714 Ma, Z., Liu, H. and Wu, B. (2014). "Structure-based drug design of catechol-O-methyltransferase inhibitors for CNS disorders." British journal of clinical pharmacology **77**(3): 410-420.

715 Lerner, C., Jakob-Roetne, R., Buettelmann, B.*, et al.* (2016). "Design of Potent and Druglike Nonphenolic Inhibitors for Catechol O-Methyltransferase Derived from a Fragment Screening Approach Targeting the S-Adenosyl-l-methionine Pocket." J Med Chem **59**(22): 10163-10175.
716 Orlowski, A., St-Pierre, J. F., Magarkar, A.*, et al.* (2011). "Properties of the membrane binding component of catechol-O-methyltransferase revealed by atomistic molecular dynamics simulations." J Phys Chem B **115**(46): 13541-13550.
717 Lotta, T., Vidgren, J., Tilgmann, C.*, et al.* (1995). "Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme." Biochemistry **34**(13): 4202-4210.
718 Bates, G. W., Edman, C. D., Porter, J. C.*, et al.* (1978). "Catechol-O-methyltransferase activity in erythrocytes of pregnant women." Am J Obstet Gynecol **131**(5): 555-557.
719 Paravati, S., Rosani, A. and Warrington, S. J. (2024). Physiology, Catecholamines. StatPearls. Treasure Island (FL), StatPearls Publishing

720 Garcha, A. S. and Cohen, D. L. (2015). "Catecholamine excess: pseudopheochromocytoma and beyond." Adv Chronic Kidney Dis **22**(3): 218-223.
721 Becker, S. and Schweinhardt, P. (2012). "Dysfunctional neurotransmitter systems in fibromyalgia, their role in central stress circuitry and pharmacological actions on these systems." Pain Res Treat **2012**: 741746.
722 Potvin, S., Grignon, S. and Marchand, S. (2009). "Human evidence of a supra-spinal modulating role of dopamine on pain perception." Synapse **63**(5): 390-402.
723 Schlereth, T. and Birklein, F. (2008). "The sympathetic nervous system and pain." Neuromolecular Med **10**(3): 141-147.
724 Landau, R., Liu, S. K., Blouin, J. L.*, et al.* (2013). "The effect of OPRM1 and COMT genotypes on the analgesic response to intravenous fentanyl labor analgesia." Anesth Analg **116**(2): 386-391.
725 Cordell, H. J., Barratt, B. J. and Clayton, D. G. (2004). "Case/pseudocontrol analysis in genetic association studies: A unified framework for detection of genotype and haplotype associations, gene-gene and gene-environment interactions, and parent-of-origin effects." Genetic epidemiology **26**(3): 167-185.
726 Yao, P., Ding, Y. Y., Wang, Z. B.*, et al.* (2015). "Effect of gene polymorphism of COMT and OPRM1 on the preoperative pain sensitivity in patients with cancer." International journal of clinical and experimental medicine **8**(6): 10036-10039.
727 Hong, S. J., Lee, S. Y., Kim, H. B.*, et al.* (2005). "IL-5 and thromboxane A2 receptor gene polymorphisms are associated with decreased pulmonary function in Korean children with atopic asthma." J Allergy Clin Immunol **115**(4): 758-763.
728 Maier, L. M., Chapman, J., Howson, J. M.*, et al.* (2005). "No evidence of association or interaction between the IL4RA, IL4, and IL13 genes in type 1 diabetes." The American Journal of Human Genetics **76**(3): 517-521.
729 Bergholdt, R., Taxvig, C., Eising, S.*, et al.* (2005). "CBLB variants in type 1 diabetes and their genetic interaction with CTLA4." Journal of leukocyte biology **77**(4): 579-585.
730 Xu, J., Lowey, J., Wiklund, F.*, et al.* (2005). "The interaction of four genes in the inflammation pathway significantly predicts prostate cancer risk." Cancer Epidemiology Biomarkers & Prevention **14**(11): 2563-2568.
731 Zhang, X., Miao, X., Guo, Y.*, et al.* (2006). "Genetic polymorphisms in cell cycle regulatory genes MDM2 and TP53 are associated with susceptibility to lung cancer." Hum Mutat **27**(1): 110-117.
732 Infante, J., Sanz, C., Fernández-Luna, J.*, et al.* (2004). "Gene–gene interaction between interleukin-6 and interleukin-10 reduces AD risk." Neurology **63**(6): 1135-1136.

733 Ye, S., Dhillon, S., Seear, R., *et al.* (2003). "Epistatic interaction between variations in the angiotensin I converting enzyme and angiotensin II type 1 receptor genes in relation to extent of coronary atherosclerosis." Heart **89**(10): 1195-1199.
734 Barratt, D. T., Coller, J. K., Hallinan, R., *et al.* (2012). "ABCB1 haplotype and OPRM1 118A > G genotype interaction in methadone maintenance treatment pharmacogenetics." Pharmacogenomics and personalized medicine **5**: 53-62.
735 Choi, S. W., Lam, D. M. H., Wong, S. S. C., *et al.* (2017). "Effects of Single Nucleotide Polymorphisms on Surgical and Postsurgical Opioid Requirements: A Systematic Review and Meta-Analysis." Clin J Pain **33**(12): 1117-1130.
736 Kowarik, M. C., Einhauser, J., Jochim, B., *et al.* (2012). "Impact of the COMT Val(108/158)Met polymorphism on the mu-opioid receptor system in the human brain: mu-opioid receptor, met-enkephalin and beta-endorphin expression." Neurosci Lett **506**(2): 214-219.
737 Campa, D., Gioia, A., Tomei, A., *et al.* (2008). "Association of ABCB1/MDR1 and OPRM1 gene polymorphisms with morphine pain relief." Clin Pharmacol Ther **83**(4): 559-566.
738 Nielsen, L. M., Olesen, A. E., Branford, R., *et al.* (2015). "Association Between Human Pain-Related Genotypes and Variability in Opioid Analgesia: An Updated Review." Pain Pract **15**(6): 580-594.
739 Ho, K. W. D., Wallace, M. R., Staud, R., *et al.* (2020). "OPRM1, OPRK1, and COMT genetic polymorphisms associated with opioid effects on experimental pain: a randomized, double-blind, placebo-controlled study." Pharmacogenomics J **20**(3): 471-481.
740 Hwang, I. C., Park, J. Y., Myung, S. K., *et al.* (2014). "OPRM1 A118G gene variant and postoperative opioid requirement: a systematic review and meta-analysis." Anesthesiology **121**(4): 825-834.
741 Ren, Z. Y., Xu, X. Q., Bao, Y. P., *et al.* (2015). "The impact of genetic variation on sensitivity to opioid analgesics in patients with postoperative pain: a systematic review and meta-analysis." Pain Physician **18**(2): 131-152.
742 Saiz-Rodriguez, M., Ochoa, D., Herrador, C., *et al.* (2019). "Polymorphisms associated with fentanyl pharmacokinetics, pharmacodynamics and adverse effects." Basic Clin Pharmacol Toxicol **124**(3): 321-329.
743 Lie, Y. S. and Petropoulos, C. J. (1998). "Advances in quantitative PCR technology: 5' nuclease assays." Curr Opin Biotechnol **9**(1): 43-48.
744 Holland, P. M., Abramson, R. D., Watson, R., *et al.* (1991). "Detection of specific polymerase chain reaction product by utilizing the 5'----3' exonuclease activity of Thermus aquaticus DNA polymerase." Proc Natl Acad Sci U S A **88**(16): 7276-7280.
745 Gangisetty, O. and Reddy, D. S. (2009). "The optimization of TaqMan real-time RT-PCR assay for transcriptional profiling of GABA-A receptor subunit plasticity." J Neurosci Methods **181**(1): 58-66.
746 Little, J., Higgins, J. P., Ioannidis, J. P., *et al.* (2009). "STrengthening the REporting of Genetic Association Studies (STREGA)—an extension of the STROBE statement." Genetic Epidemiology: The Official Publication of the International Genetic Epidemiology Society **33**(7): 581-598.
747 Lahiri, D. K. and Nurnberger, J. I., Jr. (1991). "A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies." Nucleic Acids Res **19**(19): 5444.
748 Mokone, G. G., Schwellnus, M. P., Noakes, T. D., *et al.* (2006). "The COL5A1 gene and Achilles tendon pathology." Scand J Med Sci Sports **16**(1): 19-26.
749 Tengrup, I., Tennvall-Nittby, L., Christiansson, I., *et al.* (2000). "Arm morbidity after breast-conserving therapy for breast cancer." Acta Oncol **39**(3): 393-397.

750 MacDermid, J. C., Solomon, P. and Prkachin, K. (2006). "The Shoulder Pain and Disability Index demonstrates factor, construct and longitudinal validity." BMC Musculoskelet Disord **7**(1): 12.
751 Mafu, T. S., September, A. V. and Shamley, D. (2019). "KDR inferred haplotype is associated with upper limb dysfunction in breast cancer survivors of mixed ancestry." Cancer Manag Res **11**: 3829-3845.
752 Roy, J. S., MacDermid, J. C. and Woodhouse, L. J. (2009). "Measuring shoulder function: a systematic review of four questionnaires." Arthritis Rheum **61**(5): 623-632.
753 Hill, C. L., Lester, S., Taylor, A. W.*, et al.* (2011). "Factor structure and validity of the shoulder pain and disability index in a population-based study of people with shoulder symptoms." BMC Musculoskelet Disord **12**(1): 8.
754 Zigmond, A. S. and Snaith, R. P. (1983). "The hospital anxiety and depression scale." Acta Psychiatr Scand **67**(6): 361-370.
755 Bocerean, C. and Dupret, E. (2014). "A validation study of the Hospital Anxiety and Depression Scale (HADS) in a large sample of French employees." Bmc Psychiatry **14**(1): 354.
756 Bjelland, I., Dahl, A. A., Haug, T. T.*, et al.* (2002). "The validity of the Hospital Anxiety and Depression Scale. An updated literature review." J Psychosom Res **52**(2): 69-77.
757 Olsson, I., Mykletun, A. and Dahl, A. A. (2005). "The Hospital Anxiety and Depression Rating Scale: a cross-sectional study of psychometrics and case finding abilities in general practice." Bmc Psychiatry **5**(1): 46.
758 Poole, N. A. and Morgan, J. F. (2006). "Validity and reliability of the Hospital Anxiety and Depression Scale in a hypertrophic cardiomyopathy clinic: the HADS in a cardiomyopathy population." Gen Hosp Psychiatry **28**(1): 55-58.
759 Hinz, A. and Brahler, E. (2011). "Normative values for the hospital anxiety and depression scale (HADS) in the general German population." J Psychosom Res **71**(2): 74-78.
760 Wiriyakijja, P., Porter, S., Fedele, S.*, et al.* (2020). "Validation of the HADS and PSS-10 and a cross-sectional study of psychological status in patients with recurrent aphthous stomatitis." J Oral Pathol Med **49**(3): 260-270.
761 Cassiani-Miranda, C. A., Scoppetta, O. and Cabanzo-Arenas, D. F. (2022). "Validity of the Hospital Anxiety and Depression Scale (HADS) in primary care patients in Colombia." Gen Hosp Psychiatry **74**: 102-109.
762 Watson, D., Clark, L. A. and Tellegen, A. (1988). "Development and validation of brief measures of positive and negative affect: the PANAS scales." J Pers Soc Psychol **54**(6): 1063-1070.
763 Diaz-Garcia, A., Gonzalez-Robles, A., Mor, S.*, et al.* (2020). "Positive and Negative Affect Schedule (PANAS): psychometric properties of the online Spanish version in a clinical sample with emotional disorders." Bmc Psychiatry **20**(1): 56.
764 Lossio-Ventura, J. A., Song, W., Sainlaire, M.*, et al.* (2022). "Opioid2MME: Standardizing opioid prescriptions to morphine milligram equivalents from electronic health records." Int J Med Inform **162**: 104739.
765 Chen, J., Lipska, B. K., Halim, N.*, et al.* (2004). "Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain." Am J Hum Genet **75**(5): 807-821.
766 Iwersen-Bergmann, S., Plattner, S., Hischke, S.*, et al.* (2021). "Brain/blood ratios of methadone and ABCB1 polymorphisms in methadone-related deaths." Int J Legal Med **135**(2): 473-482.
767 Hui, L., DelMonte, T. and Ranade, K. (2008). "Genotyping using the TaqMan assay." Current protocols in human genetics **Chapter 2**: Unit 2 10.

768 Yu, H., Bai, L., Tang, G., *et al.* (2019). "Novel Assay for Quantitative Analysis of DNA Methylation at Single-Base Resolution." Clin Chem **65**(5): 664-673.
769 Kramer, N., Ramjith, J. and Shamley, D. (2019). "Prevalence of shoulder morbidity after treatment for breast Cancer in South Africa." Support Care Cancer **27**(7): 2591-2598.
770 Kadam, P. and Bhalerao, S. (2010). "Sample size calculation." Int J Ayurveda Res **1**(1): 55-57.
771 Arndt, V., Stegmaier, C., Ziegler, H., *et al.* (2006). "A population-based study of the impact of specific symptoms on quality of life in women with breast cancer 1 year after diagnosis." Cancer **107**(10): 2496-2503.
772 Dahl, A. A., Nesvold, I. L., Reinertsen, K. V., *et al.* (2011). "Arm/shoulder problems and insomnia symptoms in breast cancer survivors: cross-sectional, controlled and longitudinal observations." Sleep Med **12**(6): 584-590.
773 Dantas de Oliveira, N. P., Guedes, T. S., Holanda, A. M., *et al.* (2017). "Functional Disability in Women Submitted to Breast Cancer Treatment." Asian Pac J Cancer Prev **18**(5): 1207-1214.
774 Langford, D. J., Paul, S. M., West, C., *et al.* (2014). "Persistent breast pain following breast cancer surgery is associated with persistent sensory changes, pain interference, and functional impairments." J Pain **15**(12): 1227-1237.
775 Gartner, R., Jensen, M. B., Nielsen, J., *et al.* (2009). "Prevalence of and factors associated with persistent pain following breast cancer surgery." Jama **302**(18): 1985-1992.
776 Engel, J., Kerr, J., Schlesinger-Raab, A., *et al.* (2003). "Axilla surgery severely affects quality of life: results of a 5-year prospective study in breast cancer patients." Breast Cancer Res Treat **79**(1): 47-57.
777 Lauridsen, M. C., Overgaard, M., Overgaard, J., *et al.* (2008). "Shoulder disability and late symptoms following surgery for early breast cancer." Acta Oncol **47**(4): 569-575.
778 Gauderman, W. J. (2002). "Sample size requirements for matched case-control studies of gene-environment interaction." Stat Med **21**(1): 35-50.
779 Gauderman, W. and Morrison, J. (2006). QUANTO 1.1: A computer program for power and sample size calculations for genetic-epidemiology studies, http://hydra.usc.edu/gxe.
780 Quanto (2009). "Quanto [Computer Program]. Version 1. 2. 4. ."
781 Prism, G. (2020). "GraphPad Prism." GraphPad Software Inc.
782 Dell (2016). "Inc. Dell Statistica (Data Analysis Software System) [Computer Program]. Version 13." Online at: www. statsoft. com.
783 Team, R. d. C. (2013). "R: A language and environment for statistical computing."
784 Warnes, G., Leisch, F., Man, M., *et al.* (2012). "Package 'genetics'." Rochester, NY.
785 González, J., Armengol, L., Guinó, E., *et al.* (2014). "SNPassoc: SNPs-based whole genome association studies." R package version **1**: 2-9.
786 Kim, H., Mittal, D. P., Iadarola, M. J., *et al.* (2006). "Genetic predictors for acute experimental cold and heat pain sensitivity in humans." J Med Genet **43**(8): e40.
787 Kambur, O., Kaunisto, M. A., Tikkanen, E., *et al.* (2013). "Effect of catechol-o-methyltransferase-gene (COMT) variants on experimental and acute postoperative pain in 1,000 women undergoing surgery for breast cancer." Anesthesiology **119**(6): 1422-1433.
788 Bortsov, A. V., Diatchenko, L. and McLean, S. A. (2014). "Complex multilocus effects of catechol-O-methyltransferase haplotypes predict pain and pain interference 6 weeks after motor vehicle collision." Neuromolecular Med **16**(1): 83-93.

789 Lin, C. H., Chaudhuri, K. R., Fan, J. Y., *et al.* (2017). "Depression and Catechol-O-methyltransferase (COMT) genetic variants are associated with pain in Parkinson's disease." Sci Rep **7**(1): 6306.
790 Schaid, D. J., Rowland, C. M., Tines, D. E., *et al.* (2002). "Score tests for association between traits and haplotypes when linkage phase is ambiguous." Am J Hum Genet **70**(2): 425-434.
791 Sinnwell, J. P. and Schaid, D. (2011). "Statistical methods for haplotypes when linkage phase is ambiguous."
792 Vaser, R., Adusumalli, S., Leng, S. N., *et al.* (2016). "SIFT missense predictions for genomes." Nat Protoc **11**(1): 1-9.
793 Ng, P. C. and Henikoff, S. (2001). "Predicting deleterious amino acid substitutions." Genome Res **11**(5): 863-874.
794 Ng, P. C. and Henikoff, S. (2003). "SIFT: Predicting amino acid changes that affect protein function." Nucleic acids research **31**(13): 3812-3814.
795 Abrahams-October, Z., Johnson, R., Benjeddou, M., *et al.* (2022). "The determination of the effect(s) of solute carrier family 22-member 2 (SLC22A2) haplotype variants on drug binding via molecular dynamic simulation systems." Sci Rep **12**(1): 16936.
796 Adzhubei, I. A., Schmidt, S., Peshkin, L., *et al.* (2010). "A method and server for predicting damaging missense mutations." Nat Methods **7**(4): 248-249.
797 Adzhubei, I., Jordan, D. M. and Sunyaev, S. R. (2013). "Predicting functional effect of human missense mutations using PolyPhen-2." Current protocols in human genetics **Chapter 7**(1): Unit7 20.
798 Shihab, H. A., Gough, J., Cooper, D. N., *et al.* (2013). "Predicting the functional, molecular, and phenotypic consequences of amino acid substitutions using hidden Markov models." Hum Mutat **34**(1): 57-65.
799 Shihab, H. A., Rogers, M. F., Gough, J., *et al.* (2015). "An integrative approach to predicting the functional effects of non-coding and coding sequence variation." Bioinformatics **31**(10): 1536-1543.
800 Warde-Farley, D., Donaldson, S. L., Comes, O., *et al.* (2010). "The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function." Nucleic Acids Res **38**(Web Server issue): W214-220.
801 Xie, Z., Bailey, A., Kuleshov, M. V., *et al.* (2021). "Gene Set Knowledge Discovery with Enrichr." Curr Protoc **1**(3): e90.
802 Skorpen, F., Laugsand, E. A., Klepstad, P., *et al.* (2008). "Variable response to opioid treatment: any genetic predictors within sight?" Palliat Med **22**(4): 310-327.
803 Weesie, Y. M., Hek, K., Schermer, T. R. J., *et al.* (2020). "Use of Opioids Increases With Age in Older Adults: An Observational Study (2005-2017)." Front Pharmacol **11**: 648.
804 Stokes, A., Berry, K. M., Collins, J. M., *et al.* (2019). "The contribution of obesity to prescription opioid use in the United States." Pain **160**(10): 2255-2262.
805 Kim, Y. S., Kang, K. H. and Lee, H. J. (2020). "Factors Related to Pain in Patients With Retorn Rotator Cuffs: Early Postoperative Pain Predicts Pain at 12 Months Postoperatively." Orthop J Sports Med **8**(9): 2325967120947414.
806 De Cosmo, G., Congedo, E., Lai, C., *et al.* (2008). "Preoperative psychologic and demographic predictors of pain perception and tramadol consumption using intravenous patient-controlled analgesia." Clin J Pain **24**(5): 399-405.
807 Ghoneim, M. M. and O'Hara, M. W. (2016). "Depression and postoperative complications: an overview." BMC Surg **16**(1): 5.
808 Ni, K., Zhu, J. and Ma, Z. (2023). "Preoperative anxiety and postoperative adverse events: a narrative overview." Anesthesiology and Perioperative Science **1**(3): 23.

809 Taenzer, P., Melzack, R. and Jeans, M. E. (1986). "Influence of psychological factors on postoperative pain, mood and analgesic requirements." Pain **24**(3): 331-342.
810 Seebach, C. L., Kirkhart, M., Lating, J. M.*, et al.* (2012). "Examining the role of positive and negative affect in recovery from spine surgery." Pain **153**(3): 518-525.
811 Barry, D. T., Irwin, K. S., Jones, E. S.*, et al.* (2010). "Opioids, Chronic Pain, and Addiction in Primary Care." Journal of Pain **11**(12): 1442-1450.
812 Martel, M. O., Petersen, K., Cornelius, M.*, et al.* (2019). "Endogenous Pain Modulation Profiles Among Individuals With Chronic Pain: Relation to Opioid Use." Journal of Pain **20**(4): 462-471.
813 Al-Mahrezi, A. and Al-Shidhani, A. (2018). Is Chronic Post-Surgical Pain Preventable? Pain Management in Special Circumstances, IntechOpen.
814 Graham, G. G., Davies, M. J., Day, R. O.*, et al.* (2013). "The modern pharmacology of paracetamol: therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings." Inflammopharmacology **21**(3): 201-232.
815 Graham, G. G. and Scott, K. F. (2005). "Mechanism of action of paracetamol." Am J Ther **12**(1): 46-55.
816 Nesvold, I. L., Dahl, A. A., Lokkevik, E.*, et al.* (2008). "Arm and shoulder morbidity in breast cancer patients after breast-conserving therapy versus mastectomy." Acta Oncol **47**(5): 835-842.
817 Doong, S. H., Dhruva, A., Dunn, L. B.*, et al.* (2015). "Associations between cytokine genes and a symptom cluster of pain, fatigue, sleep disturbance, and depression in patients prior to breast cancer surgery." Biol Res Nurs **17**(3): 237-247.
818 Riley III, J. L., Wade, J. B., Robinson, M. E.*, et al.* (2000). "The stages of pain processing across the adult lifespan." The Journal of pain **1**(2): 162-170.
819 Ackerman, I. N., Page, R. S., Fotis, K.*, et al.* (2018). "Exploring the personal burden of shoulder pain among younger people in Australia: protocol for a multicentre cohort study." BMJ open **8**(7): e021859.
820 Vargas, C., Bilbeny, N., Balmaceda, C.*, et al.* (2018). "Costs and consequences of chronic pain due to musculoskeletal disorders from a health system perspective in Chile." Pain Rep **3**(5): e656.
821 Mullins, S., Hosseini, F., Gibson, W.*, et al.* (2022). "Physiological changes from ageing regarding pain perception and its impact on pain management for older adults." Clin Med (Lond) **22**(4): 307-310.
822 Jones, M. R., Ehrhardt, K. P., Ripoll, J. G.*, et al.* (2016). "Pain in the elderly." Current Pain and Headache Reports **20**: 1-9.
823 González-Roldán, A. M., Terrasa, J. L., Sitges, C.*, et al.* (2020). "Age-related changes in pain perception are associated with altered functional connectivity during resting state." Frontiers in Aging Neuroscience **12**: 116.
824 Gerstein, A. D., Phillips, T. J., Rogers, G. S.*, et al.* (1993). "Wound healing and aging." Dermatologic clinics **11**(4): 749-757.
825 Gordon, A. and Alsayouri, K. (2019). "Anatomy, Shoulder and Upper Limb, Axilla."
826 Gherghe, M., Bordea, C. and Blidaru, A. (2015). "Sentinel lymph node biopsy (SLNB) vs. axillary lymph node dissection (ALND) in the current surgical treatment of early stage breast cancer." Journal of medicine and life **8**(2): 176-180.
827 Dias, D., Neto, M. G., Sales, S. d. S. R.*, et al.* (2023). "Effect of Mobilization with Movement on Pain, Disability, and Range of Motion in Patients with Shoulder Pain and Movement Impairment: A Systematic Review and Meta-Analysis." Journal of Clinical Medicine **12**(23): 7416.
828 Major, D. H., Røe, Y., Småstuen, M. C.*, et al.* (2022). "Fear of movement and emotional distress as prognostic factors for disability in patients with shoulder pain: a prospective cohort study." Bmc Musculoskeletal Disorders **23**(1): 183.

829 Özden, F., Tuğay, N., Karaman, Ö. N.*, et al.* (2021). "The relationship of fear of movement with pain, range of motion and function in patients with shoulder pathologies." Bulletin of Faculty of Physical Therapy **26**(1): 2.
830 Persson, A. K. M., Pettersson, F. D. and Akeson, J. (2018). "Single Nucleotide Polymorphisms Associated with Pain Sensitivity After Laparoscopic Cholecystectomy." Pain Med **19**(6): 1271-1279.
831 Tanabe, Y., Shimizu, C., Hamada, A.*, et al.* (2017). "Paclitaxel-induced sensory peripheral neuropathy is associated with an ABCB1 single nucleotide polymorphism and older age in Japanese." Cancer Chemother Pharmacol **79**(6): 1179-1186.
832 Hajj, A., Peoc'h, K., Laplanche, J. L.*, et al.* (2015). "Genotyping test with clinical factors: better management of acute postoperative pain?" Int J Mol Sci **16**(3): 6298-6311.
833 Wang, D. and Sadee, W. (2006). "Searching for polymorphisms that affect gene expression and mRNA processing: example ABCB1 (MDR1)." AAPS J **8**(3): E515-520.
834 Zappe, K. and Cichna-Markl, M. (2020). "Aberrant DNA Methylation of ABC Transporters in Cancer." Cells **9**(10).
835 Wu, L. X., Wen, C. J., Li, Y.*, et al.* (2015). "Interindividual epigenetic variation in ABCB1 promoter and its relationship with ABCB1 expression and function in healthy Chinese subjects." British journal of clinical pharmacology **80**(5): 1109-1121.
836 Reed, K., Hembruff, S. L., Sprowl, J. A.*, et al.* (2010). "The temporal relationship between ABCB1 promoter hypomethylation, ABCB1 expression and acquisition of drug resistance." The Pharmacogenomics Journal **10**(6): 489-504.
837 Scotto, K. W. (2003). "Transcriptional regulation of ABC drug transporters." Oncogene **22**(47): 7496-7511.
838 Goldstein, L. J., Galski, H., Fojo, A.*, et al.* (1989). "Expression of multidrug resistance gene in human cancers." JNCI: Journal of the National Cancer Institute **81**(2): 116-124.
839 Goldstein, L. (1996). "MDR1 gene expression in solid tumours." European Journal of Cancer **32**(6): 1039-1050.
840 Pan, S. T., Li, Z. L., He, Z. X.*, et al.* (2016). "Molecular mechanisms for tumour resistance to chemotherapy." Clinical and Experimental Pharmacology and Physiology **43**(8): 723-737.
841 Christie, E. L., Pattnaik, S., Beach, J.*, et al.* (2019). "Multiple ABCB1 transcriptional fusions in drug resistant high-grade serous ovarian and breast cancer." Nature communications **10**(1): 1295.
842 Gomes, B. C., Honrado, M., Armada, A.*, et al.* (2020). "ABC efflux transporters and the circuitry of miRNAs: kinetics of expression in cancer drug resistance." International Journal of Molecular Sciences **21**(8): 2985.
843 Anthony, V. and Skach, W. R. (2002). "Molecular mechanism of P-glycoprotein assembly into cellular membranes." Curr Protein Pept Sci **3**(5): 485-501.
844 Kimchi-Sarfaty, C., Oh, J. M., Kim, I. W.*, et al.* (2007). "A "silent" polymorphism in the MDR1 gene changes substrate specificity." Science **315**(5811): 525-528.
845 Sauna, Z. E., Kimchi-Sarfaty, C., Ambudkar, S. V.*, et al.* (2007). "Silent polymorphisms speak: how they affect pharmacogenomics and the treatment of cancer." Cancer research **67**(20): 9609-9612.
846 Shen, L. X., Basilion, J. P. and Stanton, V. P., Jr. (1999). "Single-nucleotide polymorphisms can cause different structural folds of mRNA." Proc Natl Acad Sci U S A **96**(14): 7871-7876.
847 Duan, J., Wainwright, M. S., Comeron, J. M.*, et al.* (2003). "Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor." Hum Mol Genet **12**(3): 205-216.

848 Ding, Y., Chan, C. Y. and Lawrence, C. E. (2004). "Sfold web server for statistical folding and rational design of nucleic acids." Nucleic Acids Res **32**(Web Server issue): W135-141.
849 Spitale, R. C. and Incarnato, D. (2023). "Probing the dynamic RNA structurome and its functions." Nature Reviews Genetics **24**(3): 178-196.
850 de la Peña, J. B. I., Song, J. J. and Campbell, Z. T. (2019). "RNA control in pain: Blame it on the messenger." Wiley Interdiscip Rev RNA **10**(6): e1546.
851 Bartosova, O., Polanecky, O., Sachl, R.*, et al.* (2019). "Epidural analgesia with sufentanil in relation to OPRM1 and ABCB1 polymorphisms." Physiol Res **68**(Suppl 1): S59-S64.
852 Chou, W. Y., Yang, L. C., Lu, H. F.*, et al.* (2006). "Association of mu-opioid receptor gene polymorphism (A118G) with variations in morphine consumption for analgesia after total knee arthroplasty." Acta Anaesthesiol Scand **50**(7): 787-792.
853 Olsen, M. B., Jacobsen, L. M., Schistad, E. I.*, et al.* (2012). "Pain intensity the first year after lumbar disc herniation is associated with the A118G polymorphism in the opioid receptor mu 1 gene: evidence of a sex and genotype interaction." J Neurosci **32**(29): 9831-9834.
854 Tan, E.-c., Lim, E. C., Teo, Y.-y.*, et al.* (2009). "Ethnicity and OPRM variant independently predict pain perception and patient-controlled analgesia usage for post-operative pain." Molecular Pain **5**: 1744-8069-1745-1732.
855 Walter, C. and Lotsch, J. (2009). "Meta-analysis of the relevance of the OPRM1 118A>G genetic variant for pain treatment." Pain **146**(3): 270-275.
856 Chatti, I., Creveaux, I., Woillard, J. B.*, et al.* (2016). "Association of the OPRM1 and COMT genes' polymorphisms with the efficacy of morphine in Tunisian cancer patients: Impact of the high genetic heterogeneity in Tunisia?" Therapie **71**(5): 507-513.
857 De Gregori, M., Garbin, G., De Gregori, S.*, et al.* (2013). "Genetic variability at COMT but not at OPRM1 and UGT2B7 loci modulates morphine analgesic response in acute postoperative pain." Eur J Clin Pharmacol **69**(9): 1651-1658.
858 Befort, K., Filliol, D., Decaillot, F. M.*, et al.* (2001). "A single nucleotide polymorphic mutation in the human mu-opioid receptor severely impairs receptor signaling." J Biol Chem **276**(5): 3130-3137.
859 Landau, R. (2006). "One size does not fit all: genetic variability of μ-opioid receptor and postoperative morphine consumption." The Journal of the American Society of Anesthesiologists **105**(2): 235-237.
860 Hastie, B. A., Riley, J. L., 3rd, Kaplan, L.*, et al.* (2012). "Ethnicity interacts with the OPRM1 gene in experimental pain sensitivity." Pain **153**(8): 1610-1619.
861 Trikudanathan, G., Safo, S., Abu-El-Haija, M.*, et al.* (2021). "PANCREATITIS AND OPIOID GENE VARIANTS ARE ASSOCIATED WITH PREOPERATIVE OPIOID USE: PRELIMINARY DATA FROM THE POST COHORT." Gastroenterology **160**(6): S299-S299.
862 Wang, Y., Tan, Z., Wu, L.*, et al.* (2016). "Role of OPRM1, ABCB1 and CYP3A genetic polymorphisms on sufentanil treatment of postoperative cancer patients in China." International journal of clinical and experimental medicine **9**(7): 13250-13258.
863 Lopez Soto, E. J. and Catanesi, C. I. (2015). "Human population genetic structure detected by pain-related mu opioid receptor gene polymorphisms." Genet Mol Biol **38**(2): 152-155.
864 Sandoval-Sierra, J. V., Salgado García, F. I., Brooks, J. H.*, et al.* (2020). "Effect of short-term prescription opioids on DNA methylation of the OPRM1 promoter." Clinical Epigenetics **12**(1): 76.

865 Sheikh, S., Smotherman, C., Patel, M.*, et al.* (2022). "Characterizing OPRM1 DNA methylation in prescription opioid users with chronic musculoskeletal pain." Pain Rep **7**(6): e1046.
866 Polli, A., Godderis, L., Ghosh, M.*, et al.* (2020). "Epigenetic and miRNA Expression Changes in People with Pain: A Systematic Review." The Journal of pain **21**(7): 763-780.
867 Vidic, Z., Goricar, K., Strazisar, B.*, et al.* (2023). "Association of OPRM1, MIR23B, and MIR107 genetic variability with acute pain, chronic pain and adverse effects after postoperative tramadol and paracetamol treatment in breast cancer." Radiol Oncol **57**(1): 111-120.
868 Bergen, A. W., Kokoszka, J., Peterson, R.*, et al.* (1997). "Mu opioid receptor gene variants: lack of association with alcohol dependence." Mol Psychiatry **2**(6): 490-494.
869 Bond, C., LaForge, K. S., Tian, M.*, et al.* (1998). "Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction." Proc Natl Acad Sci U S A **95**(16): 9608-9613.
870 Lotsch, J., Zimmermann, M., Darimont, J.*, et al.* (2002). "Does the A118G polymorpbism at the mu-opioid receptor gene protect against morphine-6-glucuronide toxicity?" Anesthesiology **97**(4): 814-819.
871 Lotsch, J., Stuck, B. and Hummel, T. (2006). "The human mu-opioid receptor gene polymorphism 118A > G decreases cortical activation in response to specific nociceptive stimulation." Behav Neurosci **120**(6): 1218-1224.
872 Fernandez-de-las-Penas, C., Ambite-Quesada, S., Ortega-Santiago, R.*, et al.* (2013). "Catechol-O-Methyltransferase Val158Met Polymorphism is Associated with Pain and Disability, but not Widespread Pressure Pain Sensitivity, in Women with Carpal Tunnel Syndrome." Pain Physician **16**(5): E591-E600.
873 Henker, R. A., Lewis, A., Dai, F.*, et al.* (2013). "The associations between OPRM 1 and COMT genotypes and postoperative pain, opioid use, and opioid-induced sedation." Biol Res Nurs **15**(3): 309-317.
874 Hu, B., Zhang, X., Xu, G.*, et al.* (2018). "Association between COMT Polymorphism Val158Met and Opioid Consumption in Patients with Postoperative Pain: A Meta-Analysis." Neurosignals **26**(1): 11-21.
875 Omair, A., Lie, B. A., Reikeras, O.*, et al.* (2012). "Genetic contribution of catechol-O-methyltransferase variants in treatment outcome of low back pain: a prospective genetic association study." Bmc Musculoskeletal Disorders **13**(1): 1-9.
876 Rut, M., Machoy-Mokrzynska, A., Reclawowicz, D.*, et al.* (2014). "Influence of variation in the catechol-O-methyltransferase gene on the clinical outcome after lumbar spine surgery for one-level symptomatic disc disease: a report on 176 cases." Acta Neurochir (Wien) **156**(2): 245-252.
877 Polli, A., Hendrix, J., Ickmans, K.*, et al.* (2022). "Genetic and epigenetic regulation of Catechol-O-methyltransferase in relation to inflammation in chronic fatigue syndrome and Fibromyalgia." J Transl Med **20**(1): 487.
878 Chimusa, E. R., Meintjies, A., Tchanga, M.*, et al.* (2015). "A genomic portrait of haplotype diversity and signatures of selection in indigenous southern African populations." PLoS Genet **11**(3): e1005052.
879 Pfennig, A., Petersen, L. N., Kachambwa, P.*, et al.* (2023). "Evolutionary Genetics and Admixture in African Populations." Genome Biol Evol **15**(4).
880 Gaudet, M. M., Bensen, J. T., Schroeder, J.*, et al.* (2006). "Catechol-O-methyltransferase haplotypes and breast cancer among women on Long Island, New York." Breast Cancer Res Treat **99**(2): 235-240.

881 Qin, X., Peng, Q., Qin, A.*, et al.* (2012). "Association of COMT Val158Met polymorphism and breast cancer risk: an updated meta-analysis." Diagn Pathol **7**(1): 136.
882 Kumar, P., Singh, G. and Rai, V. (2020). "Evaluation of COMT Gene rs4680 Polymorphism as a Risk Factor for Endometrial Cancer." Indian J Clin Biochem **35**(1): 63-71.
883 Peterson, N. B., Trentham-Dietz, A., Garcia-Closas, M.*, et al.* (2010). "Association of COMT haplotypes and breast cancer risk in caucasian women." Anticancer Res **30**(1): 217-220.
884 Wang, G. S. and Cooper, T. A. (2007). "Splicing in disease: disruption of the splicing code and the decoding machinery." Nat Rev Genet **8**(10): 749-761.
885 Matic, M., Simons, S. H., van Lingen, R. A.*, et al.* (2014). "Rescue morphine in mechanically ventilated newborns associated with combined OPRM1 and COMT genotype." Pharmacogenomics **15**(10): 1287-1295.
886 Colloca, L., Wang, Y., Martinez, P. E.*, et al.* (2019). "OPRM1 rs1799971, COMT rs4680, and FAAH rs324420 genes interact with placebo procedures to induce hypoalgesia." Pain **160**(8): 1824-1834.
887 Gilbert-Diamond, D. and Moore, J. H. (2011). "Analysis of gene-gene interactions." Current protocols in human genetics **Chapter 1**: Unit1 14.
888 Tompkins, D. A. and Campbell, C. M. (2011). "Opioid-induced hyperalgesia: clinically relevant or extraneous research phenomenon?" Curr Pain Headache Rep **15**(2): 129-136.
889 Mercer, S. L. and Coop, A. (2011). "Opioid analgesics and P-glycoprotein efflux transporters: a potential systems-level contribution to analgesic tolerance." Current topics in medicinal chemistry **11**(9): 1157-1164.
890 Oude Elferink, R. P. and Paulusma, C. C. (2007). "Function and pathophysiological importance of ABCB4 (MDR3 P-glycoprotein)." Pflugers Arch **453**(5): 601-610.
891 Wilson, N. C., Choudhury, A., Carstens, N.*, et al.* (2017). "Organic Cation Transporter 2 (OCT2/SLC22A2) Gene Variation in the South African Bantu-Speaking Population and Functional Promoter Variants." Omics-a Journal of Integrative Biology **21**(3): 169-176.
892 Freissmuth, M., Casey, P. J. and Gilman, A. G. (1989). "G proteins control diverse pathways of transmembrane signaling." Faseb j **3**(10): 2125-2131.
893 Lohmann, K., Masuho, I., Patil, D. N.*, et al.* (2017). "Novel GNB1 mutations disrupt assembly and function of G protein heterotrimers and cause global developmental delay in humans." Hum Mol Genet **26**(6): 1078-1086.
894 Ngo Thai Bich, V., Hongu, T., Miura, Y.*, et al.* (2018). "Physiological function of phospholipase D2 in anti-tumor immunity: regulation of CD8(+) T lymphocyte proliferation." Sci Rep **8**(1): 6283.
895 Lane, H. Y., Tsai, G. E. and Lin, E. (2012). "Assessing gene-gene interactions in pharmacogenomics." Mol Diagn Ther **16**(1): 15-27.
896 Tang, C., Deng, Y., Shao, S.*, et al.* (2023). "Long noncoding RNA UCA1 promotes the expression and function of P-glycoprotein by sponging miR-16-5p in human placental BeWo cells." Faseb j **37**(1): e22657.
897 Hou, W., Li, H., Jiang, W.*, et al.* (2016). "Simian Immunodeficiency Virus Impacts MicroRNA-16 Mediated Post-Transcriptional Regulation of mu Opioid Receptor in CEM x174 Cells." J Cell Biochem **117**(1): 84-93.
898 Yang, C., Mai, H., Peng, J.*, et al.* (2020). "STAT4: an immunoregulator contributing to diverse human diseases." International journal of biological sciences **16**(9): 1575-1585.

899 Darnell, J. E., Jr., Kerr, I. M. and Stark, G. R. (1994). "Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins." Science **264**(5164): 1415-1421.
900 Yamamoto, K., Miura, O., Hirosawa, S.*, et al.* (1997). "Binding sequence of STAT4: STAT4 complex recognizes the IFN-gamma activation site (GAS)-like sequence (T/A)TTCC(C/G)GGAA(T/A)." Biochem Biophys Res Commun **233**(1): 126-132.
901 Speidel, J. T., Xu, M. and Abdel-Rahman, S. Z. (2018). "Promoter Haplotypes of the ABCB1 Gene Encoding the P-Glycoprotein Differentially Affect Its Promoter Activity by Altering Transcription Factor Binding." DNA Cell Biol **37**(12): 973-981.
902 Bali, A., Randhawa, P. K. and Jaggi, A. S. (2015). "Stress and opioids: role of opioids in modulating stress-related behavior and effect of stress on morphine conditioned place preference." Neurosci Biobehav Rev **51**: 138-150.
903 Collins, D., Randesi, M., da Rosa, J. C.*, et al.* (2018). "Oprm1 A112G, a single nucleotide polymorphism, alters expression of stress-responsive genes in multiple brain regions in male and female mice." Psychopharmacology (Berl) **235**(9): 2703-2711.
904 Ducat, E., Ray, B., Bart, G.*, et al.* (2013). "Mu-opioid receptor A118G polymorphism in healthy volunteers affects hypothalamic-pituitary-adrenal axis adrenocorticotropic hormone stress response to metyrapone." Addict Biol **18**(2): 325-331.
905 Lopez, J. P., Brivio, E., Santambrogio, A.*, et al.* (2021). "Single-cell molecular profiling of all three components of the HPA axis reveals adrenal ABCB1 as a regulator of stress adaptation." Sci Adv **7**(5): eabe4497.
906 Miller, W. L. (2018). "The Hypothalamic-Pituitary-Adrenal Axis: A Brief History." Horm Res Paediatr **89**(4): 212-223.
907 Lunde, C. E. and Sieberg, C. B. (2020). "Walking the Tightrope: A Proposed Model of Chronic Pain and Stress." Front Neurosci **14**: 270.
908 Chouchi, M., Klaa, H., Ben-Youssef Turki, I.*, et al.* (2019). "ABCB1 Polymorphisms and Drug-Resistant Epilepsy in a Tunisian Population." Dis Markers **2019**: 1343650.
909 Kannan, P., John, C., Zoghbi, S. S.*, et al.* (2009). "Imaging the function of P-glycoprotein with radiotracers: pharmacokinetics and in vivo applications." Clin Pharmacol Ther **86**(4): 368-377.
910 Hung, C. C., Chen, C. C., Lin, C. J.*, et al.* (2008). "Functional evaluation of polymorphisms in the human ABCB1 gene and the impact on clinical responses of antiepileptic drugs." Pharmacogenetics and genomics **18**(5): 390-402.
911 Ajmi, M., Boujaafar, S., Zouari, N.*, et al.* (2018). "Association between ABCB1 polymorphisms and response to first-generation antiepileptic drugs in a Tunisian epileptic population." Int J Neurosci **128**(8): 705-714.
912 Bartenstein, P. A., Duncan, J. S., Prevett, M. C.*, et al.* (1993). "Investigation of the opioid system in absence seizures with positron emission tomography." J Neurol Neurosurg Psychiatry **56**(12): 1295-1302.
913 Wang, J., Lin, Z. J., Liu, L.*, et al.* (2017). "Epilepsy-associated genes." Seizure **44**: 11-20.
914 Benavides, R., Vsevolozhskaya, O., Cattaneo, S.*, et al.* (2020). "A functional polymorphism in the ATP-Binding Cassette B1 transporter predicts pharmacologic response to combination of nortriptyline and morphine in neuropathic pain patients." Pain **161**(3): 619-629.
915 Hajj, A., Halepian, L., Osta, N. E.*, et al.* (2017). "OPRM1 c.118A>G Polymorphism and Duration of Morphine Treatment Associated with Morphine Doses and Quality-of-Life in Palliative Cancer Pain Settings." Int J Mol Sci **18**(4).
916 Lloyd, R. A., Hotham, E., Hall, C.*, et al.* (2017). "Pharmacogenomics and Patient Treatment Parameters to Opioid Treatment in Chronic Pain: A Focus on Morphine, Oxycodone, Tramadol, and Fentanyl." Pain Med **18**(12): 2369-2387.

917 Matic, M., Jongen, J. L., Elens, L.*, et al.* (2017). "Advanced cancer pain: the search for genetic factors correlated with interindividual variability in opioid requirement." Pharmacogenomics **18**(12): 1133-1142.
918 Rakvag, T. T., Ross, J. R., Sato, H.*, et al.* (2008). "Genetic variation in the catechol-O-methyltransferase (COMT) gene and morphine requirements in cancer patients with pain." Mol Pain **4**(1): 64.
919 Rakvåg, T. T., Klepstad, P., Baar, C.*, et al.* (2005). "The Val158Met polymorphism of the human catechol-O-methyltransferase (COMT) gene may influence morphine requirements in cancer pain patients." Pain **116**(1-2): 73-78.
920 Berthele, A., Platzer, S., Jochim, B.*, et al.* (2005). "COMT Val108/158Met genotype affects the mu-opioid receptor system in the human brain: evidence from ligand-binding, G-protein activation and preproenkephalin mRNA expression." NeuroImage **28**(1): 185-193.
921 Young, E. E., Kelly, D. L., Shim, I.*, et al.* (2017). "Variations in COMT and NTRK2 Influence Symptom Burden in Women Undergoing Breast Cancer Treatment." Biol Res Nurs **19**(3): 318-328.
922 Bu, G., Cui, L., Lv, C.*, et al.* (2020). "Opioid Peptides and Their Receptors in Chickens: Structure, Functionality, and Tissue Distribution." Peptides **128**: 170307.
923 Corbett, A. D., Henderson, G., McKnight, A. T.*, et al.* (2006). "75 years of opioid research: the exciting but vain quest for the Holy Grail." Br J Pharmacol **147 Suppl 1**(Suppl 1): S153-162.
924 McCarberg, B. and Peppin, J. (2019). "Pain Pathways and Nervous System Plasticity: Learning and Memory in Pain." Pain Med **20**(12): 2421-2437.
925 Kandel, E. R. (1991). "Nerve cells and behavior." Principles of neural science **3**: 18-32.
926 Reeves, K. C., Shah, N., Munoz, B.*, et al.* (2022). "Opioid Receptor-Mediated Regulation of Neurotransmission in the Brain." Front Mol Neurosci **15**: 919773.
927 Kelkar, P. and Parry, G. J. (2003). "Mononeuritis multiplex in diabetes mellitus: evidence for underlying immune pathogenesis." J Neurol Neurosurg Psychiatry **74**(6): 803-806.
928 Kadian, R., Shankar Kikkeri, N. and Kumar, A. (2024). Basilar Migraine. StatPearls. Treasure Island (FL), StatPearls Publishing

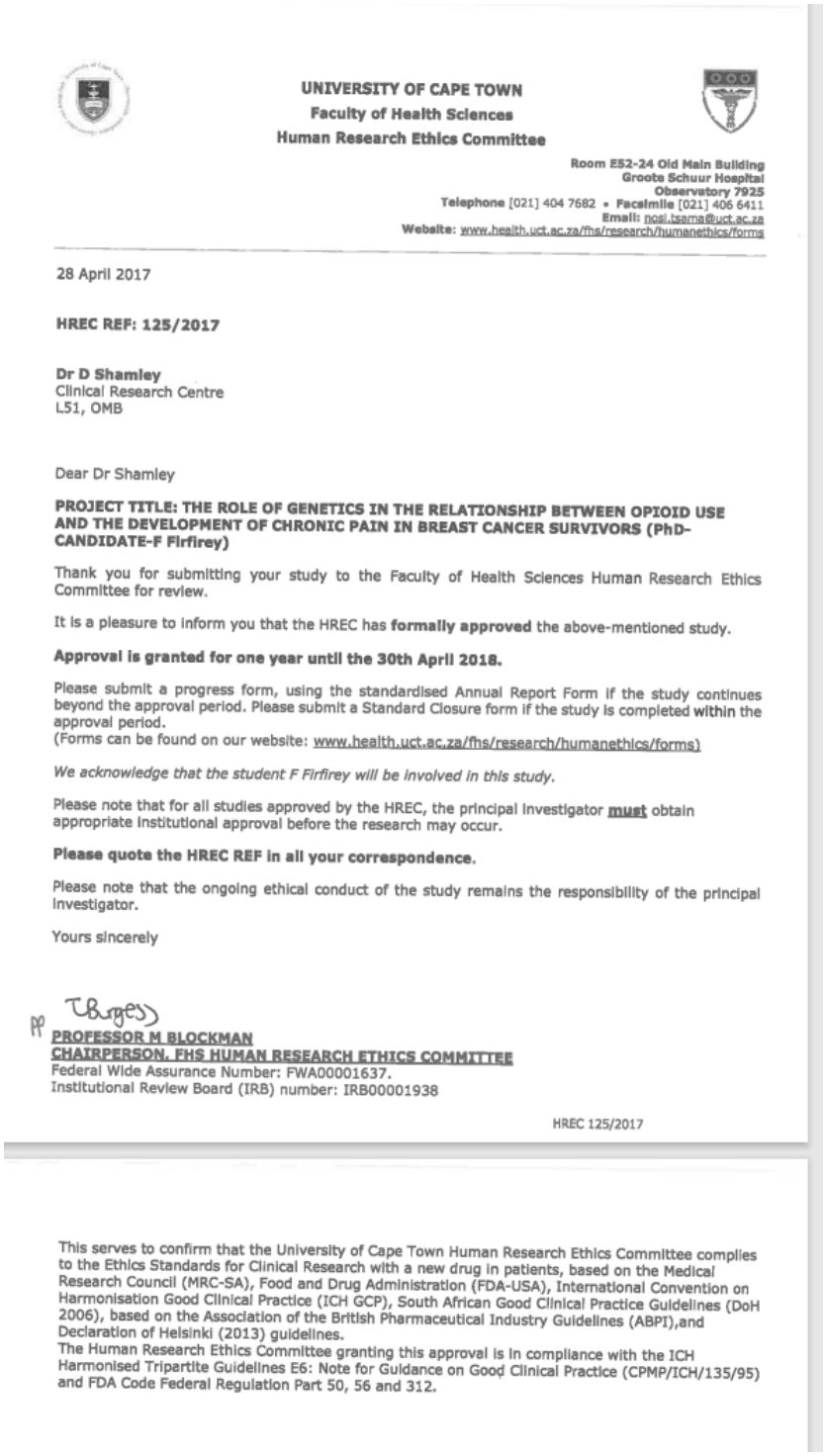
929 Leitão, M., Lopes, S., Pereira, D.*, et al.* (2022). "Genetic Polymorphisms as Predictors of Survival in Breast Cancer: Future Lessons in Historical Data." Cureus **14**(1): e21410.
930 Lee, M. G., Kim, H. J., Lee, K. H.*, et al.* (2016). "The Influence of Genotype Polymorphism on Morphine Analgesic Effect for Postoperative Pain in Children." Korean J Pain **29**(1): 34-39.
931 Baber, M., Chaudhry, S., Kelly, L.*, et al.* (2015). "The pharmacogenetics of codeine pain relief in the postpartum period." Pharmacogenomics J **15**(5): 430-435.
932 Gray, K., Adhikary, S. D. and Janicki, P. (2018). "Pharmacogenomics of analgesics in anesthesia practice: A current update of literature." J Anaesthesiol Clin Pharmacol **34**(2): 155-160.
933 Nishioka, M., Bundo, M., Koike, S.*, et al.* (2013). "Comprehensive DNA methylation analysis of peripheral blood cells derived from patients with first-episode schizophrenia." J Hum Genet **58**(2): 91-97.
934 Qian, Q. J., Yang, L., Wang, Y. F.*, et al.* (2010). "Gene-gene interaction between COMT and MAOA potentially predicts the intelligence of attention-deficit hyperactivity disorder boys in China." Behavior genetics **40**(3): 357-365.
935 Cockburn, D. M. (1991). "Tuberous sclerosis (Bourneville's disease): an illustrated review." Clinical and Experimental Optometry **74**(2): 49-50.

936 Boujelbene, N., Cosinschi, A., Boujelbene, N.*, et al.* (2011). "Pure seminoma: A review and update." Radiation Oncology **6**(1): 90.
937 Pont-Sunyer, C., Martí, M. J. and Tolosa, E. (2010). "Focal limb dystonia." Eur J Neurol **17 Suppl 1**: 22-27.
938 Yap, F. Y., Skalski, M. R., Patel, D. B.*, et al.* (2017). "Hypertrophic Osteoarthropathy: Clinical and Imaging Features." RadioGraphics **37**(1): 157-195.
939 Bellini, M. I., Lori, E., Forte, F.*, et al.* (2022). "Thyroid and renal cancers: A bidirectional association." Front Oncol **12**: 951976.
940 Curran, M. and Wiseman, L. (2001). "Fulvestrant." Drugs **61**(6): 807-813; discussion 814.
941 Vultaggio-Poma, V., Sarti, A. C. and Di Virgilio, F. (2020). "Extracellular ATP: A Feasible Target for Cancer Therapy." Cells **9**(11).
942 Chiu, Y. H., Hsu, C. Y., Lu, M. L.*, et al.* (2020). "Augmentation Strategies for Clozapine-Resistant Patients with Schizophrenia." Curr Pharm Des **26**(2): 218-227.
943 Lu, L., Zhang, J., Fan, W.*, et al.* (2021). "Deregulated 14-3-3zeta and methionine adenosyltransferase alpha1 interplay promotes liver cancer tumorigenesis in mice and humans." Oncogene **40**(39): 5866-5879.
944 Liu, N., Nelson, B. R., Bezprozvannaya, S.*, et al.* (2014). "Requirement of MEF2A, C, and D for skeletal muscle regeneration." Proc Natl Acad Sci U S A **111**(11): 4109-4114.
945 Zhu, B., Carmichael, R. E., Solabre Valois, L.*, et al.* (2018). "The transcription factor MEF2A plays a key role in the differentiation/maturation of rat neural stem cells into neurons." Biochem Biophys Res Commun **500**(3): 645-649.
946 Durham, J. T., Brand, O. M., Arnold, M.*, et al.* (2006). "Myospryn is a direct transcriptional target for MEF2A that encodes a striated muscle, α-actinin-interacting, costamere-localized protein." Journal of Biological Chemistry **281**(10): 6841-6849.
947 Wang, Y. N., Yang, W. C., Li, P. W.*, et al.* (2018). "Myocyte enhancer factor 2A promotes proliferation and its inhibition attenuates myogenic differentiation via myozenin 2 in bovine skeletal muscle myoblast." Plos One **13**(4): e0196255.
948 Wu, R., Wang, J., Yao, J.*, et al.* (2018). "MEF2A regulates Calpain 3 expression in L6 myoblasts." Gene **668**: 204-210.
949 Liu, B., Ou, W. C., Fang, L.*, et al.* (2023). "Myocyte Enhancer Factor 2A Plays a Central Role in the Regulatory Networks of Cellular Physiopathology." Aging Dis **14**(2): 331-349.
950 Chen, X., Gao, B., Ponnusamy, M.*, et al.* (2017). "MEF2 signaling and human diseases." Oncotarget **8**(67): 112152-112165.
951 Xiong, Y., Wang, L., Jiang, W.*, et al.* (2019). "MEF2A alters the proliferation, inflammation-related gene expression profiles and its silencing induces cellular senescence in human coronary endothelial cells." BMC Mol Biol **20**(1): 8.
952 Gupta, P. and Kumar, R. (2023). "GTP cyclohydroxylase1 (GCH1): Role in neurodegenerative diseases." Gene **888**: 147749.
953 Mnika, K., Pule, G. D., Dandara, C.*, et al.* (2016). "An Expert Review of Pharmacogenomics of Sickle Cell Disease Therapeutics: Not Yet Ready for Global Precision Medicine." Omics-a Journal of Integrative Biology **20**(10): 565-574.

7 APPENDICES

7.1 APPENDIX A: RESEARCH DOCUMENTATION

7.1.1 Ethical Approval and Renewal



UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 404 7682 • Facsimile [021] 406 6411
Email: nosi.tsama@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

28 April 2017

HREC REF: 125/2017

Dr D Shamley
Clinical Research Centre
L51, OMB

Dear Dr Shamley

PROJECT TITLE: THE ROLE OF GENETICS IN THE RELATIONSHIP BETWEEN OPIOID USE AND THE DEVELOPMENT OF CHRONIC PAIN IN BREAST CANCER SURVIVORS (PhD-CANDIDATE-F Firfirey)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th April 2018.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student F Firfirey will be involved in this study.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval before the research may occur.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

pp T Burgess
PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

HREC 125/2017

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI),and Declaration of Helsinki (2013) guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HUMAN RESEARCH ETHICS COMMITTEE
- 5 JUL 2023
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

UNIVERSITY OF CAPE TOWN
FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30/6/2024
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	5/7/2023

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)			
HREC REF Number	312/2012	Current Ethics Approval was granted until	30/06/2023
Protocol title	Correlating clinical disease state of the shoulder after treatment for breast cancer with biomarkers of inflammation and angiogenesis and their associated genetic variants.		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?		✓ Yes	☐ No
If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.		HREC359/2019 HREC125/2017	
Principal Investigator	A/Prof Delva Shamley		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	28/02/24
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	8/2/2023

HUMAN RESEARCH ETHICS COMMITTEE
08 FEB 2023
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	27 January 2023		
HREC REF Number	125/2017	Current Ethics Approval was granted until	28/02/2023
Protocol title	The role of genetics in the relationship between opioid use and the development of chronic pain in breast cancer survivors		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes	x No	
If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Prof Delva Shamley		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Department / Office Internal Mail Address	Human Biology

1.1 Does this protocol receive US Federal funding?	☐ Yes	x No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval? **Note:** Any annual approvals for **Full Committee** review MUST be submitted on the monthly HREC submission dates. (Please send electronic copy for full committee review to hrec-submission@uct.ac.za)	☐ Yes	☐ No

If yes in 1.2 please complete section 1.3 below for invoicing purposes

1.3 Ethics Renewal Fee

Please **(tick ✓)**appropriate box for billing purposes:

Submission Type	***Description***	***New fee (Vat Incl.)***	***tick ✓***
Research funded solely from UCT departmental/ divisional/group budget	Annual evaluation of research progress report for re-certification	**R0,00**	☐
Non-sponsored student research for degree purposes at UCT/Other Universities & Colleges	Annual evaluation of research progress report for re-certification	**R0,00**	☐
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R7000,00**	☐
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Expedited review	**R3 710.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National grant funded research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R6000.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National Grant funded research for Annual evaluation of research progress report for re-certification for Expedited review	**R1 500,00**	☐

NB: Protocols funded by UCT (e.g. departmental funding / student research) and by certain grant funding organizations (e.g. MRC, NRF, CANSA,) are exempt from these charges.

Please provide details for Invoicing, either complete section 1 or 2 :

1. Invoice billing – Directly to Sponsor

Sponsor's name	
Billing Address of Sponsor:	
Vat Number:	

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Contact person	
Telephone number	
Email Address	
2. **Internal Journal Billing:**	
Fund Number:	
Cost Centre Number:	
Account Holder Name:	
Division of Account Holder:	

2. List of documentation for approval

3. Protocol status (tick ✓)

☐	Open Enrolment
✓	Closed to enrolment (tick ✓)
☐	Research-related activities are ongoing
☐	Research-related activities are complete, long-term follow-up only
✓	Research-related activities are complete, data analysis only
☐	Main study is complete but sub-study research-related activities are ongoing
☐	Study is closed → Please submit a Study Closure Form (FHS010)

4. Enrolment

Number of participants enrolled to date	80
Number of participants enrolled, since last HREC Progress report (continuing review)	0
Additional number of participants still required	0

5. Refusals

Total number of refusals (participants invited to join the study, but refused to take part)	-

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

6. Cumulative summary of participants

Total number of participants who provided consent	81
Number of participants determined to be ineligible (i.e. after screening)	10
Number of participants currently active on the study	41
Number of participants completed study (without events leading to withdrawal)	55
Number of participants withdrawn at participants' request (i.e. changed their mind)	4
Number of participants withdrawn by PI due to toxicity or adverse events	0
Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance)	4
Number of participants lost to follow-up. Please comment below on reasons for loss of follow-up.	22
Student could not get final visits for these patients. After calling, and scheduling, participants would not show up, or simply refused to meet at all. In some cases, individuals had to work or, felt too overwhelmed with participating further and having to deal with personal issues. However, since multiple visits were collected, this group remains part of the active group	
Number of participants no longer taking part for reasons not listed above. Please provide reasons below:	18
Participants were not actual patients in GSH, some were diagnosed at GSH but were sent to another hospital for their procedure, and there were some who had decided to go to a private facility	

7. Progress of study

Please provide a brief summary of the research to date including the overall progress and the progress since the last annual report as well as any relevant comments/issues you would like to report to the HREC:

- Sample and data collections were completed on patients (where patients were willing to come for the last follow up). All clinical data was collected and captured.
- Genetic laboratory work is completed in the cohort.
- During 2019, student was faced with some personal challenges. Much of the analysis had been on pause for this year, and analysis and writing were incomplete.
- During 2020-2021, with the unexpected COVID-19 -19 pandemic, much time has been missed, however, analysis and writing is being completed
- For the year 2022, the student has submitted and published two manuscripts.
- Additionally, the students' thesis is underway with progress is as follows:

Chapter 1: Literature review at 50%

Chapter 2 : Materials and methods (100%) under review

Chapter 3: Results (100%) under review

Chapter 4: Discussion (70%) in progress

Chapter 5: Pilot at 50%

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Chapter 6: Conclusion (30%) in progress For the year 2023, the student aim to complete all chapters

8. Protocol violations and exceptions (tick ✓ all that apply)

☐	No prior violations or exceptions have occurred since the original approval
☐	Prior violations or exceptions have been reported since the last review and have already been acknowledged or approved
☐	Unreported minor violations that have occurred since the last review, as well as significant deviations not yet reported, are attached for review

9. Amendments (tick ✓ all that apply)

✓	No Prior amendments have been made since the original approval
☐	Prior amendments have been reported since the last review and have already been approved
☐	New protocol changes/ amendments are requested as part of this continuing review (See note below)

Note: If new protocol changes are being requested in this review, please complete an amendment form (FHS006).
Specific changes in the amended protocol and consent/assent forms must be **bolded**, *italicised* or tracked and all changes must include a rationale.

10. Adverse events

10.1 Please provide below or attach a narrative summary of serious adverse events and/ or unanticipated problems since the last progress report. Please indicate changes made to the protocol and informed consent document(s) as a result (if not already reported to the HREC). Please comment on whether causality to any study procedure or intervention could be established.
To date, none of the above-mentioned changes or events have occurred

10.2 Have participants received appropriate treatment/ follow-up/ referral when indicated (e.g. in the case of abnormal or incidental clinical findings, distress or anxiety)?		
☐ Yes	☐ No	✓ Not applicable
If yes, please describe:		

11. Summary of Monitoring and Audit Activities (tick ✓)

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

11.1 Was this study monitored or audited by an external agency (e.g. SAHPRA, FDA)?		
☐ Yes	☐ No	✓☐ Not applicable

11.2 Did a Data and Safety Monitoring Board publish a report?		
☐ Yes	☐ No	✓☐ Not applicable

11.3 If yes, please identify the agency and attach a summary of the findings.					
Agency Name		Report attached	☐ Yes	☐ No	☐ Not applicable
		DSMB report attached	☐ Yes	☐ No	☐ Not applicable

11.4 Has there been any agency, institutional or other inquiry into non-compliance in this study, or any finding of non-compliance concerning a member of the research team?	
☐ Yes	☐ No
If yes, please explain:	

12. Level of risk (tick ✓)

12.1 In light of your experience of this research, please indicate whether the level of risk to participants has:	
☐	Increased
☐	Decreased
✓☐	Shown no change
If there has been a change, please explain:	

12.2 Please provide a narrative summary of recent relevant literature that may have a bearing on the level of risk.

13. Insurance

Please confirm that valid no fault insurance is still in place? **(tick ✓)**			
☐ Yes	☐ No		☐ Not Applicable – N/A
If yes, please complete the following:			
Insurer's name:			
Policy no.		*Coverage Period:	
For UCT sponsored studies please liaise the Insurance office via fhs.sponsorship@uct.ac.za regarding the required documentation and information required obtain a renewed UCT No-fault Insurance Certificate.			

14. Statement of conflict of interest

Has there been any change in the conflict of interest status of this protocol since the original approval? (tick ✓)	
☐ Yes	✓☐ No
If yes, please explain and if necessary, attach a revised conflict of interest statement (Section #7 in the New Protocol Application Form FHS013):	

15. Signature

My signature certifies that the above is complete and correct.			
Signature of PI		Date	06/02/23

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

7.1.2 Recruitment Form

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

Date: 11/10/16

Version: 01

TITLE OF THE RESEARCH PROJECT: Pain management in breast cancer study

PRINCIPAL INVESTIGATOR: Dr Delva Shamley 021 4065066

CO-INVESTIGATORS: A/Professor Alison September 021 6504574

Dr Wynand Smythe

ADDRESS: Clinical Research Centre, University of Cape Town, Old Main Building, L51, Groote Schuur Hospital, Observatory, 7925; Division of Exercise Science and Sports Medicine, Department of Human Biology, University of Cape Town, PO Box 115, Newlands, 7725

Invitation to participate: We are inviting you to partake in a research project focused on morphine drugs and pain management which you will experience as you have been diagnosed for breast cancer and will be treated in the future. Please read the information in this letter very carefully as it explains the details of the project and how you could be involved. Please ask your doctor to explain anything that you do not understand. Only you can decide if you want to take part and, if you choose to say no or to withdraw from the study at any time, it will not affect your medical treatment in any way, not now or in the future.

What is the study about? Chronic pain is a side effect experienced in breast cancer survivors that affects 60% of women. These people develop chronic pain with reduced quality of life, shoulder disability, depression, and fatigue. Chronic pain is treated with many opioid drugs, morphine being the main one and the ability to metabolise morphine is important. It has been seen that each person responds differently or not at all to drugs based on their genetic background. There is evidence suggesting that patients who experience severe acute post-surgery pain are at risk for developing chronic pain and this may be a predictor. This study will look at patients who experience severe pain during and after the breast cancer treatment. It will examine the genetic effect on drug metabolism by looking at changes in genes responsible for the drug break down. We want to see if people who are non-responders to morphine drugs may be at risk for developing chronic pain in breast cancer survivors.

Ethical Approval: This study has been approved by the University of Cape Town Human Research Ethics Committee. This study will be carried out according to the Declaration of Helsinki which is a set of guidelines informing researchers working with people how to do their research so that the people are always treated in the best possible way. It promotes respect for the subjects and protects their health and their rights.

Who will be doing the research? All the researchers and health professionals who will be involved are experts in their field and are based at the clinical research centre at the University of Cape Town and the Division of Exercise science and sports medicine, Cape Town. All new scientists to join the group will be trained and supervised by senior staff members.

Who is paying for this study? The study is funded by a University of Cape Town Development Research Grant

Can we trust that there is no conflict of interest? No, there are no conflicts of interest. The findings of the study are purely for the benefit of the breast cancer survivors and will add to the knowledge of the development of chronic pain post treatment for breast cancer.

What if something goes wrong? The University of Cape Town promises that in the event of you suffering in health or well-being caused directly by you participating, it will provide immediate medical care and assistance. This research study is covered by an insurance policy taken out by the University of Cape Town with Marsh Pty. Policy number: BOWLT1600031.

Procedures and Time involved: Once we receive your reply slip, we will phone you to confirm your eligibility for inclusion in the study. We will then make an appointment to meet you at the Oncology clinic at Groote Schuur hospital, Cape Town during your pre-operative visit. This appointment will require about 1 hour of your time. This will start with a 15-minute information session about the aim of the research project. We will read through the information sheet and answer your questions about the project. If you are interested in taking part in our research project, you will be asked to read and sign a consent form. If you agree to take part, we will continue as follows:

1. A member of the research team will assist you to complete a questionnaire related to your personal details, your history of breast cancer. Your history of treatment by physiotherapists, your history of exercising.

2. You will fill in a short questionnaire about your current level of pain and functional ability of the arm and shoulder. You may not be experiencing any difficulty, but we will still use your data to compare to those who are having difficulty.

3. A health professional will draw two small samples of blood (about 1-2 tablespoons, about 10ml) from a vein in the arm.

4. The blood will be stored at the CRC, Groote Schuur, University of Cape Town. Aliquots will be transported to the Division of Exercise Science and Sports Medicine in Newlands for genetic analysis. DNA analysis will be carried out at the institute of ESSM.

How long will my blood/DNA be stored? There is no limit as to how long the sample can be stored because we will constantly be screening the sample the different genes involved in the metabolism of opioid drugs use in treatment for breast cancer pain. However, you have the right to decide if you do not want your sample to be kept for a period longer than 10 years.

Will I get results from my tests? This is a pilot study which may determine the genetic association with pain relief during treatment for breast cancer. It may take a few years before we can draw any conclusions from these results and as such, we will not be sending out any results to participants.

Who can take part: To take part in this study you must be older than 18 years of age. You must have been diagnosed for unilateral breast cancer. You must not have a history of neck, shoulder, problems prior to cancer and you must not have had a mastectomy.

Who will know that I am taking part in this project: Your identity will be kept secret, and your personal details will be safely stored in the office of the principal investigator, at the Clinical Research Centre, Groote Schuur, University of Cape Town. Your samples will be coded with a reference number so that your name and other details will not be known to laboratory staff and will not be used in any reports or publications written about this study.

The only person who will know that you are taking part is your Oncology consultant.

Will I get paid for taking part: You will not be paid to take part in the study.

Are there any risks involved in the process: Drawing blood is a standard procedure. There may be a little discomfort and pain and a small risk of bruising. If you are still concerned after a few days about a study-related injury, then please contact Dr Shamley (telephone numbers are given on page 1 of this document).

What benefits are there for me: There are no direct benefits from the study. This study does not aim to provide a cure for chronic pain, but it may provide information and insight in pain management and opioid metabolism that will be useful in the future to understand what causes or determines the shift of acute pain to chronic pain and how to improve treatment.

Do I have to take part? NO. It is entirely up to you if you want to take part in this study. You may decide that you do not want to take part or decide to withdraw from the project at any time. This decision will not affect any current treatment you may be receiving.

What if I have other questions now or later about the study or my rights: If you have questions about the study that are not answered in these Information Sheets, please ask the interviewer to answer them. If you have any concerns related to this project in the future, you may contact:

The study investigators at the telephone numbers given on the first page.

Professor Marc Blockman, Chair, UCT Human Research Ethics Committee, Faculty of Health Sciences, may be contacted by research subjects to discuss their rights. Office Tel: 021-4066492; Email address:marc.blockman@uct.ac.za

Dr Shamley, Director, UCT Clinical Research Centre, may be contacted: Office Tel: 021 4065066; Email address: delva.shamley@uct.ac.za

7.1.3 Consent Form

Date:

Version: 01

Project Title: Pain management in breast cancer study

AGREEMENT TO PARTICIPATE

I, _____, have read (or had read to me) the Information Sheet for the study named 'Opioid metabolism and pain management post treatment for breast cancer'.

My role in the study is as a research volunteer to help the investigators collect information about the opioid metabolism and genes that may be involved in the development of chronic pain after treatment for breast cancer. I understand that the purpose of this study is not to cure chronic pain and the genetic information may or may not be useful in designing better ways to treat these complications in the future. My questions have been answered to my satisfaction in a language that I understood. By signing this consent form I do not waive any of my rights.

I, ……., AGREE to take part in the study "Pain management in breast cancer study".

(initial boxes where appropriate)

I agree to give a blood sample for protein studies.

I agree to give a blood sample for pharmacogenetic studies related to this study only.

I agree to my DNA being stored for a maximum period of 10 years.

I authorise my doctor to provide relevant clinical details to the

Department of Human Biology, UCT.

Research Volunteer:

Signature: _____

Date: _____

Name: _____

Please Print

Volunteer: Please sign both copies of this page

Interviewer Obtaining Consent:

Signature: _____

Date: _____ Name: _____

Please Print

Interviewer: Please sign both copies of this page and hand one copy back to the research volunteer.

7.2 APPENDIX B: OUTCOME MEASURES

7.2.1 Shoulder Pain and Disability Index (SPADI)

Study no:
Date:
Visit No:

Shoulder Pain and Disability Index

Please place a mark on the line that best represents your experience during the last week attributable to your shoulder problem.

Pain scale
How severe is your pain?
Circle the number that best describes your pain where: 0 = no pain and 10 = the worst pain imaginable.

At its worst?	0	1	2	3	4	5	6	7	8	9	10
When lying on the involved side?	0	1	2	3	4	5	6	7	8	9	10
Reaching for something on a high shelf?	0	1	2	3	4	5	6	7	8	9	10
Touching the back of your neck?	0	1	2	3	4	5	6	7	8	9	10
Pushing with the involved arm?	0	1	2	3	4	5	6	7	8	9	10

Total pain score¬¬¬¬¬¬_____ /50 x 100 = %
(Note: If a person does not answer all questions divide by the total possible score, e.g., if 1 question missed divide by 40)

Disability scale
How much difficulty do you have?
Circle the number that best describes your experience where: 0 = no difficulty and 10 = so difficult it requires help

Washing your hair?	0	1	2	3	4	5	6	7	8	9	10
Washing your back?	0	1	2	3	4	5	6	7	8	9	10
Putting on an undershirt or jumper?	0	1	2	3	4	5	6	7	8	9	10
Putting on a shirt that buttons down the front?	0	1	2	3	4	5	6	7	8	9	10
Putting on your pants?	0	1	2	3	4	5	6	7	8	9	10
Placing an object on a high shelf?	0	1	2	3	4	5	6	7	8	9	10
Carrying a heavy object of 10 pounds (4.5 kilograms)	0	1	2	3	4	5	6	7	8	9	10

Removing something from your back pocket? 0 1 2 3 4 5
6 7 8 9 10

Total disability score: _____/ 80 x 100 = %
(Note: If a person does not answer all questions divide by the total possible score, e.g., if 1 question missed divide by 70)

Total SPADI score: _____ 130 x 100 = %

(Note: If a person does not answer all questions divide by the total possible score, e.g., if 1 question missed divide by 120)

Minimum Detectable Change (90% confidence) = 13 points
(Change less than this may be attributable to measurement error)
Source: Roach et al. (1991). Development of a shoulder pain and disability index.

7.2.2 Hospital Anxiety and Depression Scale (HADS)

Hospital Anxiety and Depression Scale
Study no:
Date:
Visit No:

Yes, definitely Yes sometimesNo, not much No, not at all.

1. I wake early and then sleep badly for the rest of the night.
   3 2 1 0
2. I get very frightened or have panic feelings for apparently no reason at all.
   3 2 1 0
3. I feel miserable and sad.
   3 2 1 0
4. I feel anxious when I go out of the house on my own.
   3 2 1 0
5. I have lost interest in things
   3 2 1 0
6. I get palpitations, or sensations of ‘butterflies’ in my stomach or chest.
   3 2 1 0
7. I have a good appetite.
   0 1 2 3
8. I feel scared or frightened.
   3 2 1 0
9. I feel life is not worth living.
   3 2 1 0
10. I still enjoy the things I used to.

11. I am restless and can’t keep still
    3 2 1 0
12. I am more irritable than usual.
    3 2 1 0
13. I feel as if I have slowed down.
    3 2 1 0
14. Worrying thoughts constantly go through my mind. 3 2 1 0

7.2.3 Positive and Negative Affect Scale (PANAS)

Positive and negative affect scale
Study no:
Date:
Visit No:
This scale consists of a number of words that describe different feelings and emotions. Read each item and then mark the appropriate answer in the space next to that word. Indicate to what extent you feel this way generally, that is, how you feel most of the time:

0 1 2 3 4 5
not at all very slightly or a little moderately quite a bit extremely

_____ interested _____ irritable.

_____ distressed _____ alert.

_____ excited _____ ashamed

_____ upset _____ inspired.

_____ strong _____ nervous

_____ guilty _____ determined.

_____ scared _____ attentive.

_____ hostile _____ jittery

_____ enthusiastic _____ active

_____ proud _____ afraid

7.3 APPENDIX C: DNA EXTRACTION MATERIALS

7.3.1 DNA Extraction Reagents

Last modified March 09/2021

1. TKM1 Buffer (pH 7.6)

	Final Conc.	**MW**	**For 500ml**	**For 1000ml**
Tris-Base*	10mM	121.00	0.6056	1.2112
KCl	10mM	74.56	0.3728	0.7456
$MgCl_2.6H_2O$	10mM	203.20	1.016	2.032
EDTA	2mM	372.24	0.372	0.744
dH_2O			to 500ml	To 1000ml

- Autoclave
- Make up 1 volume which includes 2.5% NP40 and 1 volume without NP40.

2. TKM2 Buffer (pH 7.6)

	Final Conc.	**MW**	**For 200ml**
Tris-Base*	10mM	121.00	0.242
KCl	10mM	74.56	0.149
$MgCl_2.6H_2O$	10mM	203.20	0.406
EDTA	2mM	372.24	0.1488
NaCl	0.4M	58.44	4.675
dH_2O			to 200 ml

- Autoclave

3. 10% SDS

	Final Conc.	**MW**	**For 200ml**
SDS	10%		20
dH_2O			to 200 ml

- Autoclave

4.1X TE buffer (pH 8.0)

	Final Conc.	**MW**	**For 100ml**
Tris-Base*	10mM	121.00	0.121
EDTA	1mM	372.24	0.037
dH_2O			to 100 ml

- Autoclave

5. 5M $NaClO_4$

	Final Conc.	**MW**	**For 100ml**
$NaClO_4$	5M	122.4	61.2
dH_2O			to 100 ml

- Autoclave

. Other Chemicals and Reagents

- Chloroform (molecular grade)
- NP40
- Absolute ethanol

7.3.2 DNA Extraction from Whole Blood Protocol

1. Draw 5mls of blood into an EDTA vacutainer tube (Purple top).
2. Blood can be stored at 4ºC up to 1 week before the DNA is extracted.
3. Transfer the blood to a sterile 15ml polypropylene tube.
4. Add 2 volumes (10ml) of TKM1 buffer containing 2.5% NP40.
5. Mix by inverting several times and incubate at room temperature for 10 minutes in order to enhance the haemolysis of red blood cells.
6. Centrifuge at 3000rpm (1200Xg) at room temperature for 10 minutes.
7. Decant off the supernatant containing leaving the white pellet at the bottom of the tube.
8. Add 1 volume (5ml) of TKM1 buffer (without NP40).
9. Invert and vortex the solution.
10. Centrifuge at 3000rpm (1200Xg) at room temperature for 10 minutes.
11. Decant the supernatant leaving the white pellet in the bottom of the tube.
12. Repeat steps 7-10 until the pellet in the bottom of the tube is clean and white.
13. Add 800ul of TKM2 buffer and 50ul of the 10% SDS solution.
14. Vortex and then **mix using a blue pipette tip** to assist in the lyses of the white blood cells.
15. Incubate for 60 minutes at 55ºC in a water bath. Make sure the pellet is totally dissolved before moving on.
16. Add 150ul of 5M $NaClO_4$.
17. Add 500ul of molecular biology grade chloroform.
18. Vortex the solution.
19. Transfer the solution to sterile 1.5ml microfuge tubes.
20. Centrifuge at 1300rpm at room temperature for 5 minutes.
21. Carefully transfer 500ul of the top aqueous phase to a new sterile microfuge tube.
22. Add 1ml of absolute ethanol.
23. Invert until DNA precipitates.
24. Centrifuge at 1300rpm at room temperature for 5-10 minutes.
25. Carefully tip off supernatant leaving the pellet in the bottom of the tube.
26. Allow pellet to air dry completely.
27. Add 200ul of 1XTE buffer.
28. Incubate the tubes at 65ºC for 15 minutes in a heating block.
29. Store DNA at 4ºC.

* Tris-Base and Tris-HCL have different MW

Reference: Lahiri et al.(1991) Nucleic acids research 19:54444

7.4 APPENDIX D: SUPPLEMENTARY RESULTS

7.4.1 Genetic Association

Supplemental Table 1: A priori power calculation to determine a sufficient sample size (n) for candidate genes with odds ratios 1.5-2.5 at 80% power using a literary inferred baseline risk.

N, number of participants with Moderate – High *pain, disability, and combined (pain and disability)*, required for 80% study power										
		Dominant			Recessive			Log Additive		
MAF	OR	Pain (~37%)	Disability (~34%)	Combined (~45%)	Pain (~37%)	Disability (~34%)	Combined (~45%)	Pain (~37%)	Disability (~34%)	Combined (~45%)
0,1	1,5	452	448	460	6867	6765	7076	385	381	395
	2	154	152	159	2320	2261	2442	133	130	138
	2,5	89	87	93	1333	1288	1426	78	76	82
0,2	1,5	308	307	310	1777	1753	1828	221	21	225
	2	106	105	107	602	587	631	77	76	80
	2,5	61	61	62	346	335	369	46	45	48
0,3	1,5	288	289	288	839	828	860	171	170	173
	2	100	100	99	285	279	297	60	60	62
	2,5	58	58	57	164	160	173	36	36	37
0,4	1,5	317	319	314	515	510	526	151	151	152
	2	110	111	108	176	173	182	54	53	54
	2,5	63	64	62	101	99	106	32	32	32
0,5	1,5	394	397	388	374	371	379	146	146	146
	2	136	138	133	128	126	131	52	52	52
	2,5	78	80	76	74	73	76	31	31	31

Note: Abbreviations: MAF, Minor Allele Frequency; OR Odds Ratio

Supplemental Table 2: A priori power calculation to determine a sufficient sample size (n) for candidate genes with odds ratios 1.5-2.5 at 80% power using SA BCS prevalence.

Sample size required: N, number of participants with Moderate – High *pain, disability, and combined (pain and disability)*, required for 80% study power										
		Dominant			Recessive			Log Additive		
MAF	**OR**	**Pain (28%)**	**Disability (17%)**	**Combined (22%)**	**Pain (28%)**	**Disability (17%)**	**Combined (22%)**	**Pain (28%)**	**Disability (17%)**	**Combined (22%)**
0,1	1,5	441	428	434	6595	6284	6426	373	359	365
	2	148	141	144	2162	1980	2063	126	118	121
	2,5	84	79	81	1213	1076	1138	73	66	69
0,2	1,5	305	301	303	1712	1636	1670	215	209	212
	2	104	102	103	563	520	539	74	71	73
	2,5	60	59	59	317	28	299	44	41	42
0,3	1,5	289	290	290	811	780	794	168	165	166
	2	100	101	101	269	251	259	59	57	58
	2,5	58	59	58	152	138	144	35	34	34
0,4	1,5	321	326	324	502	486	493	150	148	149
	2	112	116	114	168	159	163	53	52	53
	2,5	65	68	67	95	88	92	32	31	31
0,5	1,5	402	413	408	366	358	362	146	146	146
	2	142	149	146	124	119	121	52	52	52
	2,5	83	89	86	71	67	69	31	31	31

Note: Abbreviations: MAF, Minor Allele Frequency; OR Odds Ratio

Supplemental Table 3: Post hoc analysis of study statistical power by literature inferred baseline risk.

		Dominant	Recessive	Log Additive	Dominant	Recessive	Log Additive	Dominant	Recessive	Log Additive
		Pain (37%) n=68			Disability (34%) n=48			Combined (45%) n=55		
***ABCB1* rs1045642 A=0.3952**	1,5	26%	17%	47%	19%	14%	35%	22%	15%	39%
	2,0	60%	41%	**88%**	46%	31%	75%	52%	33%	**80%**
	2,5	**83%**	62%	**98%**	68%	49%	**93%**	75%	51%	**95%**
***ABCB1* rs1128503 A=0.4161**	1,5	25%	18%	47%	19%	14%	36%	21%	15%	40%
	2,0	58%	44%	**89%**	44%	33%	76%	51%	36%	**81%**
	2,5	**82%**	66%	**98%**	67%	52%	**93%**	74%	55%	**96%**
***OPRM1* rs1799971 G=0.2234**	1,5	27%	9%	37%	20%	8%	28%	22%	8%	30%
	2,0	63%	18%	78%	48%	14%	63%	53%	15%	68%
	2,5	**85%**	28%	**94%**	72%	22%	**85%**	76%	23%	**88%**
***OPRM1* rs540825 A=0.1178**	1,5	21%	6%	24%	16%	6%	19%	18%	6%	20%
	2,0	50%	9%	57%	38%	8%	44%	41%	8%	47%
	2,5	74%	12%	**80%**	59%	10%	66%	63%	10%	69%
***COMT* rs6269 G=0.3568**	1,5	27%	15%	45%	20%	12%	34%	23%	13%	38%
	2,0	62%	36%	**87%**	48%	27%	74%	54%	29%	79%
	2,5	**85%**	55%	**98%**	71%	43%	**92%**	77%	45%	**95%**
***COMT* rs4633 T=0.3716**	1,5	26%	16%	46%	20%	13%	35%	22%	14%	38%
	2,0	61%	38%	**88%**	47%	29%	75%	53%	31%	**80%**
	2,5	**84%**	1%	**98%**	70%	45%	**92%**	77%	48%	**95%**
***COMT* rs4818 G=0.2969**	1,5	27%	0%	42%	21%	10%	32%	23%	11%	35%
	2,0	64%	27%	**84%**	49%	21%	71%	55%	22%	75%
	2,5	**86%**	43%	**97%**	72%	33%	**90%**	78%	35%	**93%**
***COMT* rs4680 A=0.3692**	1,5	26%	16%	46%	20%	13%	35%	22%	15%	39%

	2,0	61%	37%	**88%**	47%	28%	75%	52%	33%	**80%**
	2,5	**84%**	58%	**98%**	70%	45%	**92%**	75%	52%	**95%**

Notes: *Frequencies were reported from the NCBI portal (National Centre for Biotechnology Information [http://www.ncbi.nlm.nih.gov/]). The number of unmatched participants in the no – low category per participant in the moderate – high category was 2. Abbreviations: SNP, Single nucleotide polymorphism; MAF, Minor Allele Frequency; OR, Odds Ratio; n, number of participants with moderate – high pain, disability and combined (pain and disability).

Supplemental Table 4: Post hoc analysis of study statistical power by SA BCS prevalence.

		Dominant	Recessive	Log Additive	Dominant	Recessive	Log Additive	Dominant	Recessive	Log Additive
		Pain (28%) n=68			Disability (17%) n=48			Combined (22%) n=55		
***ABCB1* rs1045642 A=0.3952**	1,5	25%	18%	47%	19%	14%	36%	21%	15%	40%
	2,0	59%	42%	**89%**	44%	33%	77%	50%	36%	**82%**
	2,5	**82%**	65%	**98%**	66%	53%	**94%**	72%	58%	**96%**
***ABCB1* rs1128503 A=0.4161**	1,5	25%	19%	47%	18%	15%	36%	21%	16%	40%
	2,0	57%	45%	**89%**	43%	35%	77%	48%	39%	**82%**
	2,5	**80%**	68%	**98%**	64%	56%	**94%**	71%	61%	**96%**
***OPRM1* rs1799971 G=0.2234**	1,5	27%	10%	37%	21%	8%	29%	23%	9%	32%
	2,0	63%	19%	79%	49%	16%	66%	54%	17%	71%
	2,5	**86%**	30%	**95%**	72%	25%	**88%**	78%	27%	**91%**
***OPRM1* rs540825 A=0.1178**	1,5	21%	6%	25%	17%	6%	20%	18%	6%	21%
	2,0	52%	9%	60%	40%	8%	48%	44%	8%	52%
	2,5	75%	12%	**82%**	63%	11%	72%	68%	11%	76%
***COMT* rs6269 G=0.3568**	1,5	26%	16%	46%	20%	13%	35%	22%	14%	39%
	2,0	61%	37%	**88%**	46%	29%	76%	52%	32%	**81%**
	2,5	**84%**	58%	**98%**	68%	48%	**93%**	75%	51%	**96%**
***COMT* rs4633 T=0.3716**	1,5	26%	16%	46%	20%	13%	35%	22%	14%	39%
	2,0	61%	39%	**88%**	46%	31%	76%	51%	34%	**81%**
	2,5	**83%**	61%	**98%**	67%	50%	**93%**	74%	54%	**96%**
***COMT* rs4818 G=0.2969**	1,5	27%	13%	43%	21%	11%	33%	23%	11%	36%
	2,0	64%	29%	**85%**	49%	23%	73%	55%	25%	78%
	2,5	**86%**	46%	**97%**	72%	37%	**92%**	78%	40%	**94%**
***COMT* rs4680 A=0.3692**	1,5	26%	16%	46%	20%	13%	35%	22%	14%	39%

	2,0	61%	39%	**88%**	46%	31%	76%	51%	33%	**81%**
	2,5	**83%**	60%	**98%**	68%	50%	**93%**	74%	53%	**96%**

Supplemental Table 5: Clinical characteristics of a subset of South African Breast Cancer Survivors.

Patient	Age	BMI (Kg/m2)	Surgery type	Lymph node surgery	Neo-CT	Adjuvant CT	Adjuvant RT	Adjuvant HT	Treatment given
	53.3±13.2	33.1 (27.6-38.3)							
SW p	0.704	0.022							
1	38	28,08	MTX	ALND	Yes	No	Yes	Yes	Tamoxifen
2	57	37,88	WLE	SLNB	No	No	Yes	Yes	Tamoxifen
3	76	25,33	MTX	ALND	No	No	No	Yes	Tamoxifen
4	48	34,24	WLE	ALND	Yes	No	Yes	No	None
5	42	38,27	WLE	SLNB	No	No	Yes	Yes	Tamoxifen
6	73	33,11	WLE	SLNB	No	No	Yes	Yes	Tamoxifen
7	64	44,92	WLE	SLNB	No	No	Yes	Yes	Tamoxifen
8	63	41,02	MTX	None	No	No	No	Yes	Tamoxifen
9	47	26,45	WLE	SLNB	No	No	Yes	Yes	Tamoxifen
10	59	26,13	WLE	SLNB	No	No	No	Yes	Tamoxifen
11	47	62,47	WLE	SLNB	No	No	Yes	Yes	Tamoxifen
12	46	27,58	MTX	ALND	No	No	No	Yes	Tamoxifen
13	33	31,63	MTX	SLNB	Yes	No	No	Yes	Tamoxifen

Supplemental Table 6: Normality distribution for quantitative variables for the subset samples analyses.

Variable	Shapiro-Wilk test	P value	Kolmogorov-Smirnov test	P value
Age	0,957	0,704	0,195	>0,1000
BMI(Kg/m^2)	0,841	**0,022**	0,17	>0,1000
Total Tramadol (MME) Dose	0,85	**0,029**	0,226	0,069
Total Paracetamol (g) Dose	0,882	0,077	0,211	>0,1000
Pain (T1)	0.482	<0.001	0.372	<0.001
Disability (T1)	0.586	<0.001	0.381	<0.001
Combined (T1)	0.556	<0.001	0.372	<0.001
Pain (T2)	0,926	0,339	0,144	>0,1000
Disability (T2)	0,924	0,316	0,194	>0,1000
Combined Pain and Disability (T2)	0,95	0,638	0,126	>0,1000
Pain (T3)	0,852	**0,031**	0,179	>0,1000
Disability (T3)	0,825	**0,014**	0,209	>0,1000
Combined Pain and Disability (T3)	0,84	**0,021**	0,228	0,063
Anxiety (T1)	0,915	0,214	0,279	**0,007**
Depression (T1)	0,953	0,651	0,149	>0,1000
Anxiety (T2)	0,845	**0,032**	0,202	>0,1000
Depression (T2)	0,909	0,205	0,224	0,098
Anxiety (T3)	0,891	0,099	0,138	>0,1000
Depression (T3)	0,897	0,122	0,217	0,096
Positive Affect (T1)	0,929	0,333	0,142	>0,1000
Negative Affect (T1)	0,884	0,080	0,17	>0,1000
Positive Affect (T2)	0,985	0,997	0,101	>0,1000
Negative Affect (T2)	0,859	**0,047**	0,259	**0,026**

Positive Affect (T3)	0,9	0,134	0,208	>0,1000
Negative Affect (T3)	0,917	0,231	0,155	>0,1000

Notes: T1,Pre-operative, T2, 3 months post-operative; T3, 1 year post-operative; P-values (*P*) in bold typeset indicate significance ($P<0.05$).BMI, Body Mass Index.

Supplemental Table 7: Normality distribution for quantitative variables in the full SA BCS cohort.

Quantitative variables	N	Mean±SD	Range (min-max)	Kolmogorov-Smirnov p value	Lilliefors p value	Shapiro-Wilk W p value
Age at surgery	242	54,0±9,8	22,0-74,0	>0,20	p<0,10	0,014
Time since surgery(yr.)	240	3,4±2,5	0,4-10,0	<0,01	<0,01	0,000
Total number of nodes examined	221	10,2±6,1	0,0-34,0	>0,20	<0,01	0,000
Total number of nodes involved	121	3,4±3,5	0,0-14,0	<0,01	<0,01	0,000
Pain Score	252	20,5±24,2	0,0-100,0	<0,01	<0,01	0,000
Disability Score	252	13,2±20,5	0,0-100,0	<0,01	<0,01	0,000
SPADI Score	252	16,0±21,2	0,0-100,0	<0,01	<0,01	0,000

Supplemental Table 8: Breast cancer survivors' quantitative variables between no-low and moderate-high groups of pain, disability, and the combined (pain and disability) categories.

	Pain			Disability			Combined		
Variables	**No-Low**	**Mod-High**	***P***	**No-Low**	**Mod-High**	***P***	**No-Low**	**Mod-High**	***P***
N=252	73,0 (184)	27 (68)		81 (204)	19 (48)		78 (197)	22 (55)	
Age at surgery	55,3±9,2	50,7±10,7	**0,002**	54,8±9,3	50,6±11,3	**0,011**	55,0±9,3	50,5±10,9	**0,003**
Time since surgery (yr.)	3,5±2,5	3,1±2,4	0,116	3,5±2,5	3,2±2,3	0,482	3,5±2,5	3,1±2,5	0,133
Total nodes examined	10,5±6,0	9,5±6,3	0,155	10,5±6,3	8,9±5,1	0,177	10,6±6,3	8,6±5,2	0,066
Total nodes involved	3,6±3,7	2,8±3,1	0,145	3,7±3,7	2,1±2,5	**0,025**	3,7±3,7	2,3±2,7	**0,034**

Notes: Data presented as mean±standard deviation (Mean±SD) or % (n). P-values (*P*) in bold typeset indicate significance ($P<0.05$). Tests used for comparative analysis include the Mann-Whitney U test (Independent sample T-test); Fisher's exact test (*when n <10); Chi-squared test Abbreviations: Mod-High: Moderate-High

Supplemental Table 9: Amplification success rates for the *ABCB1*, *OPRM1* and *COMT* SNPs evaluated.

Gene	SNP	Success rate % (n=252)
ABCB1	rs1128503 G>A	98,4 (248)
	rs1045642 G>A	98,4 (248)
OPRM1	rs1799971 A>G	98,4 (248)
	rs540825 A>T	93,7 (236)
COMT	rs6269 A>G	98,4 (248)
	rs4633 C>T	98,4 (248)
	rs4818 C>G	96,8 (244)
	rs4680 G>A	97,6 (246)

Supplemental Table 10: Unadjusted- Genotype and minor allele frequency distributions, of the *ABCB1* (rs1128503 G>A, rs1045642 G>A) polymorphisms between pain, disability, and combined (pain and disability) categories.

	Pain			Disability					Pain and Disability				
Polymorphism	**No-Low**	**Mod -High**		**No-Low**	**Mod-High**				**No-Low**	**Mod-High**			
SA BCS	**(n=184)**	**(n=68)**	**OR (95% CI)**	**(n= 204)**	**(n=48)**	**OR (95% CI)**			**(n=197)**	**(n=55)**	**OR (95% CI)**		
rs1128503 G>A	(n=181)	(n=67)		(n=200)	(n=48)				(n=193)	(n=55)			
G/G	35,3 (65)	35,8 (24)	1 (0,00-0,00)	36,3 (73)	33,3 (16)	1 (0,00-0,00)			36,3 (70)	34,5 (19)	1 (0,00-0,00)		
A/G	47,4 (85)	50,7 (34)	1,08 (0,59-2,00)	46,4 (93)	54,2 (26)	1,28 (0,64-2,55)			46,1 (89)	54,5 (30)	1,24 (0,65-2,39)		
A/A	17,1 (31)	13,4 (9)	0,79 (0,33-1,89)	17,1 (34)	12,5 (6)	0,81 (0,29-2,24)			17,6 (34)	10,9 (6)	0,65 (0,24-1,78)		
An Allele	40,6 (147)	38,8 (52)	0,93 (0,60-1,42)	40,3 (161)	39,6 (38)	0,97 (0,60-1,57)			40,7 (157)	38,2 (42)	0,90 (0,57-1,42)		
P value1	0,750			0,581					0,376				
An Allele P value²	0,757			1,000					0,661				
HWE	0,758	0,765		0,66	0,545				0,551	0,39			
rs1045642 G>A	(n=181)	(n=67)		(n=200)	(n=48)				(n=193)	(n=55)			
G/G	40,4 (74)	49,3 (33)	1 (0,00-0,00)	40,4 (80)	56,2 (27)	1 (0,00-0,00)	**0,042**	243.6	38,9 (75)	58,2 (32)	1 (0,00-0,00)	**0,011**	260.0
A/G	46,4 (84)	38,8 (26)	0,69 (0,38-1,27)	45,4 (91)	39,6 (19)	0,62 (0,32-1,20)	0,457	247.1	46,6 (90)	36,4 (20)	0,52 (0,28-0,98)	0,174	264.6
A/A	12,1 (23)	11,9 (8)	0,78 (0,32-1,92)	14,1 (29)	4,2 (2)	0,20 (0,05-0,91)	**0,031**	243.0	14,5 (28)	5 (5,3)	0,25 (0,07-0,89)	0,053	262.7
An Allele	35,9 (130)	31,3 (42)	0,82 (0,52-1,27)	37,3 (149)	24,0 (23)	0,53 (0,30-0,90)			37,8 (146)	23,6 (26)	0,51 (0,30-0,84)		
P value1	0,484			**0,034**				243,0	**0,019**				260,6
An Allele P value²	0,396			**0,017**					**0,006**				
HWE	1.000	0,405		0,762	0,709				0,879	1.000			

Notes: Genotype and allele frequencies are expressed as a percentage (%) with the number of participants (n) in parentheses. Global P values[1] for genotype between groups and P values[2] for allele between groups; P-values in bold indicate significance ($P<0.05$). P values for logistic regression analysis are listed for the dominant [a], over-dominant [b], and recessive [c] models. P-values for the exact test of Hardy–Weinberg equilibrium for each of the categories are included in the Table; Abbreviations: AIC: Akaike information criterion score; Mod-High: Moderate-High; HWE: Hardy–Weinberg equilibrium.

Supplemental Table 11: Inferred *ABCB1* rs1128503 G>A – rs1045642 G>A haplotype analyses for the pain, disability and combined (pain, and disability) categories.

ABCB1	Frequency %			Unadjusted		*Adjusted		
	No-Low	Mod-High	HS	Global P	Specific P	Global P	Specific P	OR (95%CI)
Pain	**(n=184)**	**(n=68)**						
G-G	53,2	56,9	0,71	0,784	0,479	0,798	0,391	1 (0,00-0,00)
A-A	29,7	27,1	-0,62		0,534		0,406	0,85 (0,53-1,36)
A-G	10,9	11,7	0,3		0,763		0,872	1,00 (0,53-1,87)
G-A	6,2	4,3	-0,77		0,441		0,630	0,67 (0,25-1,79)
Disability	**(n= 204)**	**(n=48)**						
G-G	52,8	60,4	1,2	**0,025**	0,23	**0,027**	0,186	1 (0,00-0,00)
A-A	30,3	29,6	-1,42		0,156		0,091	0,70 (0,41-1,2)
A-G	9,9	15,6	1,76		0,078		0,076	1,32 (0,70-2,48)
G-A	6,9	0	-2,38		**0,017**		**0,029**	0 (0,00-0,00)
Pain and Disability	**(n=197)**	**(n=55)**						
G-G	52,4	60,7	1,44	**0,020**	0,15	0,019	0,107	1 (0,00-0,00)
A-A	30,9	22,5	-1,86		0,063		**0,029**	0,63 (0,37-1,06)
A-G	9,7	15,7	1,83		0,066		0,068	1,33 (0,72-2,46)
G-A	6,9	1,1	-2,14		0,032		0,057	0,16 (0,02-1,18)

Supplemental Table 12: Unadjusted- Genotype and minor allele frequency distributions, of the *OPRM1* (rs1799971 A>G, rs540825 T>A) polymorphisms between pain, disability, and combined (pain and disability) categories.

Polymorphisms	Pain			Disability			Pain and Disability		
	No-Low	Mod -High		No-Low	Mod-High		No-Low	Mod-High	
SA BCS	(n=184)	(n=68)	OR (95% CI)	(n= 204)	(n=48)	OR (95% CI)	(n=197)	(n=55)	OR (95% CI)
rs1799971 A>G	(n=181)	(n=67)		(n=200)	(n=48)		(n=193)	(n=55)	
A/A	64,6 (116)	74,6 (50)	1 (0,00-0,00)	68,6 (136)	62,5 (30)	1 (0,00-0,00)	66,8 (129)	67,3 (37)	1 (0,00-0,00)
A/G	30,3 (56)	23,9 (16)	0,66 (0,35-1,27)	27,2 (55)	35,4 (17)	1,40 (0,72-2,74)	28,5 (55)	30,9 (17)	1,08 (0,56-2,08)
G/G	5,5 (9)	1,5 (1)	0,26 (0,03-2,09)	4,4 (9)	2,1 (1)	0,50 (0,06-4,13)	4,7 (9)	1,8 (1)	0,39 (0,05-3,16)
G allele	79,6 (288)	86,6 (116)	0,60 (0,32-1,08)	18,3 (73)	19,8 (19)	1,11 (0,59-1,98)	18,9 (73)	17,3 (19)	0,90 (0,48-1,60)
P value1	0,178			0,443			0,574		
G Allele P value[2]	0,090			0,770			0,782		
HWE	0,496	1.000		0,244	0,663		0,346	1.000	
rs540825 T>A	(n=172)	(n=64)		(n=190)	(n=46)		(n=183)	(n=53)	
T/T	59,5 (102)	60,9 (39)	1 (0,00-0,00)	60,6 (115)	56,5 (26)	1 (0,00-0,00)	60,1 (110)	58,5 (31)	1 (0,00-0,00)
A/T	36,3 (63)	34,4 (22)	0,91 (0,50-1,68)	36,3 (69)	34,8 (16)	1,03 (0,51-2,05)	36,6 (67)	34,0 (18)	0,95 (0,49-1,84)
A/A	4,4 (7)	4,7 (3)	1,12 (0,28-4,55)	3,3 (6)	8,7 (4)	2,95 (0,78-11,20)	3,3 (6)	7,5 (4)	2,37 (0,63-8,91)
An allele	22,4 (77)	21,9 (28)	0,97 (0,57-1,62)	21,3 (81)	26,1 (24)	1,30 (0,73-2,26)	21,6 (79)	24,5 (26)	1,18 (0,68-2,01)
P value1	0,938			0,309			0,441		
An Allele P value[2]	1.000			0,330			0,510		
HWE	0.660	1.000		0,385	0,464		0.381	0.481	

Notes: Genotype and allele frequencies are expressed as a percentage (%) with the number of participants (n) in parentheses. Global P values[1] for genotype between groups and P values[2] for allele between groups; P-values in bold indicate significance ($P<0.05$). P values for logistic regression analysis are listed for the dominant [a], over-dominant [b], and recessive [c] models. P-values for the exact test of Hardy–Weinberg equilibrium for each of the categories are included in the Table; Abbreviations: AIC: Akaike information criterion score; Mod-High: Moderate-High; HWE: Hardy–Weinberg equilibrium.

Supplemental Table 13: Inferred *OPRM1* (rs1799971 A>G - rs540825 T>A) haplotype analyses for the pain, disability and combined (pain, and disability) categories.

OPRM1	Frequency %			Unadjusted		*Adjusted		
	No-Low	Mod-High	HS	Global P	Specific P	Global P	Specific P	OR (95%CI)
Pain	**(n=184)**	**(n=68)**						
A-T	61,2	71,4	1,77	**0,049**	0,076	**0,040**	0,569	1 (0,00-0,00)
A-A	18,4	15,2	-0,33		0,741		0,100	0,64 (0,34-1,21)
G-T	**16,4**	**6,9**	**-2,30**		**0,022**		**0,019**	**0,33 (0,14-0,75)**
G-A	4,0	6,5	0,36		0,720		0,808	1,70 (0,57-4,99)
Disability	**(n= 204)**	**(n=48)**						
A-T	64,4	61,9	-0,67	0,447	0,505	0,173	0,531	1 (0,00-0,00)
A-A	17,4	18,3	0,49		0,623		0,466	1,05 (0,52-2,11)
G-T	14,3	11,9	-0,31		0,756		0,455	0,82 (0,36-1,83)
G-A	3,9	7,9	1,47		0,143		0,064	2,28 (0,75-6,86)
Pain and Disability	**(n=197)**	**(n=55)**						
A-T	63,5	65,1	0,09	0,515	0,925	0,239	0,994	1 (0,00-0,00)
A-A	17,6	17,6	0,32		0,751		0,589	0,94 (0,48-1,80)
G-T	14,9	0,0	-0,94		0,348		0,219	0,64 (0,28-1,41)
G-A	4,0	7,0	0,92		0,355		0,207	1,89 (0,63-5,63)

Supplemental Table 14: Unadjusted- Genotype and minor allele frequency distributions, of the *COMT* (rs6269 A>G, rs4633 C>T, rs4818 C>G, rs4680 G>A) polymorphisms between pain, disability, and combined (pain and disability) categories.

	Pain			Disability			Pain and Disability		
	No-Low	Mod -High		No-Low	Mod-High		No-Low	Mod-High	
Polymorphisms	**(n=184)**	**(n=68)**	**OR (95% CI)**	**(n= 204)**	**(n=48)**	**OR (95% CI)**	**(n=197)**	**(n=55)**	**OR (95% CI)**
rs6269 A>G	(n=182)	(n=66)		(n=201)	(n=47)		(n=194)	(n=54)	
G/G	39,3 (71)	36,4 (24)	1 (0.00-0.00)	38,3 (78)	36,2 (17)	1 (0.00-0.00)	38,1 (74)	38,9 (21)	1 (0.00-0.00)
A/G	45,4 (82)	45,5 (30)	1,08 (0,58-2,02)	44,4 (90)	46,8 (22)	1,12 (0,56-2,26)	45,9 (89)	42,6 (23)	0,91 (0,47-1,77)
A/A	15,1 (29)	18,2 (12)	1,22 (0,54-2,77)	16,1 (33)	17,0 (8)	1,11 (0,44-2,83)	16,0 (31)	18,5 (10)	1,14 (0,48-2,69)
G allele	61,5 (224)	59,1 (78)	1,11 (0,72-1,69)	61,2 (246)	59,6 (56)	1,07 (0,66-1,73)	61,1 (237)	60,2 (65)	1,04 (0,65-1,64)
P value1	0,889			0,945			0,874		
G Allele P value2	0,677			0,815			0,911		
HWE	0,532	0,617		0,457	1		0,651	0,406	
rs4633 C>T	(n=182)	(n=66)		(n=201)	(n=47)		(n=194)	(n=54)	
T/T	33,3 (61)	28,8 (19)	1 (0.00-0.00)	34,3 (69)	23,4 (11)	1 (0.00-0.00)	34,5 (67)	24,1 (13)	1 (0.00-0.00)
C/T	46,4 (85)	47,0 (31)	1,17 (0,61-2,26)	44,4 (89)	57,4 (27)	1,90 (0,88-4,10)	45,4 (88)	51,9 (28)	1,64 (0,79-3,40)
C/C	19,1 (36)	24,2 (16)	1,43 (0,65-3,12)	21,2 (43)	19,1 (9)	1,31 (0,50-3,43)	20,1 (39)	24,1 (13)	1,72 (0,72-4,08)
T allele	56,9 (207)	52,3 (69)	1,20 (0,79-1,83)	56,5 (227)	52,1 (49)	1,19 (0,74-1,92)	57,2 (222)	50,0 (54)	1,34 (0,85-2,10)
P value1	0,674			0,228			0,331		
T Allele P value[2]	0,413			0,490			0,190		
HWE	0,546	0,628		0,154	0,389		0,307	1	

rs4818 C>G	(n=178)	(n=66)		(n=198)	(n=46)		(n=191)	(n=53)	
C/C	52,5 (93)	53,0 (35)	1 (0.00-0.00)	52,5 (103)	54,3 (25)	1 (0.00-0.00)	51,3 (98)	56,6 (30)	1 (0.00-0.00)
C/G	42,4 (76)	42,4 (28)	0,98 (0,55-1,75)	42,4 (85)	41,3 (19)	0,92 (0,48-1,79)	43,5 (83)	39,6 (21)	0,83 (0,44-1,55)
G/G	5,5 (9)	4,5 (3)	0,89 (0,23-3,46)	5,5 (10)	4,3 (2)	0,82 (0,17-4,00)	5,2 (10)	3,8 (2)	0,65 (0,14-3,15)
G allele	26,4 (94)	25,8 (34)	0,97 (0,59-1,55)	26,5 (105)	25,0 (23)	0,92 (0,52-1,59)	27,0 (103)	23,6 (25)	0,84 (0,48-1,50)
P value1	0,984			0,951			0,758		
G Allele P value2	0,908			0,895			0,534		
HWE	0,247	0,526		0,201	0,702		0,199	0,707	
rs4680 G>A	(n=180)	(n=66)		(n=199)	(n=47)		(n=192)	(n=54)	
G/G	40,4 (72)	27,3 (18)	1 (0.00-0.00)	39,3 (78)	25,5 (12)	1 (0.00-0.00)	40,1 (77)	24,1 (13)	1 (0.00-0.00)
A/G	46,4 (83)	50,0 (33)	1,59 (0,83-3,06)	45,4 (90)	55,3 (26)	1,88 (0,89-3,97)	45,8 (88)	51,9 (28)	1,88 (0,91-3,89)
A/A	13,1 (25)	22,7 (15)	2,40 (1,05-5,46)	15,1 (31)	19,1 (9)	1,89 (0,72-4,92)	14,1 (27)	24,1 (13)	2,85 (1,18-6,91)
A allele	36,9 (133)	47,7 (63)	1,83 (1,19-2,82)	38,2 (152)	46,8 (44)	1,42 (0,88-2,30)	37,0 (142)	50,0 (54)	1,70 (1,08-2,68)
P value1	0,101			0,203			0,051		
A Allele P value2	**0,004**			0,130			**0,019**		
HWE	0,874	1		0,55	0,564		0,877	1	

Notes: Genotype and allele frequencies are expressed as a percentage (%) with the number of participants (n) in parentheses. Global P values[1] for genotype between groups and P values[2] for allele between groups; P-values in bold indicate significance ($P<0.05$). P values for logistic regression analysis are listed for the dominant [a], over-dominant [b], and recessive [c] models. P-values for the exact test of Hardy–Weinberg equilibrium for each of the categories are included in the Table; Abbreviations: AIC: Akaike information criterion score; Mod-High: Moderate-High; HWE: Hardy–Weinberg equilibrium.

Supplemental Table 15: Inferred *COMT* (rs6269 A>G-rs4633 C>T-rs4818 C>G-rs4680 G>A) haplotype analyses for the pain, disability and combined (pain, and disability) categories.

COMT	Frequency %			Unadjusted		*Adjusted		
	No-Low	Mod-High	HS	Global P	Specific P	Global P	Specific P	OR (95%CI)
				rs6269 A>G-rs4633 C>T-rs4818 C>G-rs4680 G>A				
Pain	**(n=184)**	**(n=68)**						
G-C-C-A	34,0	9,0	1,05	0,154	0,294	0,076	0,122	1 (0,00-0,00)
A-T-G-G	22,3	0,5	-0,39		0,700		0,225	0,81 (0,45-1,47)
G-T-C-G	19,7	4,5	-1,39		0,166		0,081	0,63 (0,33-1,18)
A-T-C-G	11,9	0,6	-0,91		0,361		0,727	0,63 (0,31-1,31)
G-C-C-G	3,9	0,5	-0,90		0,367		0,383	0,39 (0,10-1,52)
G-C-G-G	2,6	0,2						1,50 (0,80-2,82)
A-T-C-A	1,6	0,7						1,50 (0,80-2,82)
A-C-C-G	1,5	0,9						1,50 (0,80-2,82)
G-T-G-T	1,4	0,1						1,50 (0,80-2,82)
A-C-C-A	1,2	0,0						1,50 (0,80-2,82)
A-C-G-G	0,0	0,2						1,50 (0,80-2,82)
G-C-G-A								
Disability	**(n= 204)**	**(n=48)**						
G-C-C-A	34,8	7,4	0,57	0,556	0,569	0,461	0,418	1 (0,00-0,00)
A-T-G-G	22,4	9,3	-0,61		0,545		0,471	0,80 (0,41-1,58)
G-T-C-G	19,4	4,1	-1,24		0,214		0,162	0,65 (0,31-1,35)
A-T-C-G	11,3	0,4	-0,29		0,775		0,894	0,82 (0,37-1,80)
G-C-C-G	3,9	0,1	0,01		0,992		0,833	0,78 (0,23-2,58)
A-T-C-A	2,2	0,9						1,37 (0,69-2,70)
G-C-G-G	1,9							1,37 (0,69-2,70)
G-T-G-G	1,2	0,4						1,37 (0,69-2,70)
A-C-C-A	1,0	0,5						1,37 (0,69-2,70)
A-C-C-G	0,9	0,0						1,37 (0,69-2,70)
A-C-G-G	0,9	0,2						1,37 (0,69-2,70)
G-C-G-A	0,0	0						
Pain and Disability	**(n=197)**	**(n=55)**						
G-C-C-A	34,0	9,9	1,22	0,139	0,222	0,073	0,112	1 (0,00-0,00)
A-T-G-G	22,9	7,7	-1,15		0,251		0,194	0,64 (0,33-1,23)
G-T-C-G	19,8	3,2	-1,63		0,102		0,065	0,54 (0,27-1,09)
A-T-C-G	11,4	0,1	-0,46		0,643		0,747	0,72 (0,34-1,52)
G-C-C-G	3,4	0,5	-0,29		0,772		0,587	0,55 (0,16-1,89)
G-C-G-G	2,7	0,4						1,41 (0,73-2,70)
A-T-C-A	1,8	0,9						1,41 (0,73-2,70)
A-C-C-G	1,7	0,0						1,41 (0,73-2,70)
G-T-G-G	1,3	0,2						1,41 (0,73-2,70)
A-C-C-A	1,0	0,2						1,41 (0,73-2,70)
A-C-G-G	0,0	0,0						1,41 (0,73-2,70)
G-C-G-A								

Supplemental Table 16: Inferred *COMT* (rs4633 C>T-rs4818 C>G-rs4680 G>A) haplotype analyses for the pain, disability and combined (pain, and disability) categories.

COMT	Frequency %			Unadjusted		*Adjusted		
	No-Low	Mod-High	HS	Global P	Specific P	Global P	Specific P	OR (95%CI)
				rs4633 C>T-rs4818 C>G-rs4680 G>A				
Pain	**(n=184)**	**(n=68)**						
C-C-A	35,3	0,2	1,13	**0,033**	0,256	**0,018**	0,106	1 (0,00-0,00)
T-C-G	31,5	3,7	-1,72		0,085		**0,046**	**0,65 (0,38-1,09)**
T-G-G	23,8	1,0	-0,55		0,580		0,180	0,77 (0,44-1,38)
C-C-G	5,4	0,8	-1,08		0,281		0,606	0,39 (0,12-1,29)
C-G-G	2,5	0,7						1,82 (0,90-3,68)
T-C-A	1,5	0,5						1,82 (0,90-3,68)
C-G-A	0,0	0,0						
Disability	**(n= 204)**	**(n=48)**						
C-C-A	35,9	9,6	0,79	0,345	0,428	0,301	0,298	1 (0,00-0,00)
T-C-G	30,6	4,2	-1,24		0,216		0,203	0,71 (0,39-1,27)
T-G-G	23,7	0,8	-0,55		0,581		0,517	0,79 (0,41-1,52)
C-C-G	4,9	0,1	-0,36		0,720		0,599	0,64 (0,21-2,00)
C-G-G	2,8	0,2						1,47 (0,71-3,05)
T-C-A	2,2	0,2						1,47 (0,71-3,05)
C-G-A	0,0	0,0						
Pain and Disability	**(n=197)**	**(n=55)**						
C-C-A	35,1	1,7	1,40	**0,036**	0,160	**0,023**	0,076	1 (0,00-0,00)
T-C-G	31,2	2,9	-1,70		0,090		0,073	0,61 (0,35-1,07)
T-G-G	24,3	8,8	-1,14		0,253		0,204	0,64 (0,34-1,20)
C-C-G	5,1	0,5	-0,64		0,522		0,401	0,47 (0,15-1,52)
C-G-G	2,6	0,8						1,64 (0,81-3,30)
T-C-A	1,7	0,3						1,64 (0,81-3,30)
C-G-A	0,0	0,0						

Supplemental Table 17: Inferred *COMT* (rs4818 C>G-rs4680 G>A), (rs4633 C>T-rs4680 G>A) and (rs6269 A>G-rs4680 G>A) haplotype analyses for the pain, disability and combined (pain, and disability) categories.

COMT	Frequency %			Unadjusted		*Adjusted		
	No-Low	Mod-High	HS	Global P	Specific P	Global P	Specific P	OR (95%CI)
rs4818 C>G-rs4680 G>A								
Pain	**(n=184)**	**(n=68)**						
C-A	36,9	7,7	2,16	0,056	0,031	**0,013**	**0,007**	1 (0,00-0,00)
C-G	36,8	6,5	-2,17		0,030		**0,009**	0,56 (0,34-0,90)
G-G	26,3	5,8	-0,13		0,893		0,828	0,77 (0,46-1,29)
G-A	0,0	0,0						
Disability	**(n= 204)**	**(n=48)**						
C-A	38,1	6,8	1,53	0,274	0,125	0,162	0,062	1 (0,00-0,00)
C-G	35,4	8,2	-1,34		0,180		0,139	0,65 (0,38-1,12)
G-G	26,5	5,0	-0,32		0,746		0,616	0,78 (0,43-1,39)
G-A	0,0	0,0						
Pain and Disability	**(n=197)**	**(n=55)**						
C-A	36,9	0,0	2,44	0,045	0,015	0,014	0,004	1 (0,00-0,00)
C-G	36,2	6,4	-1,92		0,055		0,032	0,54 (0,32-0,91)
G-G	26,9	3,6	-0,75		0,456		0,335	0,65 (0,37-1,14)
G-A	0,0	0,0						
rs4633 C>T-rs4680 G>A								
Pain	**(n=184)**	**(n=68)**						
T-G	55,3	44,8	-1,91	**0,036**	0,056	**0,014**	**0,033**	1 (0,00-0,00)
C-A	35,3	40,2	1,13		0,256		0,106	1,43 (0,92-2,23)
C-G	7,9	7,5	-0,27		0,787		0,533	1,11 (0,58-2,13)
T-A	1,5	7,5						3,09 (1,25-7,64)
Disability	**(n= 204)**	**(n=48)**						
T-G	54,3	44,9	-1,5	0,208	0,137	0,133	0,111	1 (0,00-0,00)
C-A	35,8	39,6	0,8		0,428		0,297	1,35 (0,82-2,22)
C-G	7,7	8,3	0,0		1,000		0,815	1,18 (0,58-2,40)
T-A	2,2	7,2						2,32 (0,97-5,53)
Pain and Disability	**(n=197)**	**(n=55)**						
T-G	55,5	41,7	-2,33	**0,019**	**0,020**	**0,007**	**0,012**	1 (0,00-0,00)
C-A	35,1	41,7	1,40		0,160		0,076	1,61 (1,00-2,61)
C-G	7,7	8,3	-0,02		0,981		0,763	1,28 (0,65-2,52)
T-A	1,7	8,3						3,22 (1,33-7,81)

Table Continued..... 1

				rs6269 A>G-rs4680 G>A				
Pain	**(n=184)**	**(n=68)**						
A-G	35,4	30,2	-0,79	**0,031**	0,432	**0,010**	0,447	0,77 (0,47-1,25)
G-A	33,8	37,0	0,97		0,334		0,146	1(0,00-0,00)
G-G	27,7	22,1	-1,64		0,101		**0,026**	0,67 (0,38-1,18)
A-A	3,1	10,7	2,60		**0,009**		**0,007**	2,09 (0,89-4,88)
Disability	**(n= 204)**	**(n=48)**						
A-G	35,1	28,0	-0,8	0,107	0,399	0,068	0,398	0,79 (0,45-1,38)
G-A	34,4	34,4	0,5		0,637		0,472	1(0,00-0,00)
G-G	26,8	25,2	-0,9		0,390		0,236	0,83 (0,44-1,59)
A-A	3,7	12,4	2,3		0,020		0,014	2,09 (0,88-5,00)
Pain and Disability	**(n=197)**	**(n=55)**						
A-G	35,7	27,4	-1,23	**0,014**	0,218	**0,005**	0,207	0,68 (0,40-1,15)
G-A	33,7	37,6	1,15		0,249		0,125	1(0,00-0,00)
G-G	27,4	22,6	-1,48		0,140		0,058	0,66 (0,36-1,23)
A-A	3,2	12,4	2,83		**0,005**		**0,003**	2,18 (0,92-5,17)

Supplemental Table 18: Inferred *ABCB1* (rs1045642 G>A) - *OPRM1* (rs1799971 A>G-rs540825 T>A) allele-allele combination analyses for the pain, disability and combined (pain and disability) categories.

	Frequency %			Unadjusted		*Adjusted		
	No-Low	Mod-High	HS	Global P	Specific P	Global P	Specific P	OR (95%CI)
				***ABCB1* (rs1045642 G>A) - *OPRM1* (rs1799971 A>G-rs540825 T>A)**				
Pain	**(n=184)**	**(n=68)**						
G-A-T	38,6	48,4	1,77	0,133	0,077	0,106		1 (0,00-0,00)
A-A-T	22,6	23,1	-0,09		0,928		0,868	0,83 (0,47-1,46)
G-G-T	11,7	4,2	-2,11		0,035		0,027	0,25 (0,08-0,81)
G-A-A	11,6	11,9	0,31		0,758			0,76 (0,35-1,63)
A-A-A	6,8	3,2	-1,11		0,267		0,281	0,32 (0,08-1,31)
A-G-T	4,7	2,7	-1,25		0,212		0,211	0,44 (0,10-1,99)
G-G-A	2,1	4,1						1,58 (0,51-4,88)
A-G-A	1,9	2,3						1,58 (0,51-4,88)
Disability	**(n= 204)**	**(n=48)**						
G-A-T	40,0	45,7	1,16	0,123	0,245	0,053		1 (0,00-0,00)
A-A-T	24,4	16,0	-2,14		0,032		0,026	0,60 (0,30-1,18)
G-A-A	10,6	18,5	1,60		0,109			1,42 (0,67-2,97)
G-G-T	10,0	8,4	-0,06		0,949		0,760	0,67 (0,23-1,94)
A-A-A	6,8	0,0	-1,56		0,120		0,146	0,00 (0,00-0,00)
A-G-T	4,3	4,1	-0,44		0,662		0,522	0,82 (0,18-3,73)
G-G-A	2,2	3,5						1,73 (0,52-5,78)
A-G-A	1,8	3,9						1,73 (0,52-5,78)
Pain and Disability	**(n=197)**	**(n=55)**						
G-A-T	39,1	48,2	1,84	0,061	0,066	**0,027**		1 (0,00-0,00)
A-A-T	24,4	16,7	-2,08		0,038		**0,029**	0,58 (0,31-1,09)
G-G-T	10,5	7,2	-0,64		0,525		0,396	0,51 (0,18-1,45)
G-A-A	10,3	17,8	1,63		0,103			1,29 (0,63-2,63)
A-A-A	7,3	0,0	-1,93		0,054		0,066	0,00 (0,00-0,00)
A-G-T	4,4	3,5	-0,80		0,425		0,317	0,64 (0,14-2,85)
G-G-A	2,3	3,2						1,41 (0,43-4,67)
A-G-A	1,7	3,4						1,41 (0,43-4,67)

Supplemental Table 19: Inferred *ABCB1* (rs1045642 G>A) - *OPRM1* (rs1799971 A>G) and *ABCB1* (rs1045642 G>A) - *OPRM1* (rs540825 T>A allele-allele combination analyses for the pain, disability and combined (pain and disability) categories.

COMT	Frequency %			Unadjusted		*Adjusted		
	No-Low	Mod-High	HS	Global P	Specific P	Global P	Specific P	OR (95%CI)
***ABCB1* (rs1045642 G>A)-*OPRM1* (rs1799971 A>G)**								
Pain	**(n=184)**	**(n=68)**						
G-A	50,1	60,1	1,82	0,234	0,069	0,244	0,068	1 (0,00-0,00)
A-A	29,4	26,5	-0,48		0,634		0,588	0,76 (0,47-1,24)
G-G	13,9	8,6	-1,52		0,128		0,138	0,52 (0,22-1,24)
A-G	6,5	4,8	-1,22		0,222		0,245	0,64 (0,20-2,11)
Disability	**(n= 204)**	**(n=48)**						
G-A	50,5	63,2	1,97	0,096	0,049	0,077	0,035	1 (0,00-0,00)
A-A	31,3	17,0	-2,48		0,013		0,011	0,46 (0,24-0,88)
G-G	12,3	12,8	0,65		0,513		0,549	0,87 (0,38-1,99)
A-G	6,0	7,0	-0,31		0,757		0,672	0,89 (0,27-2,88)
Pain and Disability	**(n=197)**	**(n=55)**						
G-A	49,3	65,2	2,64	**0,041**	**0,008**	**0,028**	**0,005**	1 (0,00-0,00)
A-A	31,8	17,6	-2,59		**0,010**		**0,008**	0,44 (0,24-0,80)
G-G	12,8	11,2	-0,02		0,988		0,962	0,69 (0,30-1,56)
A-G	6,1	6,1	-0,76		0,445		0,380	0,73 (0,23-2,31)
***ABCB1* (rs1045642 G>A) - *OPRM1* (rs540825 T>A)**								
Pain	**(n=184)**	**(n=68)**						
G-T	50,2	52,3	0,60	0,708	0,549	0,684	0,594	1 (0,00-0,00)
A-T	27,3	25,8	-0,57		0,566		0,515	0,91 (0,55-1,51)
G-A	13,8	16,3	0,48		0,630		0,517	1,14 (0,59-2,18)
A-A	8,6	5,5	-0,97		0,335		0,372	0,59 (0,20-1,69)
Disability	**(n= 204)**	**(n=48)**						
G-T	49,9	54,6	1,08	0,050	0,280	**0,026**	0,328	1 (0,00-0,00)
A-T	28,8	19,3	-2,16		0,031		**0,019**	0,62 (0,33-1,16)
G-A	12,9	21,4	2,00		0,046		**0,021**	1,57 (0,80-3,10)
A-A	8,5	4,7	-1,11		0,267		0,337	0,46 (0,12-1,84)
Pain and Disability	**(n=197)**	**(n=55)**						
G-T	49,5	55,7	1,44	**0,028**	0,149	**0,014**	0,168	1 (0,00-0,00)
A-T	28,9	19,8	-2,24		**0,025**		**0,014**	0,62 (0,35-1,10)
G-A	12,7	20,7	1,86		0,063		**0,030**	1,50 (0,78-2,86)
A-A	9,0	3,8	-1,57		0,117		0,150	0,34 (0,08-1,37)

Supplemental Table 20: Inferred *OPRM1* (rs1799971 A>G-rs540825 T>A)- *COMT* (rs4680 G>A) allele-allele combination analyses for the pain, disability and combined (pain and disability) categories.

	Frequency %			Unadjusted		*Adjusted		
	No-Low	Mod-High	HS	Global P	Specific P	Global P	Specific P	OR (95%CI)
***OPRM1* (rs1799971 A>G-rs540825 T>A)- *COMT* (rs4680 G>A)**								
Pain	**(n=184)**	**(n=68)**						
A-T-G	39,6	40,7	-0,36	**0,031**	0,721	**0,014**	0,494	1 (0,00-0,00)
A-T-A	21,5	30,7	2,44		**0,015**		**0,008**	1,36 (0,77-2,41)
A-A-G	12,1	8,7	-0,85		0,394		0,222	0,64 (0,24-1,75)
G-T-G	9,6	0,0	-3,02		**0,003**		**0,004**	0,00 (0,00-0,00)
G-T-A	6,9	7,0	-0,42		0,677		0,574	0,93 (0,36-2,35)
A-A-A	6,4	6,5	0,53		0,599		0,208	0,88 (0,31-2,53)
G-A-A	2,1	3,6						2,13 (0,63-7,26)
G-A-G	1,8	2,9						2,13 (0,63-7,26)
Disability	**(n= 204)**	**(n=48)**						
A-T--G	40,3	37,8	-1,05	0,348	0,293	0,231	0,207	1 (0,00-0,00)
A-T--A	24,1	23,9	0,41		0,682		0,575	1,11 (0,56-2,22)
A-A--G	11,0	12,1	0,15		0,882		0,949	1,13 (0,39-3,26)
G-T--G	8,3	1,6	-1,64		0,101		0,111	0,18 (0,02-1,52)
A-A--A	6,4	6,3	0,71		0,480		0,120	1,10 (0,34-3,57)
G-T--A	6,1	10,6	1,18		0,239		0,495	1,82 (0,69-4,83)
G-A--G	2,2	1,8						2,57 (0,71-9,35)
G-A--A	1,7	5,8						2,57 (0,71-9,35)
Pain and Disability	**(n=197)**	**(n=55)**						
A-T-G	41,1	35,5	-1,50	0,200	0,133	0,063	0,075	1 (0,00-0,00)
A-T-A	22,4	29,4	1,81		0,071		0,043	1,55 (0,82-2,93)
A-A-G	11,3	11,0	-0,31		0,758		0,570	1,09 (0,39-3,04)
G-T-G	8,4	1,6	-2,02		0,044		0,048	0,19 (0,02-1,61)
G-T-A	6,5	8,9	0,60		0,547		0,867	1,58 (0,60-4,21)
A-A-A	6,3	6,7	0,99		0,323		0,059	1,24 (0,42-3,69)
G-A-G	2,2	1,9						2,44 (0,69-8,65)
G-A-A	1,8	4,9						2,44 (0,69-8,65)

Supplemental Table 21: Inferred *OPRM1* (rs1799971 A>G)- *COMT* (rs4680 G>A) and *OPRM1* (rs540825 T>A)- *COMT* (rs4680 G>A) allele-allele combination analyses for the pain, disability and combined (pain and disability) categories.

	Frequency %			Unadjusted		*Adjusted		
	No-Low	Mod-High	HS	Global P	Specific P	Global P	Specific P	OR (95%CI)
***OPRM1* (rs1799971 A>G)-*COMT* (rs4680 G>A)**								
Pain	**(n=184)**	**(n=68)**						
A-G	51,7	49,7	-0,77	**0,026**	0,442	**0,011**	0,193	1 (0,00-0,00)
A-A	27,9	36,9	2,36		**0,018**		**0,004**	1,35 (0,85-2,15)
G-G	11,4	2,6	-2,64		**0,008**		**0,010**	0,23 (0,05-1,03)
G-A	9,0	10,8	-0,05		0,964		0,992	1,27 (0,63-2,58)
Disability	**(n= 204)**	**(n=48)**						
A-G	51,3	50,1	-0,90	0,094	0,370	0,135	0,219	1 (0,00-0,00)
A-A	30,4	30,2	0,65		0,514		0,267	1,05 (0,61-1,80)
G-G	10,5	3,3	-1,31		0,191		0,216	0,33 (0,07-1,54)
G-A	7,8	16,5	1,76		0,078		0,116	2,07 (1,02-4,20)
Pain and Disability	**(n=197)**	**(n=55)**						
A-G	52,4	46,7	-1,55	0,061	0,121	**0,027**	**0,046**	1 (0,00-0,00)
A-A	28,7	36,0	2,00		0,046		**0,010**	1,42 (0,85-2,35)
G-G	10,7	3,4	-1,78		0,075		0,085	0,36 (0,08-1,66)
G-A	8,3	13,9	1,11		0,266		0,336	1,87 (0,91-3,81)
***OPRM1* (rs540825 T>A) - *COMT* (rs4680 G>A)**								
Pain	**(n=184)**	**(n=68)**						
T-G	49,1	40,2	-1,77	0,198	0,077	0,052	0,040	1 (0,00-0,00)
T-A	28,5	37,9	1,99		0,047		0,032	1,61 (0,95-2,71)
A-G	13,9	12,0	-0,81		0,420		0,248	1,04 (0,44-2,46)
A-A	8,4	9,9	0,77		0,444		0,112	1,44 (0,63-3,26)
Disability	**(n= 204)**	**(n=48)**						
T-G	48,6	38,8	-1,76	0,343	0,078	0,079	0,048	1 (0,00-0,00)
T-A	30,1	35,1	0,95		0,343		0,394	1,46 (0,79-2,70)
A-G	13,2	14,4	0,24		0,809		0,942	1,40 (0,54-3,63)
A-A	8,1	11,7	1,45		0,147		0,014	1,81 (0,76-4,31)
Pain and Disability	**(n=197)**	**(n=55)**						
T-G	49,5	36,6	-2,38	0,096	0,017	**0,016**	**0,008**	1 (0,00-0,00)
T-A	28,9	38,8	1,93		0,054		0,053	1,81 (1,02-3,22)
A-G	13,5	13,3	-0,30		0,765		0,603	1,35 (0,54-3,41)
A-A	8,0	11,2	1,48		0,138		**0,012**	1,89 (0,81-4,38)

Supplemental Table 22: Inferred *ABCB1* (rs1045642 G>A- rs1128503 G>A) - *COMT* (rs4680 G>A) allele-allele combination analyses for the pain, disability and combined (pain and disability) categories.

	Frequency %			Unadjusted		*Adjusted		
	No-Low	Mod-High	HS	Global P	Specific P	Global P	Specific P	OR (95%CI)
***ABCB1* (rs1045642 G>A- rs1128503 G>A) - *COMT* (rs4680 G>A)**								
Pain	**(n=184)**	**(n=68)**						
G-G-G	33,5	25,2	-1,46	0,207	0,144	0,102	0,112	1 (0,00-0,00)
G-G-A	19,6	31,7	2,54		0,011			2,22 (1,15-4,28)
A-A-G	17,1	17,4	-0,55		0,583		0,306	1,44 (0,69-3,01)
A-A-A	12,6	9,7	-0,28		0,782		0,991	0,99 (0,44-2,21)
G-A-G	7,7	6,6	-0,36		0,718		0,512	1,13 (0,42-3,06)
A-G-G	4,7	3,2	-0,85		0,395		0,554	0,84 (0,22-3,15)
G-A-A	3,2	5,1					0,181	2,08 (0,64-6,71)
A-G-A	1,5	1,1						2,08 (0,64-6,71)
Disability	**(n= 204)**	**(n=48)**						
G-G-G	31,4	31,7	-0,09	0,097	0,925	0,087	0,936	1 (0,00-0,00)
G-G-A	21,4	28,7	1,67		0,094		0,116	1,30 (0,65-2,59)
A-A-G	18,9	10,4	-1,93		0,054		0,019	0,57 (0,21-1,54)
A-A-A	11,4	13,5	0,12		0,908		0,778	1,13 (0,51-2,50)
G-A-G	6,4	10,9	1,51		0,130		0,178	1,65 (0,67-4,07)
A-G-G	5,2	0,0	-2,28		0,023		0,036	0,00 (0,00-0,00)
G-A-A	3,6	4,7						0,95 (0,24-3,72)
A-G-A	1,8	0,0						0,95 (0,24-3,72)
Pain and Disability	**(n=197)**	**(n=55)**						
G-G-G	32,5	28,0	-0,83	**0,031**	0,404	**0,008**	0,378	1 (0,00-0,00)
G-G-A	20,0	32,7	2,79		**0,005**			1,90 (0,97-3,71)
A-A-G	19,0	10,5	-2,26		**0,024**		**0,006**	0,68 (0,27-1,71)
A-A-A	11,9	12,0	-0,14		0,890		0,977	1,12 (0,50-2,50)
G-A-G	6,5	10,3	1,24		0,214		0,303	1,81 (0,72-4,50)
A-G-G	5,0	1,1	-1,92		0,054		0,090	0,26 (0,03-2,14)
G-A-A	3,3	5,4					**0,028**	1,37 (0,39-4,80)
A-G-A	1,9	0,0						1,37 (0,39-4,80)

Supplemental Table 23: Inferred *ABCB1* (rs1045642 G>A) - *COMT* (rs4680 G>A) and *ABCB1* (rs1128503 G>A) - *COMT* (rs4680 G>A) allele-allele combination analyses for the pain, disability and combined (pain and disability) categories.

	Frequency %			Unadjusted		*Adjusted		
	No-Low	Mod-High	HS	Global P	Specific P	Global P	Specific P	OR (95%CI)
***ABCB1* (rs1128503 G>A) – *COMT* (rs4680 G>A)**								
Pain	**(n=184)**	**(n=68)**						
G-G	38,3	28,6	-1,71	0,092	0,087	**0,030**	0,079	1 (0,00-0,00)
G-A	24,8	23,8	-0,76		0,448		0,172	1,31 (0,68-2,53)
A-G	21,1	32,6	2,33		0,02		**0,005**	2,08 (1,12-3,84)
A-A	15,9	15	0,36		0,721		0,519	1,30 (0,69-2,45)
Disability	**(n= 204)**	**(n=48)**						
G-G	36,6	31,4	-1	0,505	0,318	0,311	0,364	1 (0,00-0,00)
G-A	25,2	21,8	-0,78		0,435		0,206	1,02 (0,48-2,17)
A-G	23,1	29	1,25		0,213		0,145	1,47 (0,74-2,89)
A-A	15,1	17,8	0,74		0,456		0,325	1,37 (0,70-2,68)
Pain and Disability	**(n=197)**	**(n=55)**						
G-G	37,5	29,2	-1,56	0,092	0,119	**0,028**	0,128	1 (0,00-0,00)
A-G	25,5	20,9	-1,27		0,203		0,066	1,07 (0,51-2,22)
G-A	21,8	32,6	2,29		0,022		**0,008**	1,94 (1,02-3,69)
A-A	15,2	17,3	0,8		0,422		0,293	1,48 (0,77-2,84)
***ABCB1* (rs1045642 G>A) – *COMT* (rs4680 G>A)**								
Pain	**(n=184)**	**(n=68)**						
G-G	41,1	31,9	-1,54	**0,032**	0,123	**0,008**	0,068	1 (0,00-0,00)
A-G	23	36,8	2,85		**0,004**		**0,001**	2,27 (1,25-4,10)
G-A	22	20,6	-0,88		0,377		0,244	1,30 (0,69-2,47)
A-A	13,9	10,8	-0,37		0,713		0,951	0,95 (0,45-1,98)
Disability	**(n= 204)**	**(n=48)**						
G-G	37,6	42,8	0,69	**0,044**	0,493	**0,019**	0,523	1 (0,00-0,00)
A-G	25,1	33,2	2,09		**0,037**		**0,018**	1,16 (0,62-2,15)
G-A	24,2	10,2	-2,72		**0,007**		**0,003**	0,38 (0,15-0,98)
A-A	13,1	13,8	-0,38		0,701		0,864	0,92 (0,43-1,94)
Pain and Disability	**(n=197)**	**(n=55)**						
G-G	38,9	38,2	-0,14	**0,004**	0,888	**0,001**	0,786	1 (0,00-0,00)
A-G	24,2	11,8	-2,85		**0,004**		**0,002**	0,53 (0,24-1,20)
G-A	23,3	38,2	3,35		**0,001**		**0**	1,75 (0,97-3,18)
A-A	13,7	11,8	-0,65		0,514		0,659	0,84 (0,39-1,83)

Supplemental Table 24: Inferred *ABCB1* (rs1045642 G>A)- *OPRM1* (rs1799971 A>G) - *COMT* (rs4680 G>A) allele combination analyses for the pain, disability and combined (pain and disability) categories.

	Frequency %			Unadjusted		*Adjusted		
	No-Low	Mod-High	HS	Global P	Specific P	Global P	Specific P	OR (95%CI)
***ABCB1* (rs1045642 G>A)- *OPRM1* (rs1799971 A>G) -*COMT* (rs4680 G>A)**								
Pain	**(n=184)**	**(n=68)**						
G-A-G	33,4	32,0	-0,60	**0,027**	0,58	**0,011**	0,392	1 (0,00-0,00)
A-A-G	18,2	18,3	-0,40		0,726		0,511	1,12(2,16-5,84)
G-A-A	16,8	28,8	3,20		**0,001**		**0,000**	1,93(1,01-3,69)
A-A-A	11,1	7,5	-0,40		0,719		0,933	6,91(1,70-2,81)
G-G-G	7,6	0,0	-2,50		**0,014**		**0,019**	0,00(0,00-0,00)
G-G-A	6,2	7,9	0,00		0,97		0,978	1,56(4,18-5,80)
A-G-G	3,8	2,3						9,35(2,92-3,00)
A-G-A	2,8	0,2						9,35(2,92-3,00)
Disability	**(n= 204)**	**(n=48)**						
G-A-G	30,4	40,6	1,10	**0,043**	0,272	**0,047**	0,328	1 (0,00-0,00)
A-A-G	20,9	9,3	-2,43		**0,015**		**0,008**	0,37(0,13-1,01)
G-A-A	19,9	22,6	1,43		0,152		0,064	0,89(0,43-1,83)
A-A-A	10,5	7,7	-0,87		0,383		0,522	0,66(0,25-1,78)
G-G-G	7,2	2,2	-0,89		0,373		0,476	0,20(0,02-1,75)
G-G-A	5,2	10,6	1,95		0,052		0,076	1,87(0,66-5,34)
A-G-G	3,2	1,2						0,78(0,19-3,21)
A-G-A	2,6	5,8						0,78(0,19-3,21)
Pain and Disability	**(n=197)**	**(n=55)**						
G-A-G	31,6	35,6	0,42	**0,022**	0,677	**0,008**	0,813	1 (0,00-0,00)
A-A-G	20,7	11,1	-2,47		**0,014**		**0,006**	0,51(0,22-1,20)
G-A-A	17,7	29,3	3,17		**0,002**		**0,000**	1,60(0,81-3,19)
A-A-A	11	6,6	-0,97		0,33		0,463	0,58(0,21-1,64)
G-G-G	7,2	2,7	-1,34		0,182		0,235	0,27(0,03-2,24)
G-G-A	5,6	8,7	1,26		0,208		0,268	1,66(0,56-4,90)
A-G-G	3,4	0,7						0,88(0,24-3,27)
A-G-A	2,6	5,2						0,88(0,24-3,27)

7.4.2 Bioinformatics

Supplemental Table 25: A summarised list of genes that form part of the functionally associated network for the *ABCB1* and *OPRM1* genes, obtained by GeneMANIA.

Gene 1	Gene 2	Weight (%)	Network group	Network
ABCB1	***ABCB4***	**2,06**	**Co-expression**	**Innocenti-Brown-2011**
	ABCB4	**3,93**	**Co-expression**	**Alizadeh-Staudt-2000**
	ABCB4	**1,11**	**Co-expression**	**Boldrick-Relman-2002**
	NEDD4	0,42	Co-expression	Arijs-Rutgeerts-2009
	EGR2	1,53	Co-expression	Perou-Botstein-2000
	ABCB4	**1,87**	**Co-expression**	**Rosenwald-Staudt-2001**
	PLD2	**1,98**	**Co-expression**	**Rosenwald-Staudt-2001**
	GNB1	**0,09**	**Genetic Interactions**	**Lin-Smith-2010**
	EGR2	12,70	Pathway	Wu-Stein-2010
	SLC22A3	38,59	Physical Interactions	IREF-reactome
	SLC22A1	38,59	Physical Interactions	IREF-reactome
	SLC22A2	**16,64**	**Physical Interactions**	**IREF-reactome**
	SLC22A3	38,59	Physical Interactions	Vastrik-Stein-2007
	SLC22A1	38,59	Physical Interactions	Vastrik-Stein-2007
	SLC22A2	**16,64**	**Physical Interactions**	**Vastrik-Stein-2007**
	NEDD4	33,03	Physical Interactions	IREF-quickgo
	NEDD4	1,79	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	ABCB4	**70,53**	**Predicted**	**Wu-Stein-2010**
	ABCC1	21,13	Predicted	Wu-Stein-2010
	ABCB4	**2,42**	**Shared protein domains**	**INTERPRO**
	ABCC1	2,37	Shared protein domains	INTERPRO
	ABCB4	**2,55**	**Shared protein domains**	**PFAM**
	ABCC1	2,55	Shared protein domains	PFAM
ABCB4	*ABCC1*	2,37	Shared protein domains	INTERPRO
	ABCC1	2,55	Shared protein domains	PFAM
GNB1	*GNAO1*	0,08	Pathway	Wu-Stein-2010
	GNAO1	0,31	Physical Interactions	IREF-reactome
	GNAO1	0,31	Physical Interactions	Vastrik-Stein-2007
	GNAO1	10,33	Predicted	I2D-BioGRID-Mouse2Human
GNG2	*FLNA*	2,22	Co-expression	Alizadeh-Staudt-2000
	FLNA	2,03	Co-expression	Rosenwald-Staudt-2001
	GNB1	0,08	Pathway	Wu-Stein-2010
	GNAO1	0,18	Pathway	Wu-Stein-2010
	GNB1	4,40	Pathway	REACTOME
	GNB1	0,04	Physical Interactions	IREF-reactome
	GNAO1	0,87	Physical Interactions	IREF-reactome
	GNB1	0,04	Physical Interactions	Vastrik-Stein-2007
	GNAO1	0,87	Physical Interactions	Vastrik-Stein-2007
	GNB1	15,05	Physical Interactions	IREF-dip

	GNB1	1,45	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	GNB1	45,11	Physical Interactions	IREF-bind-translation
	GNB1	60,78	Physical Interactions	IREF-bind
	GNB1	1,90	Physical Interactions	IREF-biogrid
	GNB1	16,58	Predicted	I2D-BioGRID-Mouse2Human
	GNAO1	21,25	Predicted	I2D-BioGRID-Mouse2Human
GRK2	*FLNA*	0,90	Co-expression	Jiang-de Kok-2017
	GNG2	0,34	Pathway	Wu-Stein-2010
	GNB1	0,15	Pathway	Wu-Stein-2010
	GNG2	0,29	Physical Interactions	IREF-reactome
	GNB1	0,10	Physical Interactions	IREF-reactome
	GNG2	0,29	Physical Interactions	Vastrik-Stein-2007
	GNB1	0,10	Physical Interactions	Vastrik-Stein-2007
	NEDD4	10,94	Physical Interactions	Persaud-Rotin-2009 A
	GNB1	0,94	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	GNB1	1,21	Physical Interactions	IREF-biogrid
	GNG2	21,28	Predicted	I2D-BioGRID-Mouse2Human
	GNB1	10,34	Predicted	I2D-BioGRID-Mouse2Human
NEDD4	*PLD2*	0,09	Genetic Interactions	Lin-Smith-2010
OPRD1	*PLD2*	1,13	Co-localization	Johnson-Shoemaker-2003
	RANBP9	0,08	Genetic Interactions	Lin-Smith-2010
	NEDD4	0,08	Genetic Interactions	Lin-Smith-2010
	ABCC1	0,10	Genetic Interactions	Lin-Smith-2010
	GRK2	1,49	Pathway	Wu-Stein-2010
	PENK	4,50	Pathway	Wu-Stein-2010
	GNG2	0,78	Pathway	Wu-Stein-2010
	GNB1	0,34	Pathway	Wu-Stein-2010
	GNAO1	0,80	Pathway	Wu-Stein-2010
	PENK	36,83	Pathway	IMID
	GNAO1	13,77	Pathway	IMID
	PENK	0,51	Physical Interactions	IREF-reactome
	GNG2	0,23	Physical Interactions	IREF-reactome
	GNB1	0,08	Physical Interactions	IREF-reactome
	PENK	0,51	Physical Interactions	Vastrik-Stein-2007
	GNG2	0,23	Physical Interactions	Vastrik-Stein-2007
	GNB1	0,08	Physical Interactions	Vastrik-Stein-2007
	GNAO1	5,93	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	GNAO1	52,87	Predicted	I2D-BIND-Mouse2Human
OPRL1	*OPRD1*	0,73	Co-expression	Roth-Zlotnik-2006
	OPRD1	1,18	Co-localization	Johnson-Shoemaker-2003
	GRK2	1,57	Pathway	Wu-Stein-2010
	GNG2	0,82	Pathway	Wu-Stein-2010
	GNB1	0,36	Pathway	Wu-Stein-2010
	GNAO1	0,84	Pathway	Wu-Stein-2010

	PENK	36,83	Pathway	IMID
	GNAO1	13,77	Pathway	IMID
	OPRD1	0,51	Physical Interactions	IREF-reactome
	PENK	0,51	Physical Interactions	IREF-reactome
	GNG2	0,23	Physical Interactions	IREF-reactome
	GNB1	0,08	Physical Interactions	IREF-reactome
	OPRD1	0,51	Physical Interactions	Vastrik-Stein-2007
	PENK	0,51	Physical Interactions	Vastrik-Stein-2007
	GNG2	0,23	Physical Interactions	Vastrik-Stein-2007
	GNB1	0,08	Physical Interactions	Vastrik-Stein-2007
	OPRD1	9,29	Shared protein domains	INTERPRO
	OPRD1	0,35	Shared protein domains	PFAM
OPRM1	*GRK2*	0,58	Co-expression	Burington-Shaughnessy-2008
	SLC22A2	**1,89**	**Co-expression**	**Wu-Garvey-2007**
	WLS	0,13	Genetic Interactions	Lin-Smith-2010
	ABCB4	**0,11**	**Genetic Interactions**	**Lin-Smith-2010**
	PENK	6,60	Pathway	Wu-Stein-2010
	GNG2	1,15	Pathway	Wu-Stein-2010
	GNB1	**0,50**	**Pathway**	**Wu-Stein-2010**
	GNAO1	1,17	Pathway	Wu-Stein-2010
	STAT6	22,78	Pathway	NCI_NATURE
	GNG2	23,22	Pathway	REACTOME
	GNB1	**23,22**	**Pathway**	**REACTOME**
	GRK2	43,78	Pathway	IMID
	PENK	23,19	Pathway	IMID
	GNAO1	8,67	Pathway	IMID
	OPRL1	0,50	Physical Interactions	IREF-reactome
	PENK	0,50	Physical Interactions	IREF-reactome
	GNG2	0,23	Physical Interactions	IREF-reactome
	GNB1	**0,08**	**Physical Interactions**	**IREF-reactome**
	GNAO1	1,86	Physical Interactions	IREF-reactome
	OPRL1	0,50	Physical Interactions	Vastrik-Stein-2007
	PENK	0,50	Physical Interactions	Vastrik-Stein-2007
	GNG2	0,23	Physical Interactions	Vastrik-Stein-2007
	GNB1	**0,08**	**Physical Interactions**	**Vastrik-Stein-2007**
	GNAO1	1,86	Physical Interactions	Vastrik-Stein-2007
	WLS	32,74	Physical Interactions	IREF-uniprotpp
	RANBP9	64,36	Physical Interactions	IREF-uniprotpp
	FLNA	38,27	Physical Interactions	IREF-uniprotpp
	WLS	13,84	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	VAPA	3,68	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	OPRL1	11,03	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	OPRD1	4,77	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES

	RANBP9	1,36	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	PLD2	**4,19**	**Physical Interactions**	**BIOGRID-SMALL-SCALE-STUDIES**
	GNAO1	3,96	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	WLS	9,55	Physical Interactions	IREF-mint
	VAPA	9,77	Physical Interactions	IREF-mint
	FLNA	18,83	Physical Interactions	IREF-matrixdb
	OPRL1	27,08	Predicted	Wu-Stein-2010
	OPRD1	31,48	Predicted	Wu-Stein-2010
	PLD2	**12,04**	**Predicted**	**Wu-Stein-2010**
	WLS	74,82	Predicted	I2D-IntAct-Mouse2Human
	VAPA	24,74	Predicted	I2D-IntAct-Mouse2Human
	WLS	66,16	Predicted	I2D-BioGRID-Mouse2Human
	VAPA	54,24	Predicted	I2D-BioGRID-Mouse2Human
	WLS	57,65	Predicted	I2D-IntAct-Rat2Human
	PLD2	**33,03**	**Predicted**	**I2D-IntAct-Rat2Human**
	OPRL1	9,29	Shared protein domains	INTERPRO
	OPRD1	9,29	Shared protein domains	INTERPRO
	OPRL1	0,35	Shared protein domains	PFAM
	OPRD1	0,35	Shared protein domains	PFAM
PENK	*EGR2*	1,29	Co-expression	Bild-Nevins-2006 B
	RANBP9	0,08	Genetic Interactions	Lin-Smith-2010
	GNG2	1,03	Pathway	Wu-Stein-2010
	GNB1	0,45	Pathway	Wu-Stein-2010
	GNAO1	1,06	Pathway	Wu-Stein-2010
	GNG2	0,23	Physical Interactions	IREF-reactome
	GNB1	0,08	Physical Interactions	IREF-reactome
	GNG2	0,23	Physical Interactions	Vastrik-Stein-2007
	GNB1	0,08	Physical Interactions	Vastrik-Stein-2007
SLC22A1	*ABCB4*	0,69	Co-expression	Roth-Zlotnik-2006
	OPRD1	2,58	Co-expression	Burington-Shaughnessy-2008
	PLD2	0,20	Genetic Interactions	Lin-Smith-2010
	SLC22A2	2,35	Shared protein domains	INTERPRO
	SLC22A2	2,92	Shared protein domains	PFAM
SLC22A2	*PLD2*	0,08	Genetic Interactions	Lin-Smith-2010
SLC22A3	*GRK2*	0,45	Co-expression	Burington-Shaughnessy-2008
	OPRD1	0,07	Genetic Interactions	Lin-Smith-2010
	SLC22A1	1,96	Shared protein domains	INTERPRO
	SLC22A2	1,93	Shared protein domains	INTERPRO
STAT6	*FLNA*	1,09	Co-expression	Rieger-Chu-2004
	EGR2	2,44	Pathway	Wu-Stein-2010
	EGR2	3,54	Pathway	NCI_NATURE
VAPA	*EGR2*	1,88	Co-expression	Wang-Cheung-2015
	GNG2	0,13	Genetic Interactions	Lin-Smith-2010
	NEDD4	0,11	Genetic Interactions	Lin-Smith-2010

	OPRD1	5,50	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
WLS	*PLD2*	1,31	Co-localization	Johnson-Shoemaker-2003
	PLD2	0,08	Genetic Interactions	Lin-Smith-2010
	GNAO1	0,07	Genetic Interactions	Lin-Smith-2010
	OPRD1	20,71	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES

Supplemental Table 26: A summarised list of genes that form part of the functionally associated network for the *OPRM1* and *COMT* genes, obtained by GeneMANIA.

Gene 1	Gene 2	Weight (%)	Network group	Network
AHCY	*PPA2*	1,24	Co-expression	Perou-Botstein-2000
	PENK	0,07	Genetic Interactions	Lin-Smith-2010
AP000812.4	*COMTD1*	2,51	Shared protein domains	INTERPRO
	COMTD1	42,69	Shared protein domains	PFAM
COMT	***RIN1***	**0,72**	**Co-expression**	**Arijs-Rutgeerts-2009**
	MAOA	41,95	Physical Interactions	IREF-reactome
	MAT1A	**22,13**	**Physical Interactions**	**IREF-reactome**
	MAT2B	18,77	Physical Interactions	IREF-reactome
	MAT2A	18,77	Physical Interactions	IREF-reactome
	AHCY	**13,19**	**Physical Interactions**	**IREF-reactome**
	MAOA	41,95	Physical Interactions	Vastrik-Stein-2007
	MAT1A	**22,13**	**Physical Interactions**	**Vastrik-Stein-2007**
	MAT2B	18,77	Physical Interactions	Vastrik-Stein-2007
	MAT2A	18,77	Physical Interactions	Vastrik-Stein-2007
	AHCY	**13,19**	**Physical Interactions**	**Vastrik-Stein-2007**
	FGF2	**50,26**	**Physical Interactions**	**Hein-Mann-2015**
	PPA2	35,85	Physical Interactions	Hein-Mann-2015
	RIN1	**35,85**	**Physical Interactions**	**Hein-Mann-2015**
	FGF2	**4,24**	**Physical Interactions**	**IREF-biogrid**
	PPA2	2,80	Physical Interactions	IREF-biogrid
	RIN1	**4,60**	**Physical Interactions**	**IREF-biogrid**
	AP000812.4	3,02	Shared protein domains	INTERPRO
	COMTD1	3,22	Shared protein domains	INTERPRO
	AP000812.4	42,69	Shared protein domains	PFAM
	COMTD1	42,69	Shared protein domains	PFAM
FGF2	*PENK*	0,46	Co-expression	Dobbin-Giordano-2005
	OPRD1	0,08	Genetic Interactions	Lin-Smith-2010
GNG2	*GNB1*	0,08	Pathway	Wu-Stein-2010
	GNB1	4,40	Pathway	REACTOME
	GNB1	0,04	Physical Interactions	IREF-reactome
	GNB1	0,04	Physical Interactions	Vastrik-Stein-2007
	GNB1	15,05	Physical Interactions	IREF-dip
	GNB1	1,45	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	GNB1	45,11	Physical Interactions	IREF-bind-translation
	GNB1	60,78	Physical Interactions	IREF-bind
	GNB1	1,90	Physical Interactions	IREF-biogrid
	GNB1	16,58	Predicted	I2D-BioGRID-Mouse2Human
GRK2	*GNG2*	0,34	Pathway	Wu-Stein-2010
	GNB1	0,15	Pathway	Wu-Stein-2010
	GNG2	0,29	Physical Interactions	IREF-reactome
	GNB1	0,10	Physical Interactions	IREF-reactome
	GNG2	0,29	Physical Interactions	Vastrik-Stein-2007

	GNB1	0,10	Physical Interactions	Vastrik-Stein-2007
	GNB1	0,94	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	GNB1	1,21	Physical Interactions	IREF-biogrid
	GNG2	21,28	Predicted	I2D-BioGRID-Mouse2Human
	GNB1	10,34	Predicted	I2D-BioGRID-Mouse2Human
MAOA	*MAT1A*	0,74	Co-expression	Innocenti-Brown-2011
	FGF2	0,12	Genetic Interactions	Lin-Smith-2010
MAT1A	*AHCY*	0,84	Co-expression	Jiang-de Kok-2017
	OPRL1	0,87	Co-expression	Wu-Garvey-2007
	MAT2A	32,78	Physical Interactions	Havugimana-Emili-2012
	MAT2B	43,97	Physical Interactions	Huttlin-Harper-2017
	MAT2A	43,97	Physical Interactions	Huttlin-Harper-2017
	MAT2B	70,71	Physical Interactions	IREF-corum
	MAT2B	41,76	Physical Interactions	Huttlin-Gygi-2015
	MAT2A	41,76	Physical Interactions	Huttlin-Gygi-2015
	MAT2A	79,62	Physical Interactions	IREF-huri
	MAT2B	9,58	Physical Interactions	IREF-biogrid
	MAT2A	7,24	Physical Interactions	IREF-biogrid
	MAT2A	87,65	Predicted	Wu-Stein-2010
	AHCY	1,13	Predicted	Stuart-Kim-2003
	MAT2A	100,00	Shared protein domains	INTERPRO
	MAT2A	100,00	Shared protein domains	PFAM
MAT2A	*AHCY*	1,95	Co-expression	Ross-Perou-2001
	AHCY	1,13	Predicted	Stuart-Kim-2003
MAT2B	*PPA2*	1,51	Co-expression	Dobbin-Giordano-2005
	MAT2A	3,13	Co-localization	Johnson-Shoemaker-2003
	MAT2A	57,64	Pathway	Wu-Stein-2010
	MAT2A	19,54	Physical Interactions	IREF-reactome
	MAT2A	19,54	Physical Interactions	Vastrik-Stein-2007
	MAT2A	7,70	Physical Interactions	Kristensen-Foster-2012
	MAT2A	100,00	Physical Interactions	IREF-quickgo
	MAT2A	20,02	Physical Interactions	Wan-Emili-2015
	MAT2A	53,33	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	MAT2A	55,05	Physical Interactions	Huttlin-Harper-2017
	MAT2A	70,71	Physical Interactions	IREF-corum
	MAT2A	55,05	Physical Interactions	Huttlin-Gygi-2015
	MAT2A	3,72	Physical Interactions	IREF-biogrid
	AHCY	1,03	Shared protein domains	INTERPRO
OPRD1	*RANBP9*	0,08	Genetic Interactions	Lin-Smith-2010
	GRK2	1,49	Pathway	Wu-Stein-2010
	PENK	4,50	Pathway	Wu-Stein-2010
	GNG2	0,78	Pathway	Wu-Stein-2010
	GNB1	0,34	Pathway	Wu-Stein-2010
	PENK	36,83	Pathway	IMID
	PENK	0,51	Physical Interactions	IREF-reactome

	GNG2	0,23	Physical Interactions	IREF-reactome
	GNB1	0,08	Physical Interactions	IREF-reactome
	PENK	0,51	Physical Interactions	Vastrik-Stein-2007
	GNG2	0,23	Physical Interactions	Vastrik-Stein-2007
	GNB1	0,08	Physical Interactions	Vastrik-Stein-2007
OPRL1	*OPRD1*	0,73	Co-expression	Roth-Zlotnik-2006
	RIN1	0,97	Co-expression	Burington-Shaughnessy-2008
	OPRD1	1,18	Co-localization	Johnson-Shoemaker-2003
	RIN1	1,11	Co-localization	Johnson-Shoemaker-2003
	GRK2	1,57	Pathway	Wu-Stein-2010
	GNG2	0,82	Pathway	Wu-Stein-2010
	GNB1	0,36	Pathway	Wu-Stein-2010
	PENK	36,83	Pathway	IMID
	OPRD1	0,51	Physical Interactions	IREF-reactome
	PENK	0,51	Physical Interactions	IREF-reactome
	GNG2	0,23	Physical Interactions	IREF-reactome
	GNB1	0,08	Physical Interactions	IREF-reactome
	OPRD1	0,51	Physical Interactions	Vastrik-Stein-2007
	PENK	0,51	Physical Interactions	Vastrik-Stein-2007
	GNG2	0,23	Physical Interactions	Vastrik-Stein-2007
	GNB1	0,08	Physical Interactions	Vastrik-Stein-2007
	OPRD1	9,29	Shared protein domains	INTERPRO
	OPRD1	0,35	Shared protein domains	PFAM
OPRM1	*GRK2*	0,58	Co-expression	Burington-Shaughnessy-2008
	WLS	0,13	Genetic Interactions	Lin-Smith-2010
	PENK	**6,60**	**Pathway**	**Wu-Stein-2010**
	GNG2	1,15	Pathway	Wu-Stein-2010
	GNB1	0,50	Pathway	Wu-Stein-2010
	STAT6	**22,78**	**Pathway**	**NCI_NATURE**
	GNG2	23,22	Pathway	REACTOME
	GNB1	23,22	Pathway	REACTOME
	GRK2	43,78	Pathway	IMID
	PENK	**23,19**	**Pathway**	**IMID**
	OPRL1	**0,50**	**Physical Interactions**	**IREF-reactome**
	PENK	**0,50**	**Physical Interactions**	**IREF-reactome**
	GNG2	0,23	Physical Interactions	IREF-reactome
	GNB1	0,08	Physical Interactions	IREF-reactome
	OPRL1	**0,50**	**Physical Interactions**	**Vastrik-Stein-2007**
	PENK	**0,50**	**Physical Interactions**	**Vastrik-Stein-2007**
	GNG2	0,23	Physical Interactions	Vastrik-Stein-2007
	GNB1	0,08	Physical Interactions	Vastrik-Stein-2007
	WLS	32,74	Physical Interactions	IREF-uniprotpp
	RANBP9	64,36	Physical Interactions	IREF-uniprotpp
	WLS	13,84	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	VAPA	3,68	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES

	OPRL1	**11,03**	**Physical Interactions**	**BIOGRID-SMALL-SCALE-STUDIES**
	OPRD1	**4,77**	**Physical Interactions**	**BIOGRID-SMALL-SCALE-STUDIES**
	RANBP9	1,36	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	WLS	9,55	Physical Interactions	IREF-mint
	VAPA	9,77	Physical Interactions	IREF-mint
	OPRL1	**27,08**	**Predicted**	**Wu-Stein-2010**
	OPRD1	**31,48**	**Predicted**	**Wu-Stein-2010**
	WLS	74,82	Predicted	I2D-IntAct-Mouse2Human
	VAPA	24,74	Predicted	I2D-IntAct-Mouse2Human
	WLS	66,16	Predicted	I2D-BioGRID-Mouse2Human
	VAPA	54,24	Predicted	I2D-BioGRID-Mouse2Human
	WLS	57,65	Predicted	I2D-IntAct-Rat2Human
	OPRL1	9,29	Shared protein domains	INTERPRO
	OPRD1	9,29	Shared protein domains	INTERPRO
	OPRL1	0,35	Shared protein domains	PFAM
	OPRD1	0,35	Shared protein domains	PFAM
PENK	*RANBP9*	0,08	Genetic Interactions	Lin-Smith-2010
	GNG2	1,03	Pathway	Wu-Stein-2010
	GNB1	0,45	Pathway	Wu-Stein-2010
	GNG2	0,23	Physical Interactions	IREF-reactome
	GNB1	0,08	Physical Interactions	IREF-reactome
	GNG2	0,23	Physical Interactions	Vastrik-Stein-2007
	GNB1	0,08	Physical Interactions	Vastrik-Stein-2007
RIN1	*STAT6*	0,78	Co-expression	Ross-Perou-2001
	STAT6	0,91	Shared protein domains	INTERPRO
VAPA	*GNG2*	0,13	Genetic Interactions	Lin-Smith-2010
	OPRD1	5,50	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
WLS	*OPRD1*	20,71	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES

Supplemental Table 27: A summarised list of genes that form part of the functionally associated network for the *ABCB1* and *COMT* genes, obtained by GeneMANIA.

Gene 1	Gene 2	Weight (%)	Network group	Network
ABCB1	*MAOA*	1,28	Co-expression	Mallon-McKay-2013
	ABCB4	2,06	Co-expression	Innocenti-Brown-2011
	ABCB4	3,93	Co-expression	Alizadeh-Staudt-2000
	ABCB4	1,11	Co-expression	Boldrick-Relman-2002
	NEDD4	0,42	Co-expression	Arijs-Rutgeerts-2009
	EGR2	1,53	Co-expression	Perou-Botstein-2000
	ABCB4	1,87	Co-expression	Rosenwald-Staudt-2001
	MAT2B	0,09	Genetic Interactions	Lin-Smith-2010
	COMTD1	0,10	Genetic Interactions	Lin-Smith-2010
	EGR2	12,70	Pathway	Wu-Stein-2010
	SLC22A3	38,59	Physical Interactions	IREF-reactome
	SLC22A1	38,59	Physical Interactions	IREF-reactome
	SLC22A2	16,64	Physical Interactions	IREF-reactome
	SLC22A3	38,59	Physical Interactions	Vastrik-Stein-2007
	SLC22A1	38,59	Physical Interactions	Vastrik-Stein-2007
	SLC22A2	16,64	Physical Interactions	Vastrik-Stein-2007
	NEDD4	33,03	Physical Interactions	IREF-quickgo
	NEDD4	1,79	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	ABCB4	70,53	Predicted	Wu-Stein-2010
	ABCC1	21,13	Predicted	Wu-Stein-2010
	ABCB4	2,42	Shared protein domains	INTERPRO
	ABCC1	2,37	Shared protein domains	INTERPRO
	ABCB4	2,55	Shared protein domains	PFAM
	ABCC1	2,55	Shared protein domains	PFAM
ABCB4	*MAT2B*	0,10	Genetic Interactions	Lin-Smith-2010
	VCP	0,07	Genetic Interactions	Lin-Smith-2010
	ABCC1	2,37	Shared protein domains	INTERPRO
	ABCC1	2,55	Shared protein domains	PFAM
ABCC1	*VCP*	3,21	Physical Interactions	Wan-Emili-2015
	VCP	0,39	Physical Interactions	IREF-biogrid
AHCY	*ABCC1*	0,79	Co-expression	Wang-Maris-2006
	VCP	0,94	Co-expression	Mallon-McKay-2013
	PPA2	1,24	Co-expression	Perou-Botstein-2000
AP000812.4	*COMTD1*	2,51	Shared protein domains	INTERPRO
	COMTD1	42,69	Shared protein domains	PFAM
COMT	*RIN1*	0,72	Co-expression	Arijs-Rutgeerts-2009
	VCP	5,98	Physical Interactions	Yu-Chow-2013
	MAOA	41,95	Physical Interactions	IREF-reactome
	MAT1A	22,13	Physical Interactions	IREF-reactome
	MAT2B	18,77	Physical Interactions	IREF-reactome
	MAT2A	18,77	Physical Interactions	IREF-reactome
	AHCY	13,19	Physical Interactions	IREF-reactome

	MAOA	41,95	Physical Interactions	Vastrik-Stein-2007
	MAT1A	22,13	Physical Interactions	Vastrik-Stein-2007
	MAT2B	18,77	Physical Interactions	Vastrik-Stein-2007
	MAT2A	18,77	Physical Interactions	Vastrik-Stein-2007
	AHCY	13,19	Physical Interactions	Vastrik-Stein-2007
	FGF2	50,26	Physical Interactions	Hein-Mann-2015
	PPA2	35,85	Physical Interactions	Hein-Mann-2015
	RIN1	35,85	Physical Interactions	Hein-Mann-2015
	COL1A2	22,44	Physical Interactions	Hein-Mann-2015
	XRN2	31,81	Physical Interactions	IREF-bind-translation
	XRN2	31,66	Physical Interactions	IREF-bind
	XRN2	37,15	Physical Interactions	Rual-Vidal-2005
	XRN2	26,34	Physical Interactions	IREF-spike
	FGF2	4,24	Physical Interactions	IREF-biogrid
	PPA2	2,80	Physical Interactions	IREF-biogrid
	RIN1	4,60	Physical Interactions	IREF-biogrid
	COL1A2	5,43	Physical Interactions	IREF-biogrid
	VCP	0,39	Physical Interactions	IREF-biogrid
	AP000812.4	3,02	Shared protein domains	INTERPRO
	COMTD1	3,22	Shared protein domains	INTERPRO
	AP000812.4	42,69	Shared protein domains	PFAM
	COMTD1	42,69	Shared protein domains	PFAM
EGR2	*COL1A2*	1,03	Co-expression	Dobbin-Giordano-2005
MAOA	*MAT1A*	0,74	Co-expression	Innocenti-Brown-2011
	COL1A2	0,67	Co-localization	Schadt-Shoemaker-2004
	FGF2	0,12	Genetic Interactions	Lin-Smith-2010
	COL1A2	0,07	Genetic Interactions	Lin-Smith-2010
	SLC22A2	25,89	Physical Interactions	IREF-reactome
	SLC22A2	25,89	Physical Interactions	Vastrik-Stein-2007
MAT1A	*ABCB4*	1,42	Co-expression	Mallon-McKay-2013
	ABCB4	0,48	Co-expression	Dobbin-Giordano-2005
	AHCY	0,84	Co-expression	Jiang-de Kok-2017
	ABCB4	1,35	Co-localization	Johnson-Shoemaker-2003
	MAT2A	32,78	Physical Interactions	Havugimana-Emili-2012
	MAT2B	43,97	Physical Interactions	Huttlin-Harper-2017
	MAT2A	43,97	Physical Interactions	Huttlin-Harper-2017
	MAT2B	70,71	Physical Interactions	IREF-corum
	MAT2B	41,76	Physical Interactions	Huttlin-Gygi-2015
	MAT2A	41,76	Physical Interactions	Huttlin-Gygi-2015
	MAT2A	79,62	Physical Interactions	IREF-huri
	MAT2B	9,58	Physical Interactions	IREF-biogrid
	MAT2A	7,24	Physical Interactions	IREF-biogrid
	MAT2A	87,65	Predicted	Wu-Stein-2010
	AHCY	1,13	Predicted	Stuart-Kim-2003
	MAT2A	100,00	Shared protein domains	INTERPRO

	MAT2A	100,00	Shared protein domains	PFAM
MAT2A	*ABCC1*	0,51	Co-expression	Bild-Nevins-2006 B
	XRN2	1,22	Co-expression	Chen-Brown-2002
	AHCY	1,95	Co-expression	Ross-Perou-2001
	ABCC1	1,81	Co-expression	Ross-Perou-2001
	AHCY	1,13	Predicted	Stuart-Kim-2003
MAT2B	*PPA2*	1,51	Co-expression	Dobbin-Giordano-2005
	MAT2A	3,13	Co-localization	Johnson-Shoemaker-2003
	MAT2A	57,64	Pathway	Wu-Stein-2010
	MAT2A	19,54	Physical Interactions	IREF-reactome
	MAT2A	19,54	Physical Interactions	Vastrik-Stein-2007
	MAT2A	7,70	Physical Interactions	Kristensen-Foster-2012
	MAT2A	100,00	Physical Interactions	IREF-quickgo
	MAT2A	20,02	Physical Interactions	Wan-Emili-2015
	MAT2A	53,33	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	MAT2A	55,05	Physical Interactions	Huttlin-Harper-2017
	MAT2A	70,71	Physical Interactions	IREF-corum
	MAT2A	55,05	Physical Interactions	Huttlin-Gygi-2015
	MAT2A	3,72	Physical Interactions	IREF-biogrid
	AHCY	1,03	Shared protein domains	INTERPRO
NEDD4	*XRN2*	0,16	Genetic Interactions	Lin-Smith-2010
SLC22A1	*MAT1A*	0,75	Co-expression	Mallon-McKay-2013
	MAT1A	0,28	Co-expression	Roth-Zlotnik-2006
	ABCB4	0,69	Co-expression	Roth-Zlotnik-2006
	MAT1A	0,36	Co-expression	Dobbin-Giordano-2005
	MAT1A	0,57	Co-localization	Johnson-Shoemaker-2003
	SLC22A2	2,35	Shared protein domains	INTERPRO
	SLC22A2	2,92	Shared protein domains	PFAM
SLC22A2	*FGF2*	1,88	Co-expression	Wu-Garvey-2007
SLC22A3	*MAT1A*	0,64	Co-expression	Jiang-de Kok-2017
	XRN2	0,12	Genetic Interactions	Lin-Smith-2010
	SLC22A1	1,96	Shared protein domains	INTERPRO
	SLC22A2	1,93	Shared protein domains	INTERPRO

Supplemental Table 28: A summarised list of genes that form part of the functionally associated network for the *ABCB1*, *OPRM1* and *COMT* genes, obtained by GeneMANIA.

Gene 1	Gene 2	Weight (%)	Network group	Network
ABCB1	*MAOA*	1,28	Co-expression	Mallon-McKay-2013
	ABCB4	2,06	Co-expression	Innocenti-Brown-2011
	ABCB4	3,93	Co-expression	Alizadeh-Staudt-2000
	ABCB4	1,11	Co-expression	Boldrick-Relman-2002
	ABCB4	1,87	Co-expression	Rosenwald-Staudt-2001
	MAT2B	0,09	Genetic Interactions	Lin-Smith-2010
	COMTD1	0,10	Genetic Interactions	Lin-Smith-2010
	SLC22A3	38,59	Physical Interactions	IREF-reactome
	SLC22A1	38,59	Physical Interactions	IREF-reactome
	SLC22A2	16,64	Physical Interactions	IREF-reactome
	SLC22A3	38,59	Physical Interactions	Vastrik-Stein-2007
	SLC22A1	38,59	Physical Interactions	Vastrik-Stein-2007
	SLC22A2	16,64	Physical Interactions	Vastrik-Stein-2007
	ABCB4	70,53	Predicted	Wu-Stein-2010
	ABCB4	2,42	Shared protein domains	INTERPRO
	ABCB4	2,55	Shared protein domains	PFAM
ABCB4	*MAT2B*	0,10	Genetic Interactions	Lin-Smith-2010
AHCY	*PENK*	0,07	Genetic Interactions	Lin-Smith-2010
AP000812.4	*COMTD1*	2,51	Shared protein domains	INTERPRO
	COMTD1	42,69	Shared protein domains	PFAM
COMT	*MAOA*	41,95	Physical Interactions	IREF-reactome
	MAT1A	22,13	Physical Interactions	IREF-reactome
	MAT2B	18,77	Physical Interactions	IREF-reactome
	MAT2A	18,77	Physical Interactions	IREF-reactome
	AHCY	13,19	Physical Interactions	IREF-reactome
	MAOA	41,95	Physical Interactions	Vastrik-Stein-2007
	MAT1A	22,13	Physical Interactions	Vastrik-Stein-2007
	MAT2B	18,77	Physical Interactions	Vastrik-Stein-2007
	MAT2A	18,77	Physical Interactions	Vastrik-Stein-2007
	AHCY	13,19	Physical Interactions	Vastrik-Stein-2007
	FGF2	50,26	Physical Interactions	Hein-Mann-2015
	FGF2	4,24	Physical Interactions	IREF-biogrid
	AP000812.4	3,02	Shared protein domains	INTERPRO
	COMTD1	3,22	Shared protein domains	INTERPRO
	AP000812.4	42,69	Shared protein domains	PFAM
	COMTD1	42,69	Shared protein domains	PFAM
FGF2	*PENK*	0,46	Co-expression	Dobbin-Giordano-2005
	OPRD1	0,08	Genetic Interactions	Lin-Smith-2010
GRK2	*GNG2*	0,34	Pathway	Wu-Stein-2010
	GNG2	0,29	Physical Interactions	IREF-reactome
	GNG2	0,29	Physical Interactions	Vastrik-Stein-2007
	GNG2	21,28	Predicted	I2D-BioGRID-Mouse2Human

MAOA	*MAT1A*	0,74	Co-expression	Innocenti-Brown-2011
	FGF2	0,12	Genetic Interactions	Lin-Smith-2010
	SLC22A2	25,89	Physical Interactions	IREF-reactome
	SLC22A2	25,89	Physical Interactions	Vastrik-Stein-2007
MAT1A	*ABCB4*	1,42	Co-expression	Mallon-McKay-2013
	ABCB4	0,48	Co-expression	Dobbin-Giordano-2005
	AHCY	0,84	Co-expression	Jiang-de Kok-2017
	OPRL1	0,87	Co-expression	Wu-Garvey-2007
	ABCB4	1,35	Co-localization	Johnson-Shoemaker-2003
	MAT2A	32,78	Physical Interactions	Havugimana-Emili-2012
	MAT2B	43,97	Physical Interactions	Huttlin-Harper-2017
	MAT2A	43,97	Physical Interactions	Huttlin-Harper-2017
	MAT2B	70,71	Physical Interactions	IREF-corum
	MAT2B	41,76	Physical Interactions	Huttlin-Gygi-2015
	MAT2A	41,76	Physical Interactions	Huttlin-Gygi-2015
	MAT2A	79,62	Physical Interactions	IREF-huri
	MAT2B	9,58	Physical Interactions	IREF-biogrid
	MAT2A	7,24	Physical Interactions	IREF-biogrid
	MAT2A	87,65	Predicted	Wu-Stein-2010
	AHCY	1,13	Predicted	Stuart-Kim-2003
	MAT2A	100,00	Shared protein domains	INTERPRO
	MAT2A	100,00	Shared protein domains	PFAM
MAT2A	*AHCY*	1,95	Co-expression	Ross-Perou-2001
	AHCY	1,13	Predicted	Stuart-Kim-2003
MAT2B	*MAT2A*	3,13	Co-localization	Johnson-Shoemaker-2003
	MAT2A	57,64	Pathway	Wu-Stein-2010
	MAT2A	19,54	Physical Interactions	IREF-reactome
	MAT2A	19,54	Physical Interactions	Vastrik-Stein-2007
	MAT2A	7,70	Physical Interactions	Kristensen-Foster-2012
	MAT2A	100,00	Physical Interactions	IREF-quickgo
	MAT2A	20,02	Physical Interactions	Wan-Emili-2015
	MAT2A	53,33	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	MAT2A	55,05	Physical Interactions	Huttlin-Harper-2017
	MAT2A	70,71	Physical Interactions	IREF-corum
	MAT2A	55,05	Physical Interactions	Huttlin-Gygi-2015
	MAT2A	3,72	Physical Interactions	IREF-biogrid
	AHCY	1,03	Shared protein domains	INTERPRO
OPRD1	*RANBP9*	0,08	Genetic Interactions	Lin-Smith-2010
	GRK2	1,49	Pathway	Wu-Stein-2010
	PENK	4,50	Pathway	Wu-Stein-2010
	GNG2	0,78	Pathway	Wu-Stein-2010
	PENK	36,83	Pathway	IMID
	PENK	0,51	Physical Interactions	IREF-reactome
	GNG2	0,23	Physical Interactions	IREF-reactome
	PENK	0,51	Physical Interactions	Vastrik-Stein-2007

	GNG2	0,23	Physical Interactions	Vastrik-Stein-2007
OPRL1	*OPRD1*	0,73	Co-expression	Roth-Zlotnik-2006
	OPRD1	1,18	Co-localization	Johnson-Shoemaker-2003
	GRK2	1,57	Pathway	Wu-Stein-2010
	GNG2	0,82	Pathway	Wu-Stein-2010
	PENK	36,83	Pathway	IMID
	OPRD1	0,51	Physical Interactions	IREF-reactome
	PENK	0,51	Physical Interactions	IREF-reactome
	GNG2	0,23	Physical Interactions	IREF-reactome
	OPRD1	0,51	Physical Interactions	Vastrik-Stein-2007
	PENK	0,51	Physical Interactions	Vastrik-Stein-2007
	GNG2	0,23	Physical Interactions	Vastrik-Stein-2007
	OPRD1	9,29	Shared protein domains	INTERPRO
	OPRD1	0,35	Shared protein domains	PFAM
OPRM1	*GRK2*	0,58	Co-expression	Burington-Shaughnessy-2008
	SLC22A2	1,89	Co-expression	Wu-Garvey-2007
	WLS	0,13	Genetic Interactions	Lin-Smith-2010
	ABCB4	0,11	Genetic Interactions	Lin-Smith-2010
	PENK	6,60	Pathway	Wu-Stein-2010
	GNG2	1,15	Pathway	Wu-Stein-2010
	GNG2	23,22	Pathway	REACTOME
	GRK2	43,78	Pathway	IMID
	PENK	23,19	Pathway	IMID
	OPRL1	0,50	Physical Interactions	IREF-reactome
	PENK	0,50	Physical Interactions	IREF-reactome
	GNG2	0,23	Physical Interactions	IREF-reactome
	OPRL1	0,50	Physical Interactions	Vastrik-Stein-2007
	PENK	0,50	Physical Interactions	Vastrik-Stein-2007
	GNG2	0,23	Physical Interactions	Vastrik-Stein-2007
	WLS	32,74	Physical Interactions	IREF-uniprotpp
	RANBP9	64,36	Physical Interactions	IREF-uniprotpp
	WLS	13,84	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	VAPA	3,68	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	OPRL1	11,03	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	OPRD1	4,77	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	RANBP9	1,36	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
	WLS	9,55	Physical Interactions	IREF-mint
	VAPA	9,77	Physical Interactions	IREF-mint
	OPRL1	27,08	Predicted	Wu-Stein-2010
	OPRD1	31,48	Predicted	Wu-Stein-2010
	WLS	74,82	Predicted	I2D-IntAct-Mouse2Human
	VAPA	24,74	Predicted	I2D-IntAct-Mouse2Human
	WLS	66,16	Predicted	I2D-BioGRID-Mouse2Human
	VAPA	54,24	Predicted	I2D-BioGRID-Mouse2Human
	WLS	57,65	Predicted	I2D-IntAct-Rat2Human

	OPRL1	9,29	Shared protein domains	INTERPRO
	OPRD1	9,29	Shared protein domains	INTERPRO
	OPRL1	0,35	Shared protein domains	PFAM
	OPRD1	0,35	Shared protein domains	PFAM
PENK	*RANBP9*	0,08	Genetic Interactions	Lin-Smith-2010
	GNG2	1,03	Pathway	Wu-Stein-2010
	GNG2	0,23	Physical Interactions	IREF-reactome
	GNG2	0,23	Physical Interactions	Vastrik-Stein-2007
SLC22A1	*MAT1A*	0,75	Co-expression	Mallon-McKay-2013
	MAT1A	0,28	Co-expression	Roth-Zlotnik-2006
	ABCB4	0,69	Co-expression	Roth-Zlotnik-2006
	MAT1A	0,36	Co-expression	Dobbin-Giordano-2005
	OPRD1	2,58	Co-expression	Burington-Shaughnessy-2008
	MAT1A	0,57	Co-localization	Johnson-Shoemaker-2003
	SLC22A2	2,35	Shared protein domains	INTERPRO
	SLC22A2	2,92	Shared protein domains	PFAM
SLC22A2	*FGF2*	1,88	Co-expression	Wu-Garvey-2007
SLC22A3	*GRK2*	0,45	Co-expression	Burington-Shaughnessy-2008
	MAT1A	0,64	Co-expression	Jiang-de Kok-2017
	OPRD1	0,07	Genetic Interactions	Lin-Smith-2010
	SLC22A1	1,96	Shared protein domains	INTERPRO
	SLC22A2	1,93	Shared protein domains	INTERPRO
VAPA	*GNG2*	0,13	Genetic Interactions	Lin-Smith-2010
	OPRD1	5,50	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
WLS	*OPRD1*	20,71	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES

8 PUBLICATIONS

Research Article

For reprint orders, please contact: reprints@futuremedicine.com

Pharmacogenomics

ABCB1 and *OPRM1* single-nucleotide polymorphisms collectively modulate chronic shoulder pain and dysfunction in South African breast cancer survivors

Firzana Firfirey*[,1], Alison V September[1,2,3] & Delva Shamley[4]

[1]Department of Human Biology, Division of Physiological Sciences, Faculty of Health Sciences, University of Cape Town, 7701, South Africa

[2]Department of Human Biology, Health through Physical Activity, Lifestyle & Sport Research Centre (HPALS), Faculty of Health Sciences, University of Cape Town, 7701, South Africa

[3]Department of Human Biology, International Federation of Sports Medicine (FIMS), Collaborative Centre of Sports Medicine, University of Cape Town, 7701, South Africa

[4]Department of Human Biology, Division of Clinical Anatomy & Biological Anthropology, Anatomy Building, Medical School, University of Cape Town, 7701, South Africa

*Author for correspondence: FirzanaFirfirey@gmail.com

Background: Chronic shoulder pain/disability is a well-recognized side effect of treatment for breast cancer, with ∼40% of patients experiencing this, despite receiving pain management. To manage acute and chronic pain, several opioids are commonly prescribed. Pharmacogenomics have implicated genes within the opioid signaling pathway, including *ABCB1* and *OPRM1*, to contribute to an individual's variable response to opioids. **Aim:** To evaluate *ABCB1* (rs1045642 G>A, rs1128503 G>A) and *OPRM1* (rs1799971 A>G, rs540825 T>A) single-nucleotide polymorphisms (SNPs) in chronic shoulder pain/disability in BCS. **Materials and methods:** TaqMan™ assays were used to genotype *ABCB1* and *OPRM1* SNPs within the BCS (N = 252) cohort. The Shoulder Pain and Disability Index was used to evaluate pain and disability features associated with shoulder pathologies. Participants end scores for each feature (pain, disability and combined [pain and disability]) were categorized into no-low (>30%) and moderate-high (≥30%) scores. Statistical analysis was applied, and significance was accepted at $p < 0.05$. **Results:** 27.0, 19.0 and 22.0% of participants reported moderate-high pain, disability and combined (pain and disability) scores, respectively. *ABCB1*:rs1045642-(A/A) genotype was significantly associated with disability (p = 0.028: no-low [14.9%] vs mod-high [4.3%]) and combined (pain and disability) (p = 0.011: no-low [15.9%] vs mod-high [5.7%]). The *ABCB1*:rs1045642-(A) allele was significantly associated with disability (p = 0.015: no-low [37.9%] vs mod-high [23.9%]) and combined (pain and disability) (p = 0.003: no-low [38.5%] vs mod-high [23.6%]). The inferred *ABCB1* (rs1045642 G>A-rs1128503 G>A): A-G (p = 0.029; odds ratio [OR]: 0.0; 95% CI: 0.0-0.0) and the *OPRM1* (rs1799971 A>G – rs540825 T>A): G-T (p = 0.019; OR: 0.33; 95% CI: 0.14-0.75) haplotypes were associated with disability and pain, respectively. Gene–gene interactions showed the *ABCB1* (rs1045642 G>A) – *OPRM1* (rs540825 T>A) combinations, (A-T) (p = 0.019; OR: 0.62; 95% CI: 0.33-1.16) and (G-A) (p = 0.021; OR: 1.57; 95% CI: 0.30-3.10) were associated with disability. **Conclusion:** The study implicated *ABCB1* with shoulder pain and disability; and haplotype analyses identified specific genetic intervals within *ABCB1* and *OPRM1* to associate with chronic shoulder pain and disability. Evidence suggests that potentially gene–gene interactions between *ABCB1* and *OPRM1* contribute to chronic shoulder pain and disability experienced in this SA cohort.

First draft submitted: 17 February 2022; Accepted for publication: 20 May 2022; Published online: 21 June 2021

Keywords: gene–gene interactions • genetic association study • mu-opioid receptor • P-glycoprotein • South African breast cancer survivors

Future Medicine

The term 'breast cancer survivors' (BCSs) is used to describe individuals with cancer 'from the point of diagnosis through the balance of his/her life' [1–3]. In BCS, 30–50% of survivors develop postoperative chronic shoulder pain and dysfunction, leading to reduced function and poor quality of life that can persist for 6–8 years after breast cancer (BC) treatment [4–8]. Chronic pain is multifactorial with biological, psychological, social and cultural contributors [9,10]. Risk factors for chronic postoperative pain in BCS include age at surgery, axillary lymph node dissection (ALND), adjuvant therapies, preoperative and severe acute postoperative pain and genetics [11,12]. Opioids are clinically used to treat both acute and chronic pain, and some of the common drugs administered for pain control in BCSs are morphine, codeine, oxycodone, tramadol, methadone and fentanyl, among others [13]. To date, several genes have been implicated to contribute to the variable response to opioids, specifically with regard to the different phases in drug pharmacology [14]. This study focused on the *ABCB1* and *OPRM1* genes that are well described to be essential in the distribution and signaling of opioids [14]

The *ABCB1* gene, also known as *MDR1,* is involved in cellular homeostasis and the ATP-dependent translocation of substrates [15,16]. The gene encodes a P-glycoprotein (P-gp) present in a variety of human tissues and is the major determinant of opioid bioavailability into the brain [17,18]. P-gp is a ubiquitous membrane transporter, with an affinity for several substrates, including opioids (e.g., morphine, methadone and fentanyl), all clinically used to treat moderate to severe pain. These substrates also have a high binding affinity to the *OPRM1* receptor, thus activating pain inhibition via the G-protein coupled receptor (GPCR) cascade.

At the blood–brain barrier (BBB), P-gp is found in the endothelial cells surrounding the lumen of the brain vasculature. P-gp function is to export opioids from the blood into the endothelial cells, and also diffusing it back into circulation. This mechanism ensures the protection against neurotoxicity but also the distribution of opioids to the central nervous system (CNS) [19,20]. Additionally, knockout studies have shown that analgesic effects vary between *mdr1*(-/-) and *mdr1*(+/+ and +/-) mice [21,22].

The rs1045642 G>A, rs2032852 T>G/A and rs1128503 G>A polymorphisms remain the most frequently studied *ABCB1* polymorphisms in pharmacogenetic studies [23–25]. Using immunohistochemistry and Western blot analysis, Hoffmeyer *et al.* [26] found the *ABCB1* rs1045642 (A/A) genotype correlated with a twofold reduction in P-gp intestinal expression. To test the effects of the rs1045642 single-nucleotide polymorphism (SNP) on P-gp activity, the authors measured duodenum plasma concentrations before and after administration of digoxin and found a greater than fourfold difference between (A/A) carriers (higher levels) and (G/G) carriers (lower levels), [26,27]. The results indicating that the *ABCB1* rs1045642 (G>A) polymorphism influences both P-gp expression and activity. Subsequent acute pain-related studies have shown the rs1045642 (A) allele to be associated with neuropathic, cancer and postoperative pediatric pain [28–31]. However, the consensus on these associations and functions of the P-gp remain inconclusive [26,32].

The *OPRM1* gene, is a GPCR that facilitates the pharmacological effects of opioids through the inhibition of cell signaling [18]. The gene encodes the mu-opioid receptor protein (MOR-1), which is widely distributed in the brain and spinal cord and acts as the primary binding site for both endogenous and exogenous opioids [28,33]. *OPRM1* rs1799971 (A118G), the most studied polymorphism, involves an asparagine to aspartate substitution (Asn40Asp), resulting in the loss of an N-linked glycosylation site in the extracellular receptor region [34–36]. The substitution thereby effects the protein stability, expression and signaling efficiency [28,37]. *OPRM1* rs540825 involves a histidine to glutamine (His > Glu) substitution within the C-terminal, intracellular domain of the receptor [38].

To date, two non cancer-related studies have shown an association between the *ABCB1* and *OPRM1* genes and risk for chronic pain [39,40]. No studies have explored the relationship between these polymorphisms and shoulder pain/disability. Understanding the genetic contribution of these polymorphisms could assist in the identification of potential predictive markers for the development of chronic postoperative shoulder pain and dysfunction.

The present study aimed to assess nongenetic and genetic risk factors for chronic shoulder pain and disability in a South African cohort of mixed-ancestry BCSs. The study objectives were to determine the genotype/allele frequency distributions for the polymorphisms in *ABCB1* and *OPRM1*, analyze inferred haplotypes between the polymorphism for each gene and investigate the gene–gene interactions between *ABCB1* and *OPRM1*.

Materials & methods

Study design

A cross-sectional study was conducted in accordance with the STREGA reporting recommendations for the reporting of genetic association studies [41]. The study forms part of a larger ongoing project that aims to investigate the association between genetic polymorphisms within the opioid metabolic pathway and the clinical phenotype of chronic shoulder pain and dysfunction in BCSs.

Participants & setting

A total of 252 women were randomly recruited from a tertiary hospital in South Africa. Volunteers were considered eligible if they were >18 years, diagnosed with unilateral BC, had undergone BC surgery ≥1 year before recruitment and self-identified as mixed race. The mixed-ancestry population in South Africa is a unique population characterized by its diversity descending from Africa, Asia and European populations [42]. Volunteers with any history of shoulder/neck pathology before diagnoses and treatment for BC, any connective tissue disorders, renal insufficiency, diabetes mellitus, hypercholesteremia, diagnoses of local recurrences or lymphedema were excluded.

Study procedure

After receiving informed consent, relevant demographic and clinical data for each participant was obtained from the medical records and participants completed the Shoulder Pain and Disability Index (SPADI). Blood samples were drawn (10 ml) from the unaffected arm using appropriately labelled EDTA vacutainer tubes and stored at -20°C. The standard DNA extraction protocol described by Lahiri and Nurnberger [43] was employed, followed by DNA quantitation using the Take 2 plate with the BioTek HT Synergy™ (Agilent Technologies, CA, USA) multi-plate reader. DNA purity and concentrations ranged from 1.2 to 2.165 (A_{260}/A_{280} ratio) and 24 ng/µl to 389 ng/µl, respectively. Deidentified DNA samples were stored for genotyping analysis at -20°C.

Study outcome measures

Self-reported outcome measure: SPADI

The SPADI index, a self-reported outcome measure, was used to assess pain and disability associated with musculoskeletal pathologies of the shoulder. The index has two domains – namely, pain and disability, each with five and eight items (total of 13 items), that describe daily activities. For each domain, the items are scored on a scale of 0 (no pain/difficulty) to 10 (worst pain/difficulty), then added and converted to a percentage as a representation of pain or disability. Furthermore, for each domain the end score (in %) was used to categorize participants into no-low (scores >30%) and moderate-high (scores ≥30%) groups. Previous literature has shown that SPADI scores of >30% can affect the activities of day-to-day living of individuals and are associated with cases presenting moderate-high pain on the visual analogue scale (VAS) scale, and for that reason, we used these parameters to categorize our groups [44–46]. Reliability and validity studies show that the SPADI index have excellent reliability (test–retest ICC: ∼0.89) with factor analysis indicating the items to represent both pain and disability characteristics observed in shoulder pathologies [45,47,48]. In this study, we evaluated pain, disability and combined (pain and disability) related to the shoulder in BCSs.

SNP selection & genotyping

The candidate genes *ABCB1* (rs1045642 G>A, rs1128503 G>A) and *OPRM1* (rs1799971 A>G, rs540825A>T), which have previously been associated with the opioid signaling (pain inhibition) pathway, were included in this study [17,31,49]. Samples were genotyped for the polymorphisms in 96-well plates using the TaqMan™ SNP genotyping assays (Thermo Fisher Scientific, Applied Biosystems, CA, USA) according to the manufacturer's instructions. Accordingly, *ABCB1* TaqMan™ SNPs are classified as drug metabolizing enzyme (DME) assays, whereas *OPRM1* SNPS are classified as standard assays (non-DME). Non-DME SNP: each sample well contained 0.2 µl of TaqMan specific primer (final concentrations of 1X), 4 µl of TaqMan genotype master mix, 2.8 µl of dH2O and 1 µl of DNA template (template [DNA] 1–10 ng) to a final volume of 8 µl. DME SNP: In these wells, each sample contained 0.4 µl of TaqMan specific primer (final concentrations of 1x), 4 µl of TaqMan genotype master mix, 2.6 µl of dH2O and 1 µl of DNA template (template [DNA] 1–10 ng). Negative controls (no DNA template) and technical replicates were used for quality control purposes. The standard PCR conditions for the *OPRM1* SNPs were activation at 95°C for 10 min (1 cycle), followed with denaturation at 92°C for 15 s and annealing/extend/acquisition at 60°C for 60 s (40 cycles), and holding at 60°C for 10 min. PCR conditions

for the *ABCB1* SNPs were activation at 95°C for 10 min (1 cycle), followed with denaturation at 95°C for 15 s and annealing/extend/acquisition at 60°C for 90 s (50 cycles), and holding at 60°C for 10 min. The PCR reactions were performed using the Quant studio 3 real-time PCR (Thermo Fisher Scientific, Applied Biosystems, CA, USA) system and genotypes were automatically called and analyzed using the Thermo Fisher Cloud genotyping analysis Software version 3.3.0-SR2-build 21. Successful genotyping was considered if the sample was amplified for all polymorphisms, except when failing to amplify after two PCR reruns. A success rate of 99% was observed for *ABCB1* rs1045642 G>A, rs1128503 G>A and *OPRM1* rs1799971 A>G, with 94% call for *OPRM1* rs540825 A>T, including the repeat typing. All laboratory work was conducted at HPALS, University of Cape Town (South Africa).

Clinical risk factors

Known clinical risk factors for chronic postoperative shoulder pain and disability were assessed for associations [46,50]. These included participants' age at the time of surgery, surgical date and type, lymph node surgery type, adjuvant therapies and BC pathology

Statistical analysis

Sample size requirement for this study was calculated using the QUANTO v 1.2.4.49 software, with an assumed, literature inferred, baseline risk of 40% for chronic pain (~36%) and disability (~30%) in BCSs [7,51–58]. A total sample of 150 participants was calculated as an effective size for the detection of an odds ratio ≥2 with an 80% power for allele frequencies of 0.1–0.5 in the dominant model (Supplementary Table 1). For the log additive and recessive models, our sample size was adequate to reach odds of 2–2.5 and 2.5 with 80% power for the minor allele frequencies 0.1–0.5 and >0.4, respectively.

Comparative analysis of demographic and clinical data was assessed using Statistica V13.5.0.17 [59]. Basic descriptive statistics were analyzed using independent sample t-test, Pearson's $\chi 2$ and Fisher's exact tests (if $n < 10$) for pain, disability and combined (pain and disability) between the no-low and moderate-high groups. The R studio V1.3.1056 running R V4.0.4 language and programming environment (www.r-project.org) was used to analyze all genotype data [60]. Differences in genotype/allele and inferred haplotype frequency distribution were compared using Pearson's $\chi 2$ and Fisher's exact tests. By using the 'genetics' v1.3.8.1.3 package, Hardy–Weinberg equilibrium (HWE) probabilities and linkage disequilibrium (LD) was determined [61]. Logistic regression analysis evaluating the association between the genotype and pain/disability characteristics was assessed using the 'SNPassoc' v2.0.2 packages [62].

To evaluate the *ABCB1* (rs1045642 G>A and rs1128503 G>A) and *OPRM1* (rs1799971 A>G and rs540825 T>A) genetic intervals, inferred haplotypes were constructed for each gene using the individual genotype data. In addition, as a proxy for gene–gene interactions, five stepwise inferred allele–allele combination constructs were generated between the *ABCB1* and *OPRM1* genes. Analysis of inferred haplotype/allelic combination frequency distribution and association between no-low and moderate-high groups was determined using the 'haplo.stats' v1.8.6. package [63,64]. Furthermore, genotype effects were evaluated on the clinical variables, age, time since surgery, total number of nodes involved and examined, invasive ductal carcinoma (IDC), tumor grade, surgery types (mastectomy [MTX] vs wide local excision [WLE]) and lymph node surgery (axillary lymph node dissection [ALND] vs sentinel lymph node biopsy [SLNB]).

Bioinformatic analysis was conducted to explore gene-associated networks and interactions between and for *ABCB1* and *OPRM1* using the GeneMANIA and Enrichr platforms. Also, using an online tool, Software for Statistical Folding of Nucleic Acids and Studies of Regulatory RNAs (SFOLD), the 2D RNA structures for the genetic intervals containing the *ABCB1* and *OPRM1* polymorphisms were predicted. All software and online programs used in this study can be downloaded from the respective sites reported in the reference list. Data are presented as either means ± standard deviation, median (interquartile range [IQR]), or as percentage (n values). All logistic regression analyses were covaried for age at surgery (including odds ratios [OR] and confidence intervals [CI] at 95%) and statistical significance was accepted a $p < 0.05$.

Results

Demographical & clinical characteristics for pain, disability & combined (pain & disability)

Table 1 summarizes the comparative analysis for demographic and clinical data with 27.0%, 19.0% and 22.0% of individuals reporting moderate-high pain, disability and combined (pain and disability), respectively. Age differed

Table 1. Clinical characteristics between pain, disability and the combined (pain and disability) categories.

Characteristics		Pain			Disability			Pain and disability		
		No-low	Mod-high	p-value	No-low	Mod-high	p-value	No-low	Mod-high	p-value
n = 252		73.0 (184)	27 (68)		81 (204)	19 (48)		78 (197)	22 (55)	
Age at surgery		55.3 ± 9.2	50.7 ± 10.7	**0.002**	54.8 ± 9.3	50.6 ± 11.3	**0.011**	55.0 ± 9.3	50.5 ± 10.9	**0.003**
Time since surgery (years)		3.5 ± 2.5	3.1 ± 2.4	0.116	3.5 ± 2.5	3.2 ± 2.3	0.482	3.5 ± 2.5	3.1 ± 2.5	0.133
Total nodes examined		10.5 ± 6.0	9.5 ± 6.3	0.155	10.5 ± 6.3	8.9 ± 5.1	0.177	10.6 ± 6.3	8.6 ± 5.2	0.066
Total nodes involved		3.6 ± 3.7	2.8 ± 3.1	0.145	3.7 ± 3.7	2.1 ± 2.5	**0.025**	3.7 ± 3.7	2.3 ± 2.7	**0.034**
Side of primary	Left	49.2 (90)	57.4 (39)	0.250	49.8 (101)	58.3 (28)	0.285	49.5 (97)	58.2 (32)	0.254
	Right	50.8 (93)	42.6 (29)		50.3 (102)	41.7 (20)		50.5 (99)	41.8 (23)	
Invasive ductal carcinoma	Yes	78.7 (144)	80.9 (55)	0.768	79.8 (162)	77.1 (37)	0.913	79.1 (155)	80.0 (44)	0.985
	No	3.3 (6)	4.4 (3)		3.5 (7)	4.2 (2)		3.6 (7)	3.6 (2)	
	Not done	18.0 (33)	14.7 (10)		16.8 (34)	18.8 (9)		17.4 (34)	16.4 (9)	
Lymphovascular invasion	Yes	35.7 (55)	29.8 (17)	0.423	35.6 (62)	27.0 (10)	0.316	35.7 (60)	27.9 (12)	0.335
	No	64.3 (99)	70.2 (40)		64.4 (112)	73.0 (27)		64.3 (108)	72.1 (31)	
Tumor grade	I	26.7 (43)	27.6 (16)	0.914	25.6 (46)	33.3 (13)	0.379	26.0 (45)	30.4 (14)	0.132
	II	49.7 (80)	48.3 (28)		51.1 (92)	41.0 (16)		51.5 (89)	41.3 (19)	
	III	21.7 (35)	20.7 (12)		21.7 (39)	20.5 (8)		21.4 (37)	21.7 (10)	
	Not known	1.9 (3)	3.5 (2)		1.7 (3)	5.1 (2)		1.2 (2)	6.5 (3)	

Data presented as mean ± standard deviation or % (n).
p-values are unadjusted and values in bold indicate significance ($p < 0.05$).
Tests used for comparative analysis includes Mann-Whitney U test (independent sample t-test); Fisher's exact test (when $n < 10$); χ^2 test.
Mod-high: Moderate-high.

significantly within pain (p = 0.002), disability (p = 0.007) and combined (pain and disability) (p = 0.002) groups. Younger participants were noted to report moderate-high pain, disability and combined (pain and disability). Younger participants also reported to having fewer number of nodes involved in the disability (p = 0.027) and combined (pain and disability) (p = 0.025) groups. No significant difference was observed for breast and lymph node surgery, or adjuvant treatments between no-low and moderate-high groups for pain, disability, or combined (pain and disability), p > 0.05 (Supplementary Table 3).

Genotype effects of the *ABCB1* & *OPRM1* polymorphisms on quantitative & categorial clinical characteristics

Genotype effect was evaluated for all clinical variables and noted associations for *ABCB1* rs1045642 G>A (p = 0.022), *ABCB1* rs1128503 G>A (p = 0.022) and *OPRM1* rs540825 T>A (p = 0.015; Supplementary Tables 5 & 7).

The *ABCB1* rs1045642 G>A polymorphism was associated with 'time since surgery', where the median (IQR) time that had passed were 3.0 (2.0–4.0) years for (G/G) and (A/A) carriers compared with 2.0 (2.0–4.0) years for (A/G) carriers (Supplementary Table 5). The *ABCB1* rs1128503 G>A polymorphism was associated with 'age at surgery', where the median (IQR) age was 56 (49–63) years for (A/G) carriers, compared with 54 (47–61) and 54 (48–2) years for (G/G) and (A/A) carriers respectively, rendering a possible sample bias (Supplementary Table 5).

The *OPRM1* rs540825 T>A polymorphism was associated with lymph node surgery, where the (A/A) (3.6% and 3.2%) genotype was less frequently observed in the ALND and SLNB surgeries, compared to the (T/T) (59.2% and 51.6%) and (A/T) (37.3% and 45.2%) genotypes, respectively (Supplementary Table 7).

No associations were observed between *OPRM1* rs1799971 A>G and the clinical variables (Supplementary Tables 5 & 7).

Genotype & allele frequency distribution of *ABCB1* & *OPRM1* polymorphisms

Table 2 summarizes the differences in the genotype and allele frequency distribution patterns between the no-low and moderate-high pain, disability and combined (pain and disability) groups.

Table 2. Genotype and minor allele frequency distributions, of the *ABCB1* (rs1045642 G>A, rs1128503 G>A) and *OPRM1* (rs1799971 A>G and rs540825 A>T) polymorphisms among pain, disability and combined (pain and disability) categories.

SNP	Pain		Disability		Pain and disability	
	No-low (n = 184)	Mod-high (n = 68)	No-low (n = 204)	Mod-high (n = 48)	No-low (n = 197)	Mod-high (n = 55)
Drug transporter						
ABCB1 rs1045642						
G/G	40.2 (70)	48.5 (32)	39.2 (76)	56.5 (26)	38.0 (71)	58.5 (31)
A/G	46.6 (81)	39.4 (26)	45.9 (89)	39.1 (18)	47.1 (88)	35.8 (19)
A/A	13.2 (23)	12.1 (8)	14.9 (29)	4.3 (2)	15.0 (28)	5.7 (3)
A allele	36.5 (127)	31.8 (42)	37.9 (147)	23.9 (22)	38.5 (144)	23.6 (25)
p-value[1]	0.367		**0.028**		**0.011**	
A allele p-value[2]	0.392		**0.015**		**0.003**	
HWE	1.000	0.405	0.762	0.709	0.879	1.000
ABCB1 rs1128503						
G/G	35.1 (61)	34.8 (23)	35.6 (69)	32.6 (15)	35.3 (66)	34.0 (18)
A/G	47.1 (82)	51.5 (34)	46.9 (91)	54.3 (25)	46.5 (87)	54.7 (29)
A/A	17.8 (31)	13.6 (9)	17.5 (34)	13.0 (6)	18.2 (34)	11.3 (6)
A allele	41.4 (144)	39.4 (52)	41.0(159)	40.2 (37)	41.4 (155)	38.7 (41)
p-value[1]	0.783		0.812		0.552	
A allele p-value[2]	0.755		0.907		0.655	
HWE	0.758	0.765	0.660	0.545	0.551	0.390
Opioid receptor						
OPRM1 rs1799971						
A/A	64.4 (112)	75.8 (50)	68.0 (132)	65.2 (30)	66.8 (125)	69.8 (37)
A/G	30.5 (53)	22.7 (15)	27.3 (53)	32.6 (15)	28.3 (53)	28.3 (15)
G/G	5.2 (9)	1.5 (1)	4.6 (9)	2.2 (1)	4.8 (9)	1.9 (1)
G allele	20.4 (71)	12.9 (17)	18.3 (71)	18.5(17)	19.0 (71)	16.0 (17)
p-value[1]	0.199		0.497		0.587	
G allele p-value[2]	0.064		1.000		0.570	
HWE	0.496	1.000	0.244	0.663	0.346	1.000
OPRM1 rs540825						
T/T	60.0 (99)	60.3 (38)	61.4 (113)	54.5 (24)	61.0 (108)	56.9 (29)
A/T	35.8 (59)	34.9 (22)	35.3 (65)	36.4 (16)	35.6 (63)	35.3 (18)
A/A	4.2 (7)	4.8 (3)	3.3 (6)	9.1 (4)	3.4 (6)	7.8 (4)
A allele	22.1 (73)	22.2 (28)	20.9 (77)	27.3 (24)	21.2 (75)	25.5 (26)
p-value1	0.990		0.275		0.435	
A allele p-value[2]	1.000		0.201		0.347	
HWE	0.660	1.000	0.385	0.464	0.381	0.481

p-values[1] for genotype between groups.
p-values[2] for allele between groups.
p-values in bold indicate significance ($p < 0.05$).
p-values for the exact test of Hardy–Weinberg equilibrium for each of the categories are included in the table.
Genotype and allele frequencies are expressed as a percentage (%) with the number of participants (n) in parentheses.
HWE: Hardy–Weinberg equilibrium; Mod-high: Moderate-high.

ABCB1

In the evaluation of pain, no associations for *ABCB1* rs1045642 G>A (p = 0.367) and rs1128503 G>A (p = 0.783) were noted (Table 2). For disability, significant frequency differences were noted for *ABCB1* rs1045642 G>A where the (A/A) genotype (p = 0.028; OR = 0.21; 95% CI: 0.05–0.93) was overrepresented in the no-low (14.9%) group compared with the moderate-high (4.3%) group (Table 2). Logistic regression showed the rs1045642 (A/A) genotype (dominant: p = 0.022; OR: 0.46; 95% CI: 0.24–0.90; recessive: p = 0.045; OR: 0.28; 95% CI: 0.06–1.21), was associated with reduced likelihood of reporting disability. In addition, the *ABCB1*

rs1045642 (A) allele (p = 0.015; OR: 0.52; 95% CI: 0.29–0.89) was overrepresented in the no-low group (37.9%), compared with the moderate-high group (23.9%), and associated with reduced likelihood of reporting disability (Table 2). *ABCB1* rs1128503 G>A showed no differences in genotype or allele frequency distribution between the no-low and moderate-high groups (p = 0.812; Table 2).

Evaluation of combined (pain and disability) showed significant differences for *ABCB1* rs1045642 G>A where the (A/A) genotype (p = 0.011; OR: 0.25; 95% CI: 0.07–0.89) was overrepresented in the no-low (15.0%) group compared with the moderate-high (5.7%) group (Table 2). Logistic regression showed the (A/A+A/G) (dominant; p = 0.004; OR: 0.40; 95% CI: 0.21-0.75) genotypes were associated with a reduced likelihood of reporting moderate-high combined (pain and disability). Once more, the *ABCB1* rs1045642 (A) (p = 0.003; OR: 0.50; 95% CI: 0.29–0.83) was overrepresented in the no-low (38.5%) group compared with the moderate-high (23.6%) group and was associated with a reduced likelihood of reporting moderate-high combined (pain and disability) (Table 2). No differences were detected for *ABCB1* rs1128503 G>A, between the no-low and moderate-high combined (pain and disability) (p = 0.552) groups.

OPRM1

Evaluating pain, we noted no significant differences in the genotype/allele frequency distribution for *OPRM1* rs1799971 A>G (p = 0.199) and rs540825 T>A (p = 0.990) between the no-low and moderate-high groups (Table 2). Although the *OPRM1* rs1799971 (G) allele (p = 0.064; OR: 0.58; 95% CI: 0.30–1.04) showed a trend toward an association.

Furthermore, when disability and combined (pain and disability) were evaluated, no significant associations were noted for *OPRM1* rs1799971 A>G (p = 0.497 and p = 0.587) and rs540825 T>A (p = 0.275 and p = 0.435; Table 2). All groups were in HWE for all polymorphisms when pain, disability and combined (pain and disability) were evaluated (Table 2).

Inferred haplotypes for *ABCB1* & *OPRM1* polymorphisms

Haplotypes were inferred and constructed for the *ABCB1* (rs1045642 G>A, rs1128503 G>A) and *OPRM1* (rs1799971 A>G, rs540825 T>A) genes, using the individual genotype data for the polymorphisms, in the pain, disability and combined (pain and disability) categories.

ABCB1 (rs1045642 G>A-rs1128503 G>A)

The inferred *ABCB1* rs1045642 G>A-rs1128503 G>A haplotype, yielded four combinations (G-G, A-A, G-A and A-G) (Figure 1A–C). For pain (p = 0.798), no significant differences were noted between the no-low and moderate-high groups (Figure 1A). For disability (p = 0.027), the (A-G) haplotype (p = 0.029; OR: 0.00; 95% CI: 0.00–0.00) was noted to be absent in the moderate-high (0.0%) group compared with the no-low group (6.9%) and significantly associated with reduced likelihood of reporting moderate-high disability (Figure 1B). Evaluating combined (pain and disability) (p = 0.019), the (A-A) haplotype was overrepresented in the no-low (30.9%) group compared with the moderate-high (22.5%) group and was associated with reduced likelihood (p = 0.029; OR: 0.63; 95% CI: 0.37–1.06) of reporting moderate-high combined (pain and disability) (Figure 1C).

OPRM1 (rs1799971 A>G-rs540825 T>A)

The inferred *OPRM1* rs1799971 A>G-*OPRM1* rs540825 T>A haplotype analyses generated four combinations, (A-T, A-A, G-T and G-A) (Figure 1D–F). For pain (p = 0.040) the inferred (G-T) haplotype (p = 0.019; OR: 0.33; 95% CI: 0.14-0.75) was overrepresented in the no-low (16.4%) group compared with the moderate-high (6.9%) group and was associated with a reduced likelihood for reporting moderate-high pain (Figure 1D). On the contrary, no differences in haplotype frequency distribution patterns were noted for disability (p = 0.173) or the combined (pain and disability) (p = 0.239; Figure 1E & F).

Stepwise allele-allele interaction between *ABCB1* & *OPRM1* polymorphisms

Individual genotype data for the *ABCB1* (rs1045642 G>A-rs1128503 G>A) and *OPRM1* (rs1799971 A>G-rs540525 T>A) polymorphisms were used to construct allele–allele combinations for *ABCB1* and *OPRM1* as a proxy for potential gene–gene interactions.

ABCB1 (rs1045642 G>A-rs1128503 G>A)–*OPRM1* (rs1799971 A>G-rs540525 T>A) generated 16 combinations, of which eight yielded frequencies >3%. Evaluation of the allele–allele combination frequencies noted no

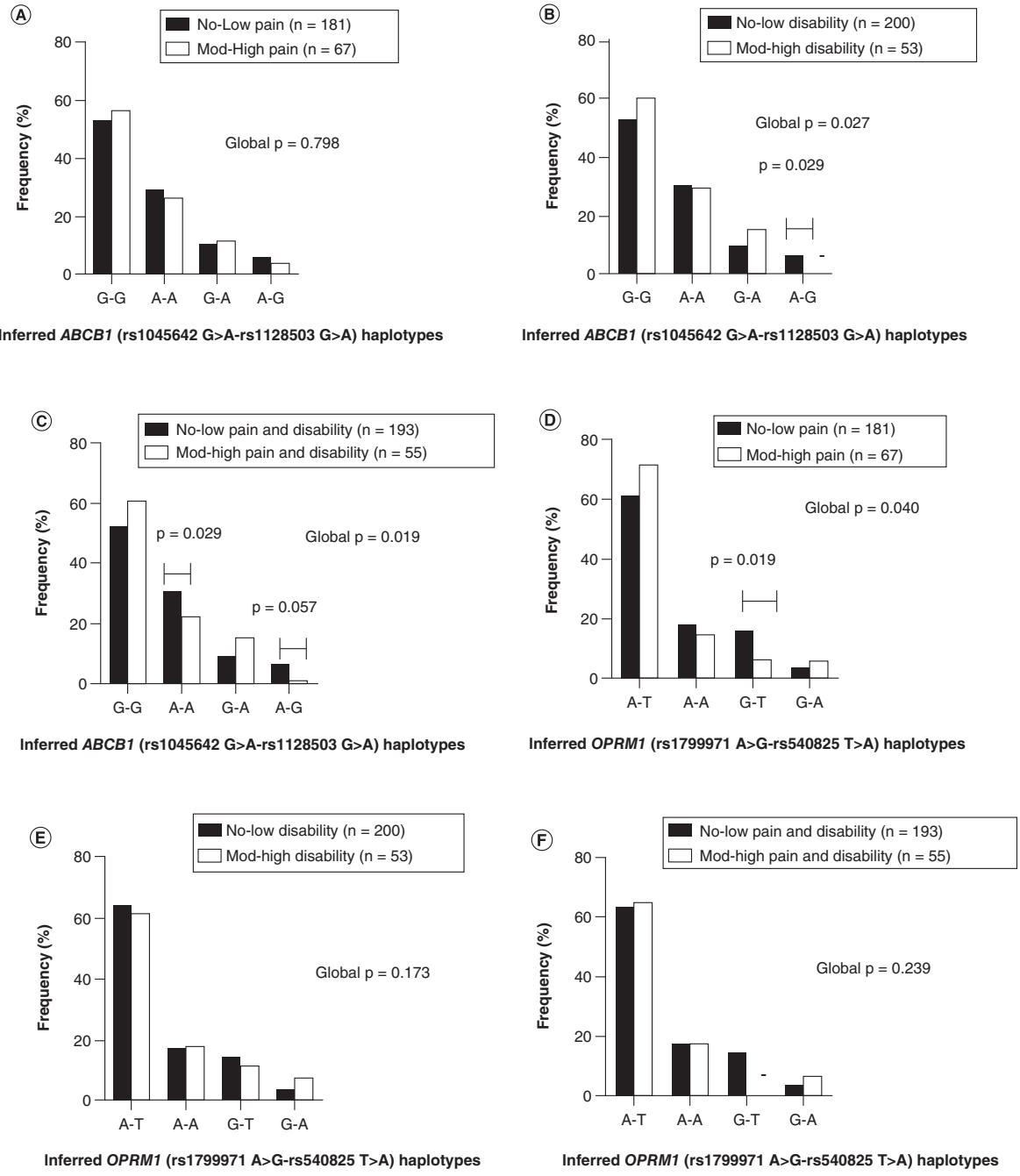


Figure 1. The inferred frequency distributions for the *ABCB1* (rs1045642 G>A–rs1128503 G>A) and *OPRM1* (rs1799971 A>G-rs540825 T>A) haplotypes in the no-low (black bars) and moderate-high (white bars) groups. For **(A & D)** pain, **(B & E)**, disability and **(C & F)** combined (pain and disability) in South African breast cancer survivors. Depicted are statistically significant differences in the inferred haplotype frequencies between the two groups with the number of participants in parenthesis (n) and (-) presenting no frequency detected for a haplotype. All p-values shown are adjusted for age.

significant differences between the no-low and moderate-high groups for pain, disability and combined (pain and disability) ($p > 0.05$; Supplementary Figure 1). Next, the *ABCB1* (rs1045642 G>A-rs1128503 G>A)–*OPRM1* (rs540525 T>A) generated eight allele–allele combinations of which six combinations yielded frequencies >5%; however, no significant differences were noted between the no-low and moderate-high pain, disability and combined (pain and disability) groups (Supplementary Figure 2).

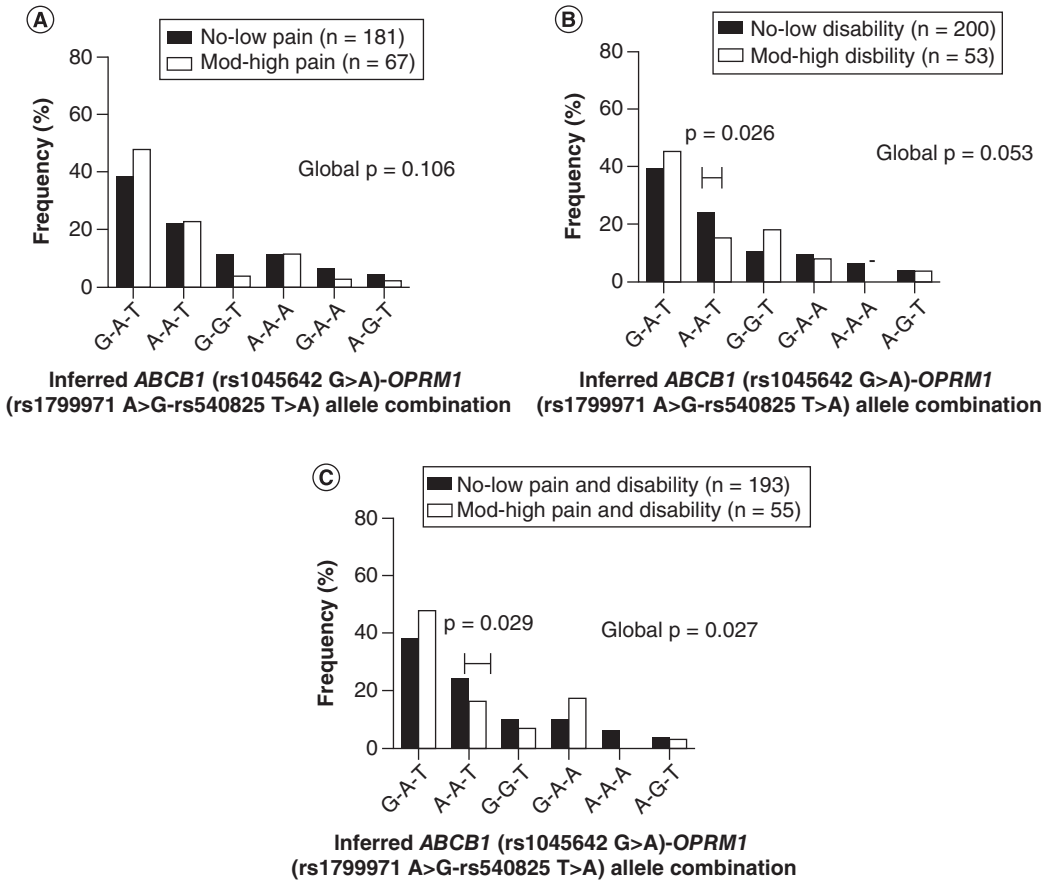


Figure 2. The inferred frequency distributions for the *ABCB1* (rs1045642 G>A)–*OPRM1* (rs1799971 A>G-rs540825 T>A) allele–allele combinations in the no-low (black bars) and moderate-high (white bars) groups. (A) Pain in South African breast cancer survivors. **(B)** Disability in South African breast cancer survivors. **(C)** Combined (pain and disability) in South African breast cancer survivors. Depicted are statistically significant differences in the inferred haplotype frequencies between the two groups with the number of participants in parenthesis (n) and (-) presenting no frequency detected for a haplotype.
All p-values shown are adjusted for age.

ABCB1 (rs1045642 G>A)–*OPRM1* (rs1799971 A>G-rs540825 T>A) generated six (G-A-T, A-A-T, G-G-T, G-A-A, A-A-A and A-G-T) allele combinations with frequencies >5% (Figure 2). No significant differences in the frequency distribution patterns were noted for these allele–allele combinations when pain scores were evaluated (p = 0.106; Figure 2A). In the disability (p = 0.053) and combined (pain and disability) scores (p = 0.027), the (A-A-T) combination was overrepresented in the no-low (24.4% and 24.4%) compared with the moderate-high (16.0% and 16.7%) groups, respectively (Figure 2B & C). Furthermore, the (A-A-T) combination was associated with a reduced likelihood of reporting moderate-high disability (p = 0.026; OR: 0.60; 95% CI: 0.30–1.18) and combined (pain and disability) scores (p = 0.029; OR: 0.58; 95% CI: 0.18–1.45).

Evaluation of *ABCB1* (rs1045642 G>A) – *OPRM1* (rs1799971 A>G) generated four combinations, (G-A, G-G, A-G and A-A) (Figure 3A–C). No significant differences were noted for either pain (p = 0.244) or disability (p = 0.077) (Figure 3A & B). For combined (pain and disability) (p = 0.028), the (G-A) combination was underrepresented in the no-low (49.3%) compared with the moderate-high (65.2%) group (Figure 3C). The (A-A) combination was also overrepresented in the no-low (31.8%) compared with the moderate-high (17.6%) group (Figure 3C). Moreover, the (G-A) (p = 0.005; OR: 1.00) and (A-A) (p = 0.008; OR: 0.44; 95% CI: 0.24–0.80) combinations were associated with equal odds and reduced likelihoods of reporting moderate-high combined (pain and disability), respectively.

Evaluation of the *ABCB1* (rs1045642 G>A) – *OPRM1* (rs540825 T>A) identified four allele–allele combinations (G-T, A-T, G-A and A-A) (Figure 3D–F). No significant differences in allele-frequencies were noted

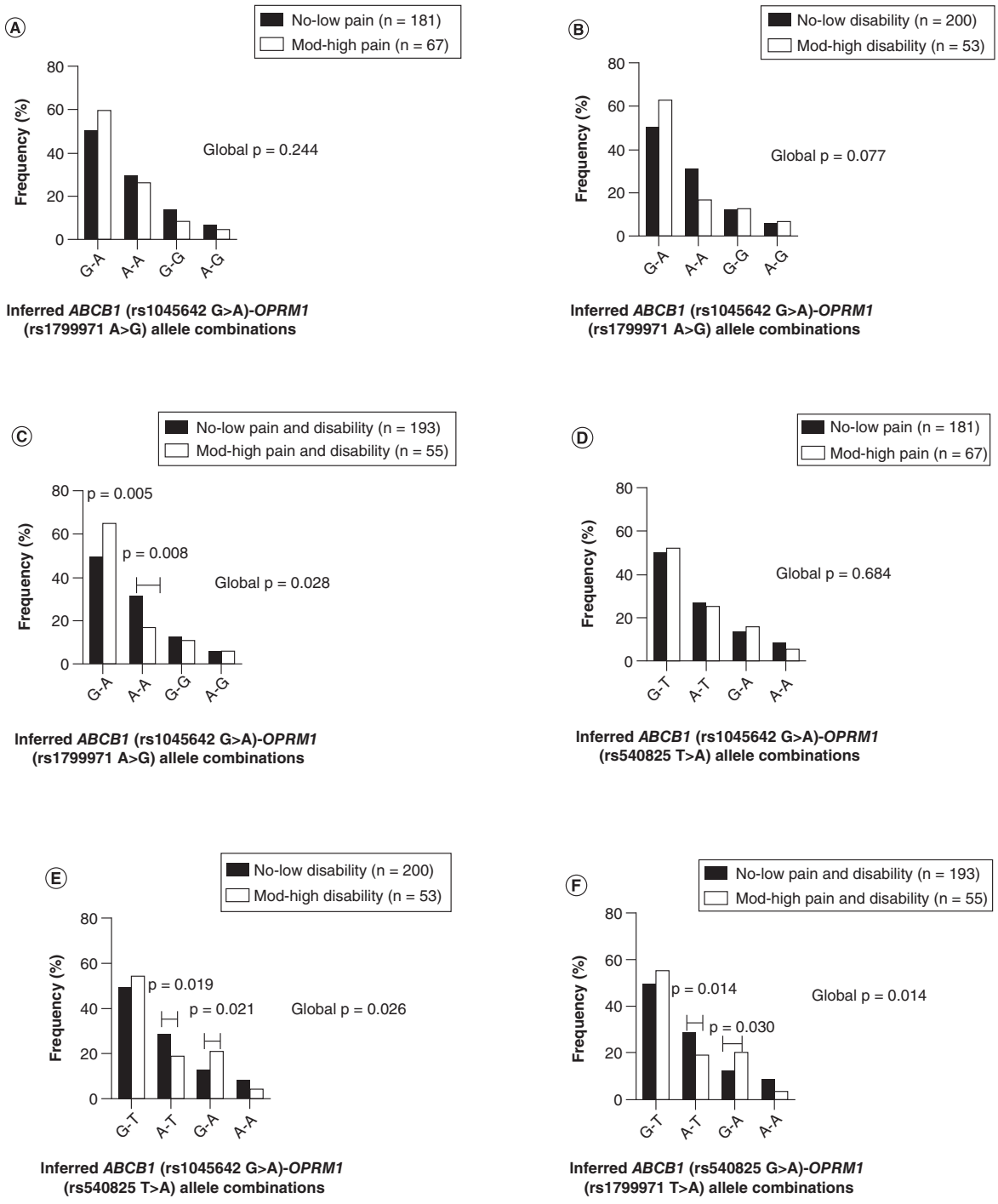


Figure 3. The inferred frequency distributions for the *ABCB1* (rs1045642 G>A)–*OPRM1* (rs1799971 A>G) and *ABCB1* (rs1045642 G>A)–*OPRM1* (rs540825 T>A) allele–allele combinations in the no-low (black bars) and moderate-high (white bars) groups. (A & D) Pain in South African breast cancer survivors. **(B & E)** Disability in South African breast cancer survivors. **(C & F)** Combined (pain and disability) in South African breast cancer survivors. Depicted are statistically significant differences in the inferred haplotype frequencies between the two groups with the number of participants in parenthesis (n).
All p-values shown are adjusted for age.

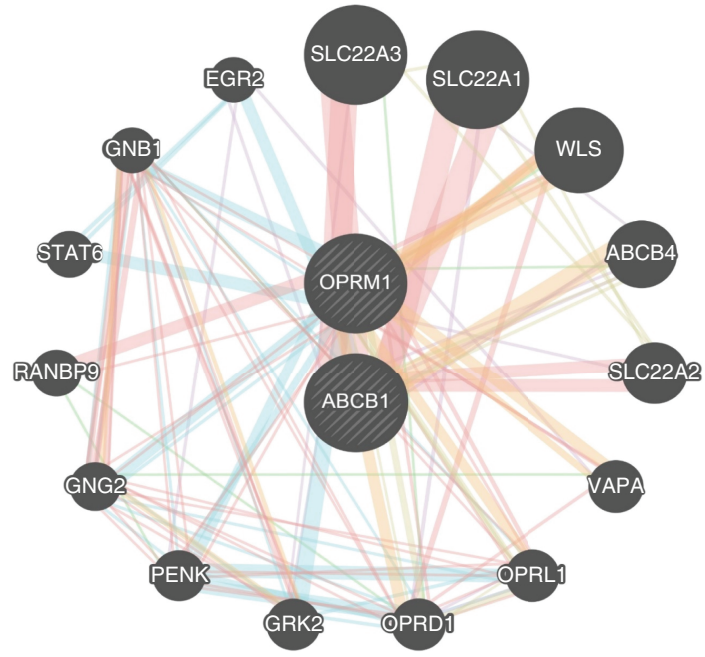


Figure 4. GeneMANIA Network analysis for the *ABCB1* and *OPRM1* genes. Physical interaction (pink), co-expressed (purple), predicted (orange), co-localization (dark blue), genetic interactions (dark green), pathway (light blue) and shared protein domain (light green) network for 15 genes.

between the no-low and moderate-high groups for pain (p = 0.684; Figure 3D). For disability (p = 0.026) the (A-T) combination (p = 0.019; OR: 0.62; 95% CI: 0.33–1.16) was overrepresented in the no-low (28.8%) group compared with the moderate-high (19.3%) group and associated with reduced likelihoods of reporting disability (Figure 3E). The (G-A) combination (p = 0.021; OR: 1.57; 95% CI: 0.30–3.10) was underrepresented in the no-low (12.9%) compared with the moderate-high (21.4%) group and associated with increased likelihood for reporting disability (Figure 3E). For combined (pain and disability), the frequencies for the (A-T) (p = 0.014; OR: 0.62; 95% CI: 0.35–1.10) and (G-A) (p = 0.030; OR: 1.50; 95% CI: 0.78–2.86) allele combinations in the no-low (28.9% and 12.7%) and moderate-high (19.8% and 20.7%) groups were comparable to the frequencies observed in the disability category (Figure 3F). Once more, the (A-T) and (G-A) combinations were associated with a reduced and increased likelihoods of reporting combined (pain and disability).

Bioinformatic analysis

GeneMANIA analysis for *ABCB1* and *OPRM1*, found no direct associated networks; however, we identified three secondary gene-associated networks (Supplementary Table 8) [65]. The analysis showed the *ABCB4*, *GNB1* and *SLC22A2* genes each shared co-expressed, predicted, shared protein domains, genetic interactions, pathway and physical interaction networks with *ABCB1* and *OPRM1* (Figure 4).

The Enrichr web-based application was used to screen *ABCB1* and *OPRM1* against several libraries of gene sets for transcriptional and regulatory factors, biological processes, pathways, diseases/drugs and phenotypes [66]. In the transcription library, the results showed *ABCB1* and *OPRM1* are both predicted targets for the microRNA-875-5p (Targetscan microRNA 2017; Supplementary Table 9). In the pathway library, the *ABCB1* and *OPRM1* gene products are associated with T-cell receptor regulation of apoptosis (Bioplanet 2019 Pathways) and Epilepsi (Elsevier Pathways Collection; Supplementary Table 9). Gene ontology (GO) libraries showed both genes are associated with the regulation of response to stress (GO0080134, Biological Process 2021; Supplementary Table 9). In addition, in the library for diseases and drugs, the genes are shown to be associated with Huntington's disease, a neurodegenerative disease (HDsigDB Human 2021; Supplementary Table 9).

SFOLD analysis of *ABCB1* rs1045642 G>A indicated that compared to the (G) allele, the (A) allele resulted in the loss of a hydrogen bond at nucleotide position 44 in the 5′-3′ direction and resulted in a predicted increased multibranch loop (Supplementary Figure 3). In addition, the substitution noted a 3-point (-20.10 to -17.10) change in the energy reaction representing protein stability. Analysis of the rs1128503 SNP containing structure indicated no distinct changes, however, the reaction showed a changed in energy between the (G) and (A) alleles (Supplementary Figure 3). SFOLD analysis of *OPRM1* rs1799971 A>G showed that compared with the (A) allele, the (G) allele resulted in the loss of an internal loop (Supplementary Figure 4). Furthermore, the A>G substitution

resulted in predicted 5-point change (ΔG°_{37} = -33.20 to -38.10) in the energy reaction, with the (G) allele noting a more negative ΔG°_{37}, compared with the (A) allele. The secondary predicted changes for *OPRM1* rs540825 T>A, indicated no structural differences between these two alleles; however, a change in the energy reaction was noted (ΔG°_{37} = -22.70 to -19.70; Supplementary Figure 4).

Discussion

This study is the first to evaluate associations between polymorphisms in genes involved in the opioid metabolism and pain pathway, and chronic shoulder pain and dysfunction in BCSs of mixed ancestry from South Africa. The findings of our study confirms that age is a risk factor, and infers that polymorphisms in the *ABCB1* and *OPRM1* genes may play a role in the development of chronic shoulder pain and dysfunction in BCSs.

Younger age was associated with an increased likelihood for reporting moderate-high pain, disability and combined (pain and disability) (Table 1). This result is in alignment with previous research reporting the association between age and persistent pain in BCSs [12,67]. It is proposed that changes in pain perception is age related; compared with older adults (>65 years), younger adults (18–44 years) report greater pain due to psychological stress [68]. Younger adults with chronic pain suffer physiological and mental health issues, and the disruption of daily routines, including the ability to work full time that can lead to a financial burden [69,70].

With more than 70% of participants having received an ALND, it was noteworthy that no significant association was found between ALND and shoulder pain, disability or combined (pain and disability). ALND is the surgical removal of lymph nodes in the axilla, which is a secondary measure to the detection of a tumor positive sentinel lymph node [71,72]. Moreover, it has been described as a risk factor for the development of persistent pain post-surgery for BC [12].

Evaluation of the genetic contribution of *ABCB1* gene polymorphisms in modulating shoulder disability and movement-related pain in this BCS cohort showed that the *ABCB1* rs1045642 (A/A) genotype and (A) allele were significantly associated with 80/75% and 48/50% reduced likelihood of reporting moderate-high disability/combined (pain and disability), respectively. Haplotype analyses further highlighted the *ABCB1* genetic locus in modulating pain and disability where the *ABCB1* (rs1045642 G>A – rs1128503 G>A) inferred (A-G) haplotype was associated with reduced likelihood of reporting disability. The (A-G) haplotype was absent in the moderate-high group, which inferred a reduced (100%) likelihood for reporting disability. The findings of our study are correlative to other studies reporting lower pain, although there are few data on postoperative pain [28,73,74]. The functional rs1045642 (A) and rs1128503 (A) alleles have steadily been associated with requiring fewer opioids compared with (G) allele carriers and with rs1045642 (A) carriers regarded as 'good responders' [17,29,35,75,76]. It is reported that the rs1045642 (A/A) genotype and (A) allele is associated with decreased protein functions and are at greater risk of opioid-related side effects that includes sweating, muscular tension, stress and sedation, compared with (G/G) and (A/G) carriers [26,77,78]. Furthermore, given its implication in opioid requirements and response studies, it is hypothesized that the (A/A) genotype have lower P-gp expression that may result in less efflux of opioids at the BBB level and therefore individuals with that genotype profile, may need fewer opioids to control pain. This hypothesis is supported by the findings of Hoffmeyer *et al.* [26], who showed a reduction in P-gp expression and altered activity related to the (A/A) genotype. More recently, allelic-specific expression analysis of liver autopsy samples noted the rs1045642 (A) allele was associated lower mRNA levels compared with the (G) allele [79].

Analyses of the *OPRM1* genetic locus showed that although no independent associations were noted for the individual polymorphisms investigated, the inferred haplotypes implicate the genetic interval spanning these polymorphisms in modulating chronic shoulder pain and disability. The *OPRM1* (rs1799971 A>G – rs540825 T>A) inferred (G-T) haplotype was associated with a 67% reduced likelihood of reporting pain (Figure 2A). Research data for the *OPRM1* (rs1799971 A>G – rs540825 T>A) locus in postoperative pain is limited. A study conducted by De Gregori *et al* [80] analyzing seven *OPRM1* polymorphisms, including both rs1799971 A>G and rs540827 T>A, observed that haplotype carriers containing the (G) and (T) alleles required more postoperative analgesia (POA) than haplotype carriers containing the (A) and (A) alleles, respectively. This observation suggests that the rs1799971 (G) and rs540825 (T) allele, in combination, required more POA may be in response to experiencing greater pain. It has been shown that the rs1799971 (G) allele increases the receptor binding affinity threefold for endogenous opioids yet reduces the expression of the receptor and leads to high morphine requirements [81]. Also, *OPRM1* rs1799971 and rs540825 polymorphisms have both been investigated in pain and opioid studies because both have been described to modify the mu-receptor properties, although inconsistencies remain [30,36,40,49,76,80,82,83].

Evaluation of the *ABCB1-OPRM1* allele combinations highlighted a combined genetic contribution to modulating disability and movement-related pain in this BCS cohort. Several associations were noted; however, the most interesting finding was for *ABCB1* (rs1045642 G>A) – *OPRM1* (rs540825 T>A) where the (A-T) allele combination was associated with reduced (38% and 38%) likelihood of reporting moderate-high disability and combined (pain and disability) (Figure 3E). Coupled with the alternate combination, (G-A), being associated with increased (57% and 50%) likelihood of reporting moderate-high disability and combined (pain and disability) (Figure 3F). Research data for pain studies surrounding the gene–gene interactions between the *ABCB1* and *OPRM1* genes, are limited. On the basis of these findings, we hypothesize that the *ABCB1* rs1045642 (A) allele may act as a potential important regulator of opioid distribution. The *OPRM1* rs540825 (T) allele is reported to require more opioids and, when inherited with the *ABCB1* rs1045642 (A) allele, resulted in reduced likelihood of reporting pain and disability [80]. This is further supported by the alternate allele combinations whereby the *OPRM1* rs540825 (A) allele that is reported to require fewer opioids are inherited with the *ABCB1* rs1045642 (G) allele, shown to have increased P-gp expression, an increased likelihood of reporting pain and disability was noted. This could be explained as nociceptive sensitization by exposure to opioids, leading to opioid-induced hyperalgesia (OIH), the paradoxical effect that may intensify preexisting pain [84]. Furthermore, it is reported that upregulation of the P-gp membrane protein may contribute to opioid tolerance [85].

Another noteworthy association was the inferred (A-A) (*ABCB1* rs1045642 G>A – *OPRM1* rs1799971 A>G) allele combination associated with a 66% reduction in the likelihood of reporting combined (pain and disability) (Figure 3C). It was interesting to note in a previous study that haplotype carriers containing the *ABCB1* rs1045642 (A) and rs1128503 (G) alleles in combination with the *OPRM1* rs1799971 (A) allele were observed to have lower methadone dosage and plasma concentrations than the alternate *ABCB1* alleles [86], allowing the conclusion that *ABCB1* (lower) and *OPRM1* (higher) polymorphisms were associated with opposing dose requirements [86]. This supports the findings of the present study that the *ABCB1* rs1045642 (A) allele, when inherited with the *OPRM1* rs1799971 (A), modulates pain scores and, in the previous study, opioid requirements.

In the bioinformatic analysis, the GeneMANIA results showed that *ABCB1* and *OPRM1* share functional and associated networks that interact. Additionally, enrichment analysis generated form the libraries in Enrichr, showed *ABCB1* and *OPRM1* share common pathways related to apoptosis, immune response and opioid signaling. Using online mRNA structure prediction tools, we examined the predicted 2D RNA structure obtained by SFOLD for the regions containing the *ABCB1* and *OPRM1* SNPs. It is reported that SNPs can give rise to varying forms of mRNA structures, that consequently may affect mRNA stability and potentially alter translation efficiency of the protein and thereby affect protein function [87-90].

For the *ABCB1* rs1045642 G>A polymorphism, the predicted secondary structure for the (A) allele showed an increased multibranch loop at that position compared with the (G) allele form. In addition, the (A) allele showed an increase in the ΔG°_{37} score indicating a less stable mRNA structure compared with the (G) allele form, which supports the findings observed in the study conducted by Wang and Sadee [79].

We hypothesize that this polymorphism could therefore potentially modulate expression and availability of the transporter, through such structural changes. Although the *ABCB1* rs1128503 (G) and (A) allele forms showed no differences in the predicted secondary mRNA structures, changes in their melting domains were noted as reflected in the energy reaction of the ΔG°_{37} scores, indicating that this polymorphism may still affect the transport through other processes; for example, it may change interactions/efficiencies of the transporter protein within the cellular environment [87–90]. For the *OPRM1* rs1799971 A>G polymorphism, the predicted secondary mRNA structure for the (G) allele, noted the loss of one (of three) internal loop at that position. Compared with the (A) allele, the (G) allele forms a hydrogen bond with the opposite nucleotide at that position. The predicted secondary structure for the (G) allele also noted a decrease in ΔG°_{37} scores, which suggests a more stable mRNA transcript compared with the (A) allele form, and therefore this polymorphism could potentially modulate the expression and availability of the mu-opioid receptor through such structural changes. For the *OPRM1* rs540825 T>A polymorphisms, no structural differences between the (T) and (A) allele forms were noted. However, like *ABCB1* rs1128503 G>A, the differences in ΔG°_{37} scores suggests that the polymorphism may still affect the receptor's binding capacity through mRNA-structure-dependent processes [87–90].

With nearly 50% of BCSs suffering chronic shoulder pain and disability, it is imperative to understand and identify risk factors influencing susceptibility [7]. Current research in chronic postoperative pain phenotypes have described severe acute postoperative pain as a risk factor [12]. Moreover, genetic association studies report the relationship between candidate gene polymorphisms and pain phenotypes are vital in the understanding of the mechanisms

underlying these differences in pain relief/perception [87]. We, therefore, hypothesize that inadequate pain relief during the acute postoperative period may influence pain severity thereby increasing the likelihood of developing chronic pain. The results found in this study show the complex nature of functional genetic polymorphisms with the potential to alter the protein structure and function.

Limitations

There are several limitations to this study. The sample was powered at <80% to detect effects sizes of OR = 1.5 (Supplementary Table 2). The study followed a hypothesis approach in which both *ABCB1* and *OPRM1* polymorphisms were previously implicated in the pain phenotype. More than two genetic polymorphisms were assessed (family-wise error rate) and, taken together with the small and underpowered (<80%) sample size, multiple testing was not adjusted for. Logistic regression analysis was applied to investigate gene–gene interactions. However, it is reported that this tends to be difficult to detect because many multilocus genotype combinations may have few or no data points, thereby hindering the outcome [88]. In our analysis, allele frequencies >3% were used to describe the gene–gene interaction between *ABCB1* and *OPRM1*. Increasing the sample size may increase the power to detect significant differences in clinically relevant characteristics with genotype/allele frequencies and allow us to consider clinically relevant confounders in the regression analyses. In addition, in this cohort ethnicity was self-reported and therefore does not hold the same strength as genomic estimates, which may undermine the population stratification of our cohort.

Conclusion

In conclusion, this study adds to the consensus that age is a risk factor for pain and that lymph node surgery could potentially identify individuals that may be susceptible to pain and dysfunction in BCSs. Furthermore, this study provides evidence of an association between the genetic polymorphisms of the *ABCB1* and *OPRM1* genes and chronic shoulder pain and disability in BCSs. Future studies incorporating bigger genetic intervals and larger population sizes are required to further scrutinize and elucidate the role of the *ABCB1* and *OPRM1* genes in chronic shoulder pain and dysfunction.

Summary points

- *ABCB1* (rs1045642 G>A; rs1128503 G>A) and *OPRM1* (rs1799971 A>G; rs540825 T>A) single-nucleotide polymorphisms were genotyped in (n = 252) South African breast cancer survivors (BCSs) of mixed ancestry.
- The Shoulder Pain and Disability Index tool was used to assess pain, disability and combined (pain and disability); results indicated that 27.0%, 19.0% and 22.0% of participants reported moderate-high pain, disability and combined (pain and disability), respectively.
- Independently, a significant association was noted for the *ABCB1* rs1045642 single-nucleotide polymorphism, with the (A/A) genotype and (A) allele associated with reduced likelihood of reporting moderate-high disability.
- For the *ABCB1* (rs1045642 G>A – rs1128503 G>A) haplotype analysis, the inferred (A-G) haplotype was significantly associated with reduced likelihoods of reporting moderate-high disability.
- The *OPRM1* (rs1799971 A>G – rs540825 T>A) inferred (G-T) haplotype was significantly associated with reduced likelihoods of reporting moderate-high pain.
- Gene–gene interaction analyses demonstrated significant associations between the *ABCB1* (rs1045642 G>A) – *OPRM1* (rs1799971 A>G – rs540825 T>A) and the *ABCB1* (rs1045642 G>A) – *OPRM1* (rs1799971 A>G) combinations for disability.
- *ABCB1* (rs1045642 G>A) – *OPRM1* (rs540825 T>A) combination analyses demonstrated that the (A-T) combination was associated with reduced likelihood of reporting moderate-high disability, and the alternate (G-A) combination was associated with increased likelihood of reporting moderate-high disability.
- The results in this study support the hypothesis that genetic polymorphisms within key pain genes can significantly influence the development of chronic postoperative pain, including movement-related pain.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/pgs-2022-0020

Author contributions
All authors contributed to study design, development and write up. All laboratory arrays and experimentation were conducted by F Firfirey.

Acknowledgments
The authors thank the study participants, the nursing and administrative staff of the Clinical Research Centre and TS Mafu for their participation and assistance in the recruitment process and data capture.

Financial & competing interests disclosure
This work was supported by the University of Cape Town (WUN scholarship and Foundation Contingency award) and the National Research Foundations (grant no. 102470), South Africa. The opinions and conclusions summarized in the study, are those of the author/s and does not necessarily reflect the opinions of the funders. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research
The authors declare that they obtained the appropriate institutional ethics and review board approvals and have followed the principles stipulated in the Declaration of Helsinki for all human or animal experimental investigations. Furthermore, informed consent was obtained from all volunteers/participants involved in this study. Ethical clearance was provided by the Human Research Ethics Committee, University of Cape Town (HREC ref: 650/2016, 125/2017).

References
Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Denlinger CS, Carlson RW, Are M *et al.* Survivorship: introduction and definition. Clinical practice guidelines in oncology. *J. Natl. Compr. Cancer Netw.* 12(1), 34–45 (2014).
2. Marzorati C, Riva S, Pravettoni G. Who is a cancer survivor? A systematic review of published definitions. *J. Cancer Educ.* 32(2), 228–237 (2017).
3. Sanft T, Denlinger CS, Armenian S *et al.* NCCN guidelines insights: survivorship, version 2.2019. *J. Natl. Compr. Cancer Netw.* 17(7), 784–794 (2019).
4. Lee TS, Kilbreath SL, Refshauge KM *et al.* Prognosis of the upper limb following surgery and radiation for breast cancer. *Breast Cancer Res. Treat.* 110(1), 19–37 (2008).
5. Hidding JT, Beurskens CH, Van Der Wees PJ *et al.* Treatment related impairments in arm and shoulder in patients with breast cancer: a systematic review. *PLoS One* 9(5), e96748 (2014).
6. Kramer N, Ramjith J, Shamley D. Prevalence of shoulder morbidity after treatment for breast cancer in South Africa. *Support Care Cancer* 27(7), 2591–2598 (2019).

•• **This study describes the prevalence and severity of shoulder pain and dysfunction associated with breast cancer treatment sequelae in South Africa**

7. Chrischilles EA, Riley D, Letuchy E *et al.* Upper extremity disability and quality of life after breast cancer treatment in the Greater Plains Collaborative clinical research network. *Breast Cancer Res. Treat.* 175(3), 675–689 (2019).
8. Shamley D. A cross-disciplinary look at shoulder pain and dysfunction after treatment for breast cancer. *Int. J. Cancer Clin. Res.* 2(1), (2015).
9. Simon LS. Relieving pain in America: a blueprint for transforming prevention, care, education, and research. *J. Pain Palliative Care Pharmacother.* 26(2), 197–198 (2012).
10. George SZ, Parr JJ, Wallace MR *et al.* Biopsychosocial influence on exercise-induced injury: genetic and psychological combinations are predictive of shoulder pain phenotypes. *J. Pain* 15(1), 68–80 (2014).
11. Andersen KG, Duriaud HM, Jensen HE *et al.* Predictive factors for the development of persistent pain after breast cancer surgery. *Pain* 156(12), 2413–2422 (2015).
12. Wang L, Guyatt GH, Kennedy SA *et al.* Predictors of persistent pain after breast cancer surgery: a systematic review and meta-analysis of observational studies. *CMAJ* 188(14), E352–E361 (2016).

13. Salz T, Lavery JA, Lipitz-Snyderman AN *et al.* Trends in opioid use among older survivors of colorectal, lung, and breast cancers. *J. Clin. Oncol.* 37(12), 1001–1011 (2019).
14. Klepstad P. Genetic variability and opioid efficacy. *Curr. Anaesth. Crit. Care* 18(3), 149–156 (2007).
15. Croop JM. P-glycoprotein structure and evolutionary homologies. *Cytotechnology* 12(1-3), 1–32 (1993).
16. Hodges LM, Markova SM, Chinn LW *et al.* Very important pharmacogene summary: *ABCB1* (MDR1, P-glycoprotein). *Pharmacogenet. Genomics* 21(3), 152–161 (2011).
17. Campa D, Gioia A, Tomei A *et al.* Association of *ABCB1*/MDR1 and *OPRM1* gene polymorphisms with morphine pain relief. *Clin. Pharmacol. Ther.* 83(4), 559–566 (2008).
18. Parchure AS, Peng YB. The impact of opioid analgesics and the pharmacogenomics of *ABCB1* in opioid dependence and pharmacotherapies: a short review. *Open Pain J.* 13(1), 7–21 (2020).

•• **This mini review describes the most recent updates in the *ABCB1* pharmacogenomics surrounding the use of opioids, the benefits and drawbacks.**

19. Schaefer CP, Tome ME, Davis TP. The opioid epidemic: a central role for the blood brain barrier in opioid analgesia and abuse. *Fluids Barriers CNS* 14(1), 32 (2017).
20. Ambudkar SV, Kim IW, Sauna ZE. The power of the pump: mechanisms of action of P-glycoprotein (*ABCB1*). *Eur. J. Pharm. Sci.* 27(5), 392–400 (2006).
21. Schinkel AH. The physiological function of drug-transporting P-glycoproteins. *Semin. Cancer Biol.* 8(3), 161–170 (1997).
22. Thompson SJ, Koszdin K, Bernards CM. Opiate-induced analgesia is increased and prolonged in mice lacking P-glycoprotein. *Anesthesiology* 92(5), 1392–1399 (2000).
23. Ho RH, Kim RB. Transporters and drug therapy: implications for drug disposition and disease. *Clin. Pharmacol. Ther.* 78(3), 260–277 (2005).
24. Fung KL, Gottesman MM. A synonymous polymorphism in a common MDR1 (*ABCB1*) haplotype shapes protein function. *Biochim. Biophys. Acta* 1794(5), 860–871 (2009).
25. Sortica Vde A, Ojopi EB, Genro JP *et al.* Influence of genomic ancestry on the distribution of SLCO1B1, SLCO1B3 and *ABCB1* gene polymorphisms among Brazilians. *Basic Clin. Pharmacol. Toxicol.* 110(5), 460–468 (2012).
26. Hoffmeyer S, Burk O, Von Richter O *et al.* Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc. Natl. Acad. Sci. U S A* 97(7), 3473–3478 (2000).
27. Greiner B, Eichelbaum M, Fritz P *et al.* The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. *J. Clin. Invest.* 104(2), 147–153 (1999).
28. Wang XS, Song HB, Chen S *et al.* Association of single nucleotide polymorphisms of *ABCB1*, *OPRM1* and COMT with pain perception in cancer patients. *J. Huazhong Univ. Sci. Technol. Med. Sci.* 35(5), 752–758 (2015).
29. Horvat CM, Au AK, Conley YP *et al. ABCB1* genotype is associated with fentanyl requirements in critically ill children. *Pediatr. Res.* 82(1), 29–35 (2017).
30. Li J, Wei Z, Zhang J *et al.* Candidate gene analyses for acute pain and morphine analgesia after pediatric day surgery: African American versus European Caucasian ancestry and dose prediction limits. *Pharmacogenomics J.* 19(6), 570–581 (2019).

• **In this genetic association study, opioid requirements, predictive dose limits and pain were evaluated in children from two ethnically different populations**

31. Benavides R, Vsevolozhskaya O, Cattaneo S *et al.* A functional polymorphism in the ATP-Binding Cassette B1 transporter predicts pharmacologic response to combination of nortriptyline and morphine in neuropathic pain patients. *Pain* 161(3), 619–629 (2020).
32. Brambila-Tapia AJ. MDR1 (*ABCB1*) polymorphisms: functional effects and clinical implications. *Rev. Invest. Clin.* 65(5), 445–454 (2013).
33. Pecina M, Love T, Stohler CS *et al.* Effects of the Mu opioid receptor polymorphism (*OPRM1* A118G) on pain regulation, placebo effects and associated personality trait measures. *Neuropsychopharmacology* 40(4), 957–965 (2015).
34. Bond C, Laforge KS, Tian M *et al.* Single-nucleotide polymorphism in the human mu opioid receptor gene alters β-endorphin binding and activity: Possible implications for opiate addiction. *Proc. Natl. Acad. Sci.* 95(16), 9608–9613 (1998).
35. Hajj A, Peoc'h K, Laplanche JL *et al.* Genotyping test with clinical factors: better management of acute postoperative pain? *Int. J. Mol. Sci.* 16(3), 6298–6311 (2015).
36. Lopez Soto EJ, Catanesi CI. Human population genetic structure detected by pain-related mu opioid receptor gene polymorphisms. *Genet. Mol. Biol.* 38(2), 152–155 (2015).
37. Huang P, Chen C, Mague SD *et al.* A common single nucleotide polymorphism A118G of the mu opioid receptor alters its N-glycosylation and protein stability. *Biochem. J.* 441(1), 379–386 (2012).
38. Garriock HA, Tanowitz M, Kraft JB *et al.* Association of mu-opioid receptor variants and response to citalopram treatment in major depressive disorder. *Am. J. Psychiatry* 167(5), 565–573 (2010).

39. Sia AT, Sng BL, Lim EC *et al.* The influence of ATP-binding cassette sub-family B member -1 (*ABCB1*) genetic polymorphisms on acute and chronic pain after intrathecal morphine for caesarean section: a prospective cohort study. *Int. J. Obstet. Anesth.* 19(3), 254–260 (2010).
40. Kolesnikov Y, Gabovits B, Levin A *et al.* Chronic pain after lower abdominal surgery: do catechol-O-methyl transferase/opioid receptor mu-1 polymorphisms contribute? *Mol. Pain* 9, 19 (2013).
41. Little J, Higgins JP, Ioannidis JP *et al.* Strengthening the Reporting of Genetic Association Studies (STREGA) – an extension of the STROBE statement. *Geneti. Epidemiol.* 33(7), 581–598 (2009).
42. De Wit E, Delport W, Rugamika CE *et al.* Genome-wide analysis of the structure of the South African Coloured population in the Western Cape. *Hum. Genet.* 128(2), 145–153 (2010).
43. Lahiri DK, Nurnberger JI Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res.* 19(19), 5444 (1991).
44. Tengrup I, Tennvall-Nittby L, Christiansson I, Laurin M. Arm morbidity after breast-conserving therapy for breast cancer. *Acta Oncol.* 39(3), 393–397 (2000).
45. Macdermid JC, Solomon P, Prkachin K. The Shoulder Pain and Disability Index demonstrates factor, construct and longitudinal validity. *BMC Musculoskelet. Disord.* 7(1), 12 (2006).
46. Mafu TS, September AV, Shamley D. KDR inferred haplotype is associated with upper limb dysfunction in breast cancer survivors of mixed ancestry. *Cancer Manag. Res.* 11, 3829–3845 (2019).
47. Roy JS, Macdermid JC, Woodhouse LJ. Measuring shoulder function: a systematic review of four questionnaires. *Arthritis Rheum.* 61(5), 623–632 (2009).
48. Hill CL, Lester S, Taylor AW *et al.* Factor structure and validity of the shoulder pain and disability index in a population-based study of people with shoulder symptoms. *BMC Musculoskelet. Disord.* 12(1), 8 (2011).
49. Bartosova O, Polanecky O, Perlik F *et al. OPRM1* and *ABCB1* polymorphisms and their effect on postoperative pain relief with piritramide. *Physiol. Res.* 64(Suppl. 4), S521–527 (2015).
50. Mafu TS, September AV, Shamley D. Regulatory VCAN polymorphism is associated with shoulder pain and disability in breast cancer survivors. *Human Genomics* 15(1), 36–36 (2021).
51. Gauderman, WJ. Sample size requirements for matched case-control studies of gene-environment interaction. *Stat. Med.* 21 35–50 (2002).
52. Nesvold IL, Dahl AA, Lokkevik E *et al.* Arm and shoulder morbidity in breast cancer patients after breast-conserving therapy versus mastectomy. *Acta Oncol.* 47(5), 835–842 (2008).
53. Lauridsen MC, Overgaard M, Overgaard J *et al.* Shoulder disability and late symptoms following surgery for early breast cancer. *Acta Oncol.* 47(4), 569–575 (2008).
54. Gartner R, Jensen MB, Nielsen J *et al.* Prevalence of and factors associated with persistent pain following breast cancer surgery. *JAMA* 302(18), 1985–1992 (2009).
55. Nascimento SLD, Oliveira RRD, Oliveira MMFD, Amaral MTPD. Complicações e condutas fisioterapêuticas após cirurgia por câncer de mama: estudo retrospectivo. *Fisioterapia Pesquisa* 19(3), 248–255 (2012).
56. Johansen S, Fossa K, Nesvold IL *et al.* Arm and shoulder morbidity following surgery and radiotherapy for breast cancer. *Acta Oncol.* 53(4), 521–529 (2014).
57. Runowicz CD, Leach CR, Henry NL *et al.* American cancer society/American society of clinical oncology breast cancer survivorship care guideline. *Cancer* 66(1), 43–73 (2016).
58. Cristina Martins Da Silva R, Rezende LF. Assessment of impact of late postoperative physical functional disabilities on quality of life in breast cancer survivors. *Tumori J.* 100(1), 87–90 (2014).
59. Dell. Inc. Dell Statistica (Data Analysis Software System) version 13 (2016). www.statsoft.com.
60. RStudio. RStudio: Integrated Development for R. Boston, MA (2020). http://www.rstudio.com/.
61. Warnes G, Leisch F, Man M, Warnes MG. Package 'Genetics'. NY, USA (2012). http://brieger.esalq.usp.br/CRAN/web/packages/genetics/genetics.pdf
62. González J, Armengol L, Guinó E *et al.* SNPassoc: SNPs-based whole genome association studies. R package version 1.9.2. 1–8 (2014). http://CRAN.R-project.org/package=SNPassoc.Rpackage version
63. Schaid DJ, Rowland CM, Tines DE *et al.* Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am. J. Human Genet.* 70(2), 425–434 (2002).
64. Sinnwell JP, Schaid D. Statistical methods for haplotypes when linkage phase is ambiguous. (2011).
65. Warde-Farley D, Donaldson SL, Comes O *et al.* The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res.* 38(Suppl. 2), W214–W220 (2010).
66. Xie Z, Bailey A, Kuleshov MV *et al.* Gene set knowledge discovery with Enrichr. *Curr. Protocols* 1(3), e90 (2021).

67. Doong SH, Dhruva A, Dunn LB *et al.* Associations between cytokine genes and a symptom cluster of pain, fatigue, sleep disturbance, and depression in patients prior to breast cancer surgery. *Biol. Res. Nurs.* 17(3), 237–247 (2015).
68. Riley Iii JL, Wade JB, Robinson ME, Price DD. The stages of pain processing across the adult lifespan. *J. Pain* 1(2), 162–170 (2000).
69. Ackerman IN, Page RS, Fotis K *et al.* Exploring the personal burden of shoulder pain among younger people in Australia: protocol for a multicentre cohort study. *BMJ Open* 8(7), e021859 (2018).
70. Vargas C, Bilbeny N, Balmaceda C *et al.* Costs and consequences of chronic pain due to musculoskeletal disorders from a health system perspective in Chile. *Pain Rep.* 3(5), e656 (2018).
71. Gordon A, Alsayouri K. Anatomy, shoulder and upper limb, axilla. StatPearls (2019). www.ncbi.nlm.nih.gov/books/NBK547723/
72. Gherghe M, Bordea C, Blidaru A. Sentinel lymph node biopsy (SLNB) vs. axillary lymph node dissection (ALND) in the current surgical treatment of early stage breast cancer. *J. Med. Life* 8(2), 176–180 (2015).
73. Persson AKM, Pettersson FD, Akeson J. Single nucleotide polymorphisms associated with pain sensitivity after laparoscopic cholecystectomy. *Pain Med.* 19(6), 1271–1279 (2018).
74. Tanabe Y, Shimizu C, Hamada A *et al.* Paclitaxel-induced sensory peripheral neuropathy is associated with an *ABCB1* single nucleotide polymorphism and older age in Japanese. *Cancer Chemother. Pharmacol.* 79(6), 1179–1186 (2017).
75. Bastami S, Gupta A, Zackrisson AL *et al.* Influence of UGT2B7, *OPRM1* and *ABCB1* gene polymorphisms on postoperative morphine consumption. *Basic Clin. Pharmacol. Toxicol.* 115(5), 423–431 (2014).
76. Gong XD, Wang JY, Liu F *et al.* Gene polymorphisms of *OPRM1* A118G and *ABCB1* C3435T may influence opioid requirements in Chinese patients with cancer pain. *Asian Pac. J. Cancer Prev.* 14(5), 2937–2943 (2013).
77. Rhodin A, Gronbladh A, Ginya H *et al.* Combined analysis of circulating beta-endorphin with gene polymorphisms in *OPRM1*, CACNAD2 and *ABCB1* reveals correlation with pain, opioid sensitivity and opioid-related side effects. *Mol. Brain* 6, 8 (2013).
78. Beer B, Erb R, Pavlic M *et al.* Association of polymorphisms in pharmacogenetic candidate genes (OPRD1, GAL, *ABCB1*, *OPRM1*) with opioid dependence in European population: a case-control study. *PLoS One* 8(9), e75359 (2013).
79. Wang D, Sadee W. Searching for polymorphisms that affect gene expression and mRNA processing: example *ABCB1* (MDR1). *AAPS J.* 8, E515–520 (2006).
80. De Gregori M, Diatchenko L, Ingelmo PM *et al.* Human genetic variability contributes to postoperative morphine consumption. *J. Pain* 17(5), 628–636 (2016).
81. Wang Y, Tan Z, Wu L *et al.* Role of *OPRM1*, *ABCB1* and CYP3A genetic polymorphisms on sufentanil treatment of postoperative cancer patients in China. *Int. J. Clin. Exp. Med.* 9(7), 13250–13258 (2016).
82. Bartosova O, Polanecky O, Sachl R *et al.* Epidural analgesia with sufentanil in relation to *OPRM1* and *ABCB1* polymorphisms. *Physiol. Res.* 68(Suppl. 1), S59–S64 (2019).
83. Zhao Z, Lv B, Zhao X, Zhang Y. Effects of *OPRM1* and *ABCB1* gene polymorphisms on the analgesic effect and dose of sufentanil after thoracoscopic-assisted radical resection of lung cancer. *Biosci. Rep.* 39(1), BSR20181211 (2019).

• **This research article describes the effects of *ABCB1* and *OPRM1*SNPs and analgesia requirements in a postoperative setting.**

84. Tompkins DA, Campbell CM. Opioid-induced hyperalgesia: clinically relevant or extraneous research phenomenon? *Curr. Pain Headache Rep.* 15(2), 129–136 (2011).
85. Mercer SL, Coop A. Opioid analgesics and P-glycoprotein efflux transporters: a potential systems-level contribution to analgesic tolerance. *Curr. Top. Med. Chem.* 11(9), 1157–1164 (2011).
86. Barratt DT, Coller JK, Hallinan R *et al. ABCB1* haplotype and *OPRM1* 118A > G genotype interaction in methadone maintenance treatment pharmacogenetics. *Pharmgenomics Pers. Med.* 5, 53–62 (2012).

•• **In this study, the authors describe an interactive effect between *ABCB1* and *OPRM1* polymorphism on methadone treatment, providing evidence of gene–gene interaction influencing drug pharmacogenomics.**

87. Shen LX Z, Basilion JP, Stanton VP *et al.* Single-nucleotide polymorphisms can cause different structural folds of mRNA. *Proc. Natl Acad. Sci. USA* 96(14), 7871–7876 (1999).
88. Duan J, Wainwright MS, Comeron JM *et al.* Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. *Hum. Mol. Genet.* 12(3), 205–216 (2003).
89. Nackley A, Shabalina S, Tchivileva I *et al.* Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. *Science* 314, 1930–1933 (2007).
90. Ding Y, Chan CY, Lawrence CE *et al.* Sfold web server for statistical folding and rational design of nucleic acids. *Nucleic Acid Res.* 32, F135–L141 (2004).

genes

MDPI

Article

Polymorphisms in *COMT* and *OPRM1* Collectively Contribute to Chronic Shoulder Pain and Disability in South African Breast Cancer Survivors'

Firzana Firfirey [1,2], Delva Shamley [3] and Alison V. September [1,2,4,*]

[1] Division of Physiological Sciences, Department of Human Biology, Faculty of Health Sciences, University of Cape Town 7700, South Africa
[2] Health through Physical Activity, Lifestyle and Sport Research Centre (HPALS), Department of Human Biology, Faculty of Health Sciences, University of Cape Town, Cape Town 7700, South Africa
[3] Division of Clinical Anatomy & Biological Anthropology, Department of Human Biology, Anatomy Building, Medical School, University of Cape Town, Cape Town 7700, South Africa
[4] International Federation of Sports Medicine (FIMS), Collaborative Centre of Sports Medicine, Department of Human Biology, University of Cape Town, Cape Town 7700, South Africa
* Correspondence: alison.september@uct.ac.za; Tel.: +27-21-650-4559

Abstract: Chronic shoulder pain and disability is a common adverse effect experienced by >40% of breast cancer survivors (BCS). Pain management protocols for acute and chronic pain include the use of opioids and opioid derivatives. Furthermore, pain-modulating genes, such as *COMT* and *OPRM1,* have been linked to the aetiology of chronic pain. This study aimed to investigate the association between genetic variants of major pain modulator genes and chronic pain/disability in BCS. Assessment of pain, disability and combined (pain and disability) symptoms were determined using the Shoulder Pain and Disability Index (SPADI). Participants were grouped according to their scores such as no-low (<30%) and moderate-high (≥30%) groups of pain, disability and combined (pain and disability). Genotyping of the *COMT* rs6269 (A > G), rs4633 (C > T), rs4818 (C > G) and the functional rs4680(G > A) SNPs within the BCS (N = 252) cohort were conducted using TaqMan® SNP assays. Genotype, allele, haplotype, and allele–allele combination frequencies were evaluated. Statistical analysis was applied, with significance accepted at $p < 0.05$. The *COMT* rs4680:A/A genotype was significantly associated with moderate-high pain ($p = 0.024$, OR: 3.23, 95% CI: 1.33–7.81) and combined (pain and disability) ($p = 0.015$, OR: 3.81, 95% CI: 1.47–9.85). The rs4680:A allele was also significantly associated with moderate-high pain ($p = 0.035$, OR: 1.58, 95% CI: 1.03–2.43) and combined (pain and disability) ($p = 0.017$, OR: 1.71, 95% CI: 1.07–2.71). For the inferred *COMT* (rs6269 A > G-rs4680 G > A) haplotype analyses, the G-G ($p = 0.026$, OR: 0.67, 95% CI: 0.38–1.18) and A-A ($p = 0.007$, OR: 2.09, 95% CI: 0.89–4.88) haplotypes were significantly associated with reduced and increased likelihoods of reporting moderate-high pain, respectively. The inferred A-A ($p = 0.003$, OR: 2.18, 95% CI: 0.92–5.17) haplotype was also significantly associated with combined (pain and disability). Gene–gene interaction analyses further showed allele–allele combinations for *COMT* (rs4680 G > A)-*OPRM1* (rs1799971 A > G) and *COMT* (rs4680 G > A)-*OPRM1*(rs540825 T > A) were associated with reporting pain and combined (pain and disability) symptoms, $p < 0.05$. The findings of this study suggest that *COMT* and *OPRM1* SNPs play a role in the development of chronic shoulder pain/disability in BCS in a unique South African cohort from the Western Cape.

Keywords: chronic shoulder pain and disability; Breast Cancer Survivors (BCS); genetic association; Gene-Gene interactions

Citation: Firfirey, F.; Shamley, D.; September, A.V., Polymorphisms in *COMT* and *OPRM1* Collectively Contribute to Chronic Shoulder Pain and Disability in South African Breast Cancer Survivors'. *Genes* **2023**, *14*, 9. https://doi.org/10.3390/genes14010009

Academic Editor: Stuart Raleigh

Received: 23 November 2022
Revised: 12 December 2022
Accepted: 16 December 2022
Published: 21 December 2022

1. Introduction

Roughly 40% of breast cancer survivors (BCS) endure chronic pain and dysfunction of the upper limb, as side effects associated with the different types of BC treatments [1]. These side effects may occur for up to six years after treatment [2]. Other side effects that have been described include lymphedema, tissue scarring, and fibrosis to name but a few [1,2]. Furthermore, several patient-related risk factors such as age, BMI, surgery type, amongst others have been associated with an increased risk [3]. Interestingly, severe acute post-operative pain is also considered a risk factor for the development of chronic pain [3]. To manage post-operative pain, clinicians largely rely on the use of opioids and/or opioid derivatives, the most frequently prescribed being morphine, codeine, tramadol, and fentanyl [4]. Genetics is more of a risk factor, which is hypothesized to explain 25% of the variability in BCS developing chronic shoulder pain and dysfunction, despite receiving treatment protocols [5,6]. Polymorphisms in the candidate genes Catechol-O-methyltransferase (*COMT*), and Opioid receptor μ 1 (*OPRM1*) gene, have been recognised as major pain modulators [7].

The *COMT* gene (chromosomal location: 22q11.21) encodes the catechol-*O*-methyltransferase enzyme. Two isoforms have been described for *COMT*, a membrane-bound (*MB- COMT*) and a soluble (*S-COMT*) form, each transcribed and regulated by two distinct promotors (Figure S1) [8]. The enzyme is essential in regulating the bioavailability of the catechols, such as dopamine (DA), epinephrine (EP), and norepinephrine (NEP), as well as catechol estrogens (ER) [9]. Catecholamines are known to act as both neurotransmitters and hormones to maintain the balance within the autonomic nervous system (ANS) [10]. Studies have shown that varying levels of catecholamines (excess/scarcity), including altered *COMT* activity, may lead to the over/under-activation of the sympathetic nervous system (SNS) [10,11]. The SNS and pain are understood to interact within this neuro-axis, implicating *COMT* enzymatic activity, particularly since altered levels of catecholamines are shown to result in persistent pain conditions [5,12,13].

Genetic studies have implicated *COMT* polymorphisms to be associated with chronic pain states [5,14]. Variation in *COMT* activity has been linked to the functional *COMT* rs4680 G > A polymorphism found on exon four at positions *MB- COMT*^{158}, and *S-COMT*^{108} [8,15]. The valine (*Val*) to methionine (*Met*) substitution is associated with a decrease enzyme thermostability and activity (up to 40% differences) explained by the hydrophobic *Met*/*Met* residues [8,16]. Three other *COMT* polymorphisms, rs6269 A > G (*intron 2*), rs4633 C > T (*His > His: exon 3*) and rs4818 C > G (*Leu > Leu: exon 4*) have been investigated in haplotype studies with rs4680 G > A [12,17]. A strong LD (D'> 0.94) is reported between the four polymorphisms [12,17]. Defining a central haploblock for *COMT*, these polymorphisms were used to characterize three major levels of pain sensitivity in healthy female volunteers, high (HPS: ACCG), average (APS: ATCA) and low (LPS: GCGG), [12,18]. The three characterised *COMT* haplotypes have been associated with altered secondary mRNA structures, and thereby different protein folding potentials and enzymatic activity [15]. The haplotypes account for 11- to 25-times the variations noted in *COMT* activity and thereby may surpass the functional significance associated with individual polymorphisms [12,15,17].

Population frequencies of *COMT* haplotypes are well described in several pain-related studies predominantly of Caucasian ancestry, and in non-breast cancer studies [12,18–20]. A study investigating the global genetic signatures of 28 *COMT* SNPs across nine geographical regions (45 populations), was conducted [21]. The study described different linkage disequilibrium (LD) patterns including the rs6269-rs4680 haploblock for each of these pain sensitivity levels [21]. The strongest LD for the rs6269-rs4818-rs740602-rs4818-rs4680 SNP pairs were observed in homogenous European, north, and south American populations [21]. In contrast, the LD across this genetic region for the African populations were not as strong. The South African (SA) mixed ancestry population is a genetically distinct cohort, with Asian, European, and African ancestral contributors [22]. Studies reporting genetic variability for mixed ancestry cohorts are limited, most notably

in BCS in sub-Saharan Africa [23]. Given the critical role of *COMT* in pharmacogenetics and pain, it is imperative to understand the population genetic structure for *COMT* SNPs within SA.

In addition, gene–gene interactions between *COMT* and *OPRM1* polymorphisms have been explored in relation to pain sensitivity in preoperative cancer, gynaecological, postoperative orthopaedic, and general surgery settings [18,24,25]. The basis for evaluating these interactions stems from studies reporting that *COMT* rs4680 G > A modulates *OPRM1* expression and receptor binding site availability in different brain structures [24]. The mu-opioid receptor (MOR1), encoded by *OPRM1,* is the main site of opioid-peptide binding and consequently influences both endogenous and exogenous analgesic responses [26]. Several studies have implicated the functional *OPRM1* rs1799971 A > G (A118G) SNP with variations in pain and opioid responsiveness [27–29]. As the most prevalent polymorphism reported for *OPRM1*, it is shown to reduce signal transduction and *OPRM1* expression [27–29]. In our previous report, the inferred *OPRM1* rs1799971 A > G-rs540825 T > A haplotype analysis implicated the G-T haplotype with a decreased risk for pain in BCS [30]. Specific *ABCB1-OPRM1* allele–allele combinations were also associated with pain and disability. Considering the complex relationship between genes and pain; the role of multiple gene interactions has been advocated in the aetiology of chronic pain development [18,31]. However, no studies have explored the role of *COMT* SNPs in the development of chronic shoulder pain following breast cancer surgery with a mixed ancestry background. In addition, none have investigated the gene–gene interactions between *COMT* and *OPRM1* in BCS with a mixed ancestry background.

The study, therefore, aimed to investigate nongenetic and genetic risk factors for chronic shoulder pain and disability in a SA cohort of mixed-ancestry BCS. Furthermore, the study aimed to describe the central haploblock distribution pattern for *COMT* and to evaluate the gene–gene interactions between *COMT* and *OPRM1*, for chronic pain and disability in BCS. The objectives were to: (i) Determine the genotype/allele frequency distributions for the *COMT* (rs6269 A > G, rs4633 C > T, rs4818 C > G, rs4680 G > A) polymorphisms, (ii) analyse inferred haplotype distribution using the two flanking SNPs of the *COMT* central haploblock, and iii) examine specific allele–allele combinations between *COMT* and *OPRM1* polymorphisms as a proxy for gene–gene interactions.

2. Materials and Methods

2.1. Study Design, Participants, and Settings

This cross-sectional genetic association study, was performed in accordance with the STREGA reporting recommendations [32]. Details of the present study design, participants, and settings were previously described [30]. Briefly, volunteers were recruited from a tertiary hospital and included if they were >18 yrs of age, diagnosed with unilateral BC for >1 yr prior to recruitment and were self-identified as SA mixed ancestry. Volunteers presenting with a history of comorbidities, or prior neck and shoulder pathologies were excluded. Following informed consent, the Shoulder Pain, and Disability Index (SPADI) questionnaire was administered to each participant. In addition, venous blood samples were collected from the unaffected arm, which was used for DNA extraction [30]. The study features a subset analysis of women (N = 252) aged between 22 yrs and 74 yrs (Mean ± SD [54 ± 9.8]) that form part of a larger ongoing project. The project investigates the association between genetic markers of pain genes and chronic shoulder pain and disability in BCS.

2.2. Instruments

2.2.1. Shoulder Pain and Disability Index (SPADI)

Shoulder pain and disability symptoms were evaluated using the SPADI index, a patient-reported questionnaire consisting of thirteen items describing daily activities [30]. Participants had to rate the daily activity on a scale of 0 (no-pain/no-difficulty) to 10 (worst

pain/difficulty) for each item under two domains. The first domain with only five items assesses pain, while the second domain with eight items assesses disability.

Item scores were subtotalled for each domain, converted to a percentage, and used to stratify participants into no-low (<30%) or moderate-high (≥30%) groups. The stratification of groups was based on earlier research that measured the effects of pain on the "activities of daily living" (ADL), demonstrating that moderate and severe pain corresponding to visual analogue scale (VAS) scores exceeding 30% and 50%, respectively to influence day-to-day activities [33,34]. In this study, we evaluated no-low and moderate-high groups of pain, disability and combined (pain and disability) scores.

2.2.2. SNP Selection and Genotyping

COMT (NP_000745.1) was selected based on previous associations described in the literature [7,31]. Following the manufacturer's instructions, TaqMan® SNP genotyping assays (Thermofisher Scientific, Applied Biosystems, Foster City, CA, USA) were used to genotype the N = 252 samples in 96-well plates. In a final volume of 8 µL, each sample reaction contained 4 µL of TaqMan® genotype master mix and 0.2 µL of TaqMan® specific primer that were diluted to one-times final concentration. The reaction also contained 2.8 µL of dH_2O and 1µl of DNA template from a concentrate of [DNA] 1–10 ng. Standard Polymerase Chain Reaction (PCR) conditions were applied for the *COMT* (rs6269 A > G; rs4633 C > T; rs4818 C > G; rs4680 G > A) SNPs, described in an earlier publication [30]. Furthermore, each reaction plate was loaded with technical repeats and negative controls (absent of DNA template) to control for experimental quality.

All PCR reactions were conducted using the Quant studio 3 Real-time PCR (Thermofisher Scientific, Applied Biosystems, Foster City, CA, USA) system. Subsequent analyses were achieved using the ThermoFisher Cloud genotyping analysis Software Version: 3.3.0-SR2-build 21. Genotyping was accepted as successful when all DNA samples were amplified for all SNPs, except when failing to amplify after two repeat runs. The following amplification success rates were recorded for the *COMT* rs6269 A > G: 98%, rs4633 C > T: 98%, rs4818 C > G: 96%, and rs4680 G > A: 97%. The genotype data for *OPRM1* previously described was used in this study [30]. All research and wet bench work was conducted at the HPALS Research Unit (Division of Physiological Sciences, Department of Human Biology, The University of Cape Town, Cape Town, South Africa).

2.3. Statistical Analysis

Using an average reported risk of 40% [30], the QUANTO v1.2.4.49 software was used to calculate the sample size, N = 150, sufficient to detect effect sizes of >2 at 80% power for minor allele frequencies of 0.1–0.5 [35]. Using Statistica V13.5.0.17 [36], an independent sample t-test, Pearson's Chi-square ($\chi 2$) and Fisher's exact tests (if n < 10), were used to analyse the frequency distributions of clinical parameters between no-low and moderate-high groups for pain, disability and combined (pain and disability). Associations were assessed for quantitative and qualitative clinical parameters (Table S1). In addition, the genotype effect on clinical parameters were assessed for the *COMT* (rs6269 A > G, rs4633 C > T, rs4818 C > G and rs4680 G > A) SNPs.

Genotype data were analyzed using the R language and programming environment R studio V1.3.1056 running R V4.0.4 [37]. Using the "genetics" (v1.3.8.1.3) package, the probabilities of Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were determined [38]. For associations between the genotype and pain/disability characteristics, logistic regression analyses were applied using the "SNPassoc" v2.0.2 package [39]. All genetic models (dominant, over-dominant, recessive) were tested, and the Akaike information criterion (AIC) score was used to identify the most significant model.

Haplotype analyses were performed by constructing inferred haplotypes using the individual genotype data for the *COMT SNPs* (rs6269 A > G; rs4680 G > A). The polymorphisms represent the genomic region spanning the central (second) haploblock described in the literature [12]. The inferred *OPRM1* (rs1799971 A > G and rs540825 T > A) haplotype

was previously described and implicated and the genotype data was used in this study [30]. The *COMT* rs4680 G > A and *OPRM1* rs1799971 A > G rs540825 T > A SNPs were used to construct stepwise inferred allele–allele combinations as a proxy for gene–gene interactions. Inferred haplotype and allele–allele combination frequency distribution patterns between the no-low and moderate-high groups, were analyzed using the "haplo.stats" (v1.8.6.) package [40].

To explore pathway associated networks between *COMT* and *OPRM1,* bioinformatic analysis was conducted using the web-based applications Enrichr (Accessed: 6 September 2022 [https://maayanlab.cloud/Enrichr/]) and GeneMANIA (Accessed: 26 October 2022 [https://genemania.org/]). The web-based and online programs used in the study are cited in the bibliography. Furthermore, study data were either expressed as means ± standard deviation (m ± sd), median (interquartile range (IQR)) or a percentage (*n* values). All logistic regression analysis was adjusted for the confounder participants' age at the time of surgery. Odds ratios [OR], confidence intervals at 95% [95% CI], and statistical significance accepted at $p < 0.05$ were reported as part of the regression analysis.

3. Results

3.1. Participants' Characteristics

Demographical and clinical characteristics were previously described [30]. The main findings noted a significant association between younger age and an increase in risk of pain ($p = 0.002$), disability ($p = 0.011$) and combined (pain and disability) ($p = 0.003$), respectively (Table S2). In addition, the study noted that younger participants had fewer nodes involved than older participants in the disability ($p = 0.025$) and combined (pain and disability) ($p = 0.034$) categories (Table S2). No associations were noted for the remaining clinical characteristics assessed (Table S3).

3.2. COMT SNP Genotype Effects on Demographical and Clinical Characteristics

A significant association between *COMT* rs6269 A > G, and the total number of nodes involved ($p = 0.008$) were noted, however, the medians (IQR) were comparable between the genotypes (Table S4). Furthermore, for rs6269, fewer A/A (10.3% and 13%) genotype carriers underwent NeoCT ($p = 0.009$), and RT ($p = 0.002$) treatments, compared to G/G (44.8% and 39.6%) and A/G (44.8% and 47.4%) genotype carriers (Table S5). The *COMT* rs4818 C > G was associated with lymph node surgery ($p = 0.002$), where fewer G/G (4.5% and 3.2%) genotype carriers underwent ALND and SLNB treatments, compared to the C/C (48.6% and 74.2%) and C/G (46.9% and 22.6%) genotype carriers (Table S6).

3.3. COMT SNP Frequencies

The allele frequency distribution of the *COMT* (rs6269 A > G, rs4633 C > T, rs4818 C > G and rs4680 G > A) polymorphisms revealed distinct differences between the SA BCS cohort and the reported global population frequencies (Figure 1). The *COMT* rs6269 (G) and rs4633 (T) minor alleles, were prevalent in the SA BCS cohort (60.9% and 55.6%) compared to the global population (35.7% and 37.2%, $p < 0.001$) (Figure 1A,B). The *COMT* rs4818 (G) and rs4680 (A) minor alleles frequencies, were similar between the SA BCS (26.2% and 39.8%) cohort and the global population (29.7% and 36.9%, $p > 0.05$) (Figure 1C,D). Furthermore, linkage disequilibrium analysis of the BCS cohort noted a strong LD for the *COMT* rs4818-rs4680 pair ($D' = 0.99$), whereas an LD decay ($D' < 0.9$) was noted for the remaining *COMT* SNP pairs (Figure S1C).

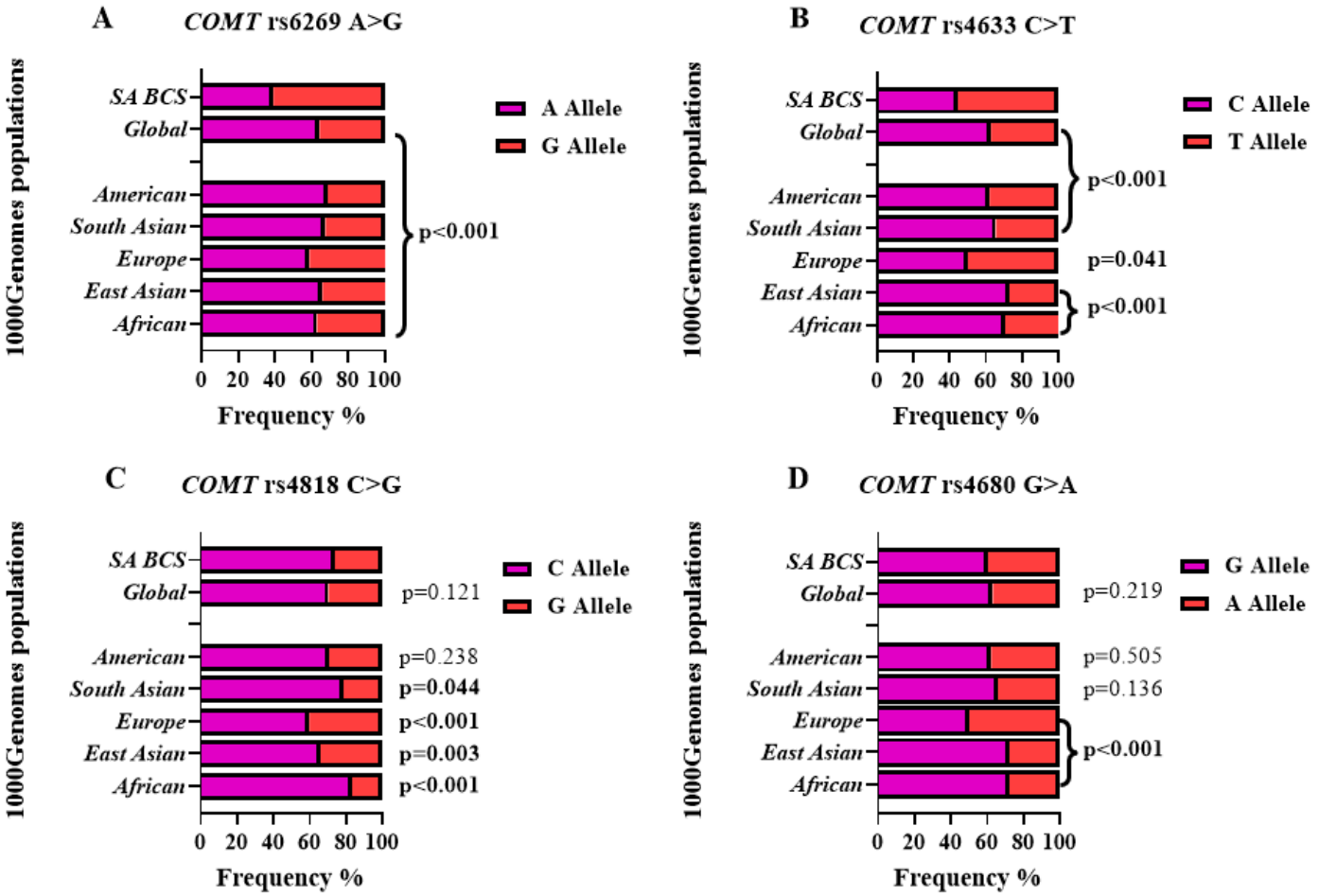


Figure 1. The global distribution and prevalence of allele frequencies for the *COMT* SNPs (rs6269 A > G, rs4633 C > T, rs4818 C > G, and rs4680 G > A) were obtained from the public database, NCBI-1000Genomes project (Accessed: 23 August 2021 [https://www.ncbi.nlm.nih.gov/snp/]). Displayed are the minor allele frequencies for (**A**) rs6269 A > G, (**B**) rs4633 C > T, (**C**) rs4818 C > G, and (**D**) rs4680 G > A are shown for the SA BCS cohort in comparison to the global populations. *P* values describe the comparison in frequency distribution between the SA BCS cohort, and the global and super populations. Significant *p values (p < 0.05)* are in **bold** type set**.**

3.4. Genotype and Allele Frequency Distribution of COMT

Table 1 summarises the distribution patterns of the genotype and allele frequencies of the *COMT* SNPs, between the no-low and moderate-high groups for pain, disability and combined (pain and disability). No significant ($p > 0.05$) associations were noted for the genotype and allele frequencies of *COMT* rs6269 A > G, rs4633 C > T and rs4818 C > G, between the no-low and moderate-high groups of pain, disability and combined (pain and disability) scores (Table 1).

However, in the pain score category, the *COMT* rs4680 A/A genotype was significantly observed in the moderate-high (21.5%) group, compared to the no-low (12.7%) group (Table 1). The A/A ($p = 0.024$, OR: 3.23, 95% CI: 1.33–7.81, AIC: 268.7) genotype was significantly associated with increased risk for reporting moderate-high pain. In the dominant model, the rs4680 A/A ($p = 0.015$, OR: 2.19, 95% CI: 1.14–4.21, AIC: 268.3) genotype was observed to be significantly disproportionate between the no-low and moderate-high groups. In the recessive model, the A/A ($p = 0.050$, OR: 2.17, 95% CI: 1.01–4.67, AIC: 270.4) genotype displayed the same distribution pattern as observed in the dominant model, however only a trend of association was noted. Based on the AIC scores, the dominant model exhibited the most significant model for *COMT* rs4680 G > A. In alignment with this finding, the *COMT* rs4680 A allele was significantly observed in the moderate-high

(46.9%) group, compared to the no-low (35.8%) group. The A ($p = 0.035$, OR: 1.58, 95% CI: 1.03–2.43) allele was significantly associated with an increased likelihood of reporting moderate-high pain (Table 1).

No significant associations were noted between *COMT* rs4680 G > A and the disability category, $p > 0.05$.

In the category of combined scores (pain and disability), the *COMT* rs4680 A/A genotype was significantly observed in the moderate-high (23.1%) group compared to the no-low (12.9%) group (Table 1). The A/A ($p = 0.015$, OR: 3.81, 95% CI: 1.47–9.85, AIC: 240.3) genotype was significantly associated with an increased likelihood of reporting moderate-high pain. The dominant ($p = 0.009$, OR: 2.51, 95% CI: 1.22–5.17, AIC: 240.0) and recessive ($p = 0.041$, OR: 2.36, 95% CI: 1.06–5.24, AIC: 241.6) models displayed a significant association for the rs4680 A/A genotype. The A/A-A/G (dominant) and A/A (recessive) genotypes distribution were significantly disproportionate between the no-low and moderate-high groups. Once more, based on the AIC score, the dominant model exhibited the most significant model for the *COMT* rs4680 G > A SNP. Similarly, the *COMT* rs4680 A allele was significantly observed moderate-high (49.0%) group, compared to the no-low (36.0%) group. The A ($p = 0.017$, OR: 1.71, 95% CI: 1.07–2.71) allele was significantly associated with an increased likelihood of reporting moderate-high combined (pain and disability)(Table 1).

Table 1. Genotype and minor allele frequency distributions, of the *COMT* (rs6269 A > G; rs4633 C > T; rs4818 C > G; rs4680 G > A) polymorphisms between groups for pain, disability and combined (pain and disability) scores.

	Pain			**Disability**			**Pain and Disability**		
Polymorphisms	**No-Low (n = 184)**	**Mod-High (n = 68)**	**AIC**	**No-Low (n = 204)**	**Mod-High (n = 48)**	**AIC**	**No-Low (n = 197)**	**Mod-High (n = 55)**	**AIC**
COMT									
rs6269 A > G									
G/G	38.9 (68)	35.4 (23)		38.5 (75)	35.6 (16)		37.8 (71)	38.5 (20)	
A/G	45.1 (79)	46.2 (30)		45.1 (88)	46.7 (21)		46.3 (87)	42.3 (22)	
A/A	16.0 (28)	18.5 (12)		16.4 (32)	17.8 (8)		16.0 (30)	19.2 (10)	
G allele	61.4 (215)	58.5 (76)		61.0 (238)	58.9 (53)		60.9 (229)	59.6 (62)	
p value [1]	0.848			0.937			0.787		
G Allele p value [2]	0.600			0.721			0.821		
HWE	0.532	0.617		0.457	1.000		0.651	0.406	
rs4633 C > T									
T/T	34.3 (60)	29.2 (19)		34.9 (68)	24.4 (11)		35.1 (66)	25 (13)	
C/T	46.9 (82)	47.7 (31)		44.6 (87)	57.8 (26)		45.7 (86)	51.9 (27)	
C/C	18.9 (33)	23.1 (15)		20.5 (40)	17.8 (8)		19.1 (36)	23.1 (12)	
T allele	57.7 (202)	53.1 (69)		57.2 (223)	53.3 (48)		58.0 (218)	51.0 (53)	
p value[1]	0.557			0.178			0.261		
T Allele p value[2]	0.407			0.556			0.220		
HWE	0.546	0.628		0.154	0.389		0.307	1.000	
rs4818 C > G									
C/C	52.0 (89)	52.3 (34)		51.6 (99)	54.5 (24)		50.8 (94)	56.9 (29)	
C/G	43.3 (74)	43.1 (28)		43.8 (84)	40.9 (18)		44.3 (82)	39.2 (20)	
G/G	4.7 (8)	4.6 (3)		4.7 (9)	4.5 (2)		4.9 (9)	3.9 (2)	

G allele	26.3 (90)	26.2 (34)			26.6 (102)	25.0 (22)	27.0 (100)	23.5 (24)		
p value [1]	0.480				0.880		0.618			
G Allele p value [2]	1.000				0.893		0.527			
HWE	0.247	0.526			0.201	0.702	0.199	0.707		
rs4680 G > A										
G/G	41.0 (71)	27.7 (18)	**0.015** [a]	**268.3**	39.9 (77)	26.7 (12)	40.9 (76)	25.0 (13)	**0.009** [a]	**240.0**
A/G	46.2 (80)	50.8 (33)	0.382 [b]	273.4	45.6 (88)	55.6 (25)	46.2 (86)	51.9 (27)	0.342 [b]	245.9
A/A	12.7 (22)	21.5 (14)	0.050 [c]	270.4	14.5 (28)	17.8 (8)	12.9 (24)	23.1 (12)	**0.041** [c]	242.6
A allele	35.8 (124)	46.9 (61)			37.3 (144)	45.6 (41)	36.0 (134)	49.0 (51)		
p value [1]	**0.024**			268.7	0.113		**0.015**			240.3
A Allele p value [2]	**0.035**				0.152		**0.017**			
HWE	0.874	1.000			0.550	0.564	0.877	1.000		

Notes: Genotype and allele frequencies are expressed as a percentage (%) with the number of participants (n) in parentheses. Global *p* values [1] signifies *p*-values for genotypes between groups, *p* values [2] signifies *p* values for alleles between groups; Significant ($p < 0.05$) *p*-values are indicated in **bold** typeset. *p* values for logistic regression analysis are listed for the dominant [a], over-dominant [b], and recessive [c] models. Included are the *p*-values of the Hardy–Weinberg equilibrium exact test for each of the categories included. Abbreviations: AIC: Akaike information criterion score; Mod-High: Moderate-High; HWE: Hardy–Weinberg equilibrium.

3.5. Inferred COMT Haplotypes

A *COMT* haplotype was constructed for the genomic region spanning the central haploblock using the individual genotype data of (rs6269 A > G, rs4680 G > A) (Figure S1 B). Evaluation of the inferred *COMT* (rs6269 A > G-rs4680 G > A) haplotype, yielded four combinations A-G, G-A, G-G, and A-A (Figure 2).

In the pain scores' category, the G-G haplotype combination was significantly observed in the no-low (27.7%) group, compared to the moderate-high (22.1%) group. The inferred G-G ($p = 0.026$, OR: 0.67, 95% CI: 0.38–1.18) haplotype was significantly associated with a reduced likelihood of reporting moderate-high pain (Figure 2A). In addition, the A-A haplotype combination was significantly observed in the moderate-high (10.7%) group compared to the no-low (3.1%) group. The inferred A-A ($p = 0.007$, OR: 2.09, 95% CI: 0.89–4.88) haplotype was significantly associated with increased likelihood of reporting moderate-high pain (Figure 2A).

No significant differences in distribution patterns were noted in the disability category (Figure 2B). In the combined (pain and disability) scores' category, the A-A haplotype was significantly observed in the moderate-high (12.4%) group, compared to the no-low (3.2%) group. The inferred A-A ($p = 0.003$, OR: 2.18, 95% CI: 0.92–5.17) haplotype was significantly associated with increased likelihood of reporting moderate-high combined (pain and disability)(Figure 2C*).*

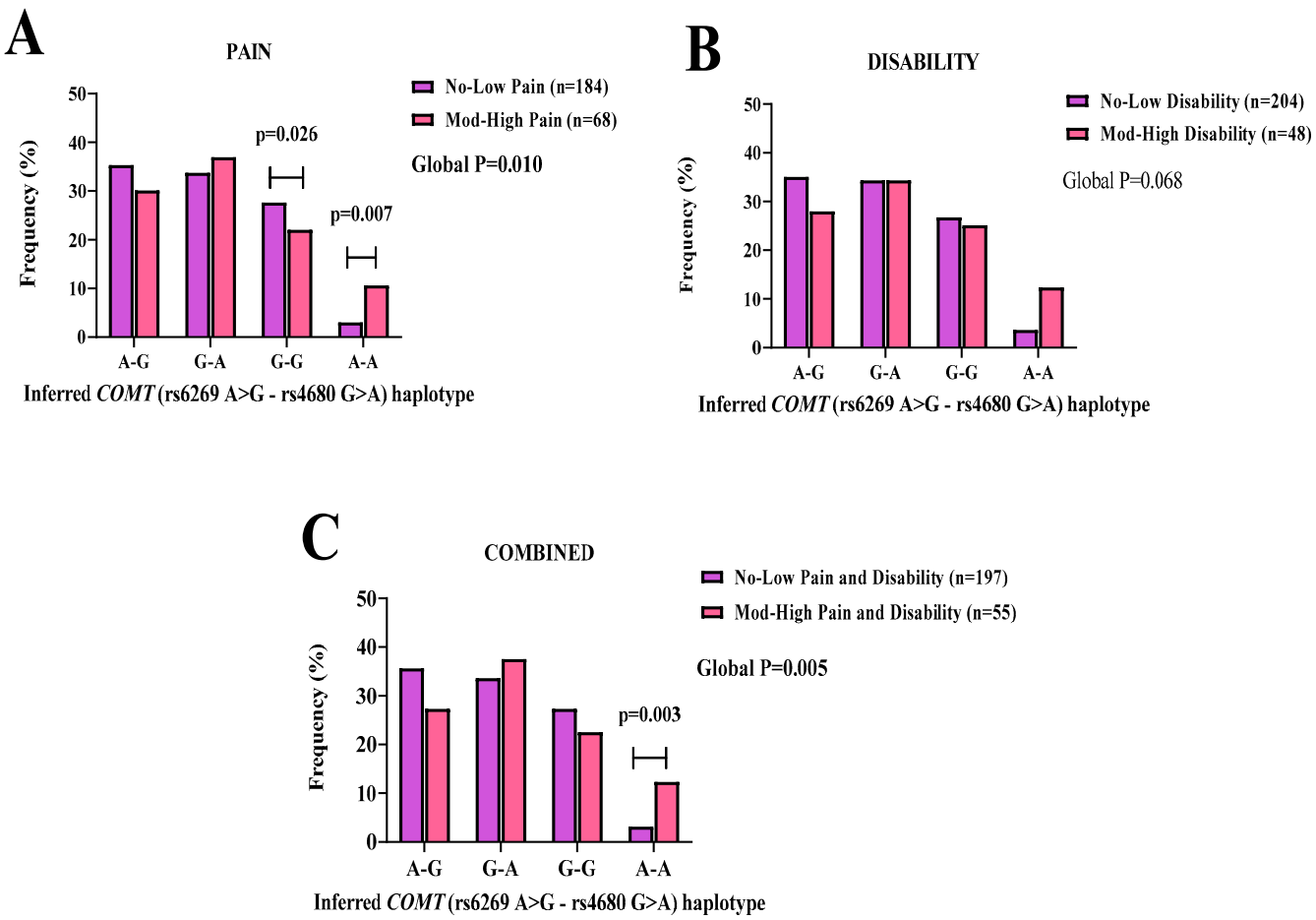


Figure 2. The inferred *COMT* (rs6269 A > G-rs4680 G > A) allele–allele combinations' frequency distribution patterns are displayed for (**A**) pain, (**B**) disability, and (**C**) combined (pain and disability) symptoms in SA BCS. No-low (purple bars) and moderate-high (pink bars) groups are displayed with the number of participants in parenthesis (n). Statistically significant ($p < 0.05$) frequency differences are noted with an age-adjusted *p*-value in **bold.**

3.6. COMT-OPRM1 Allelic Combinations

COMT-OPRM1 allele–allele combinations were generated using the individual genotype data for *COMT* (rs4680 G > A) and *OPRM1* (rs1799971 A > G, rs540825 T > A) polymorphisms.

Evaluating pain ($p = 0.011$) scores, for *COMT* (rs4680 G > A)-*OPRM1* (rs1799971 A > G), the allele combination A-A was significantly observed in the moderate-high (36.9%) group compared to the no-low (27.9%) group. (Figure 3A). The allele combination G-G was significantly observed in the no-low (11.4%) group compared to the moderate-high (2.6%) group (Figure 3A). The A-A ($p = 0.004$, OR: 1.35, 95% CI: 0.85–2.15) and G-G ($p = 0.010$, OR: 0.23, 95% CI: 0.05–1.03) allele combinations were significantly associated with increased and reduced likelihoods of reporting moderate-high pain (Figure 3A).

No significant associations were noted between this allelic combination and disability ($p = 0.135$) scores (Figure 3B).

In the combined (pain and disability) ($p = 0.027$) scores' category, the allele combination G-A, was significantly observed in the no-low (52.4%) group compared to the moderate-high (46.7%) group. The G-A ($p = 0.046$, OR: 1.00) combination was associated with equal likelihoods of reporting moderate-high combined (pain and disability)(Figure 3C). In addition, the allele combination A-A, was significantly observed in the moderate-high

(36.0%) group, compared to the no-low (28.7%) group. The A-A (p = 0.010, OR: 1.42, 95% CI: 0.85–2.35) combination was significantly associated with an increased likelihood of reporting moderate-high combined (pain and disability)(Figure 3C).

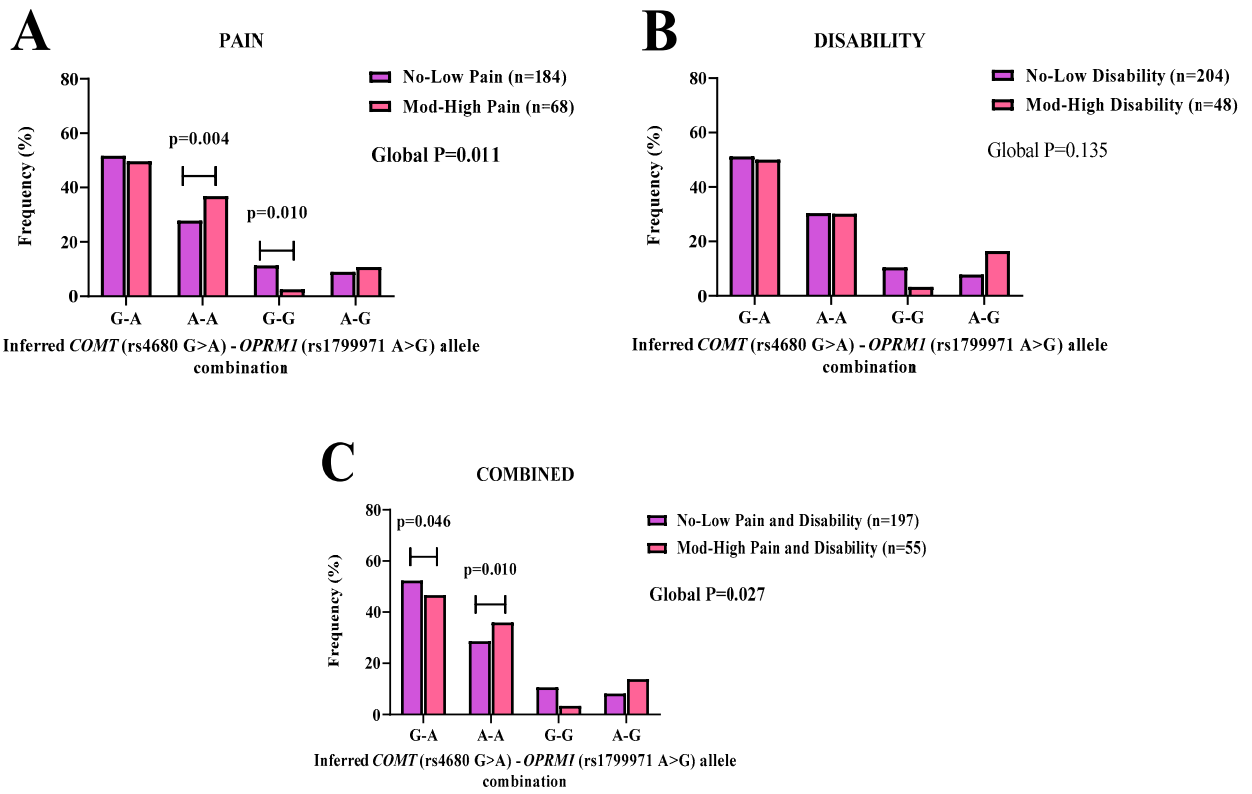


Figure 3. The inferred *COMT* (rs4680 G > A)-*OPRM1* (rs1799971 A > G) allele–allele combinations' frequency distribution patterns are shown for (**A**) pain, (**B**) disability, and (**C**) combined (pain and disability) symptoms in SA BCS. No-low (purple bars) and moderate-high (pink bars) groups are displayed with the number of participants in parenthesis (n). Statistically significant (p < 0.05) frequency differences are noted with an age-adjusted p-value in **bold.**

No significant associations were noted between *COMT* (rs4680 G > A)–*OPRM1* (rs540825 T > A) and pain (p = 0.052) or disability (p = 0.079)(Figure 4A,B).

In the combined (pain and disability) (p = 0.016) scores' category, the allele combination G-T was significantly observed in the no-low (49.5%) group, compared to the moderate-high (36.6%) group. The G-T (p = 0.008, OR: 1.00) combination was associated with equal likelihood of reporting moderate-high combined (pain and disability) (Figure 4C). Whereas the allele combination A-A was significantly observed in the moderate-high (11.2%) group, compared to the no-low (8.0%) group. The A-A (p = 0.012, OR: 1.89, 95% CI: 0.81–4.38) was significantly associated with increased likelihood of reporting moderate-high combined (pain and disability) (Figure 4C).

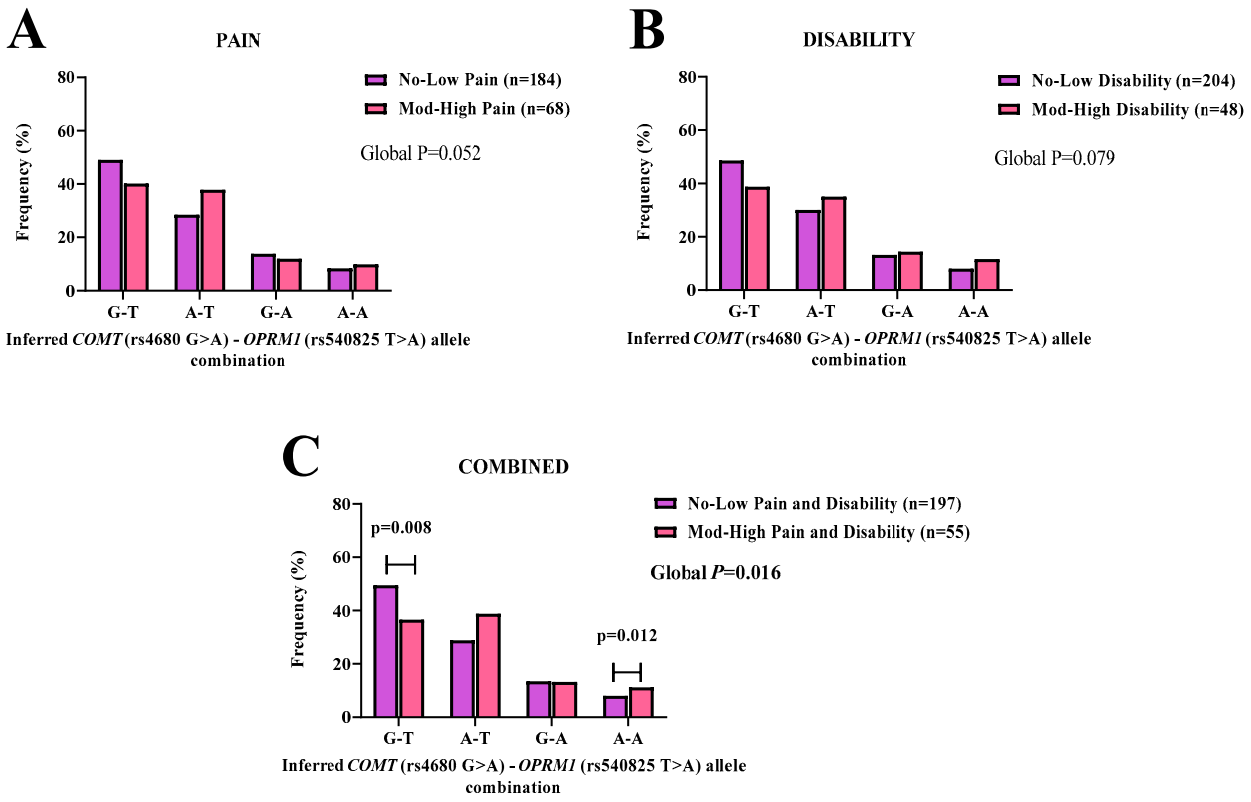


Figure 4. The inferred *COMT* (rs4680 G > A)-*OPRM1* (rs540825 T > A) allele–allele combinations' frequency distribution patterns are shown for (**A**) pain, (**B**) disability, and (**C**) combined (pain and disability) symptoms in SA BCS. No-low (purple bars) and moderate-high (pink bars) groups are displayed with the number of participants in parenthesis (n). Statistically significant ($p < 0.05$) frequency differences are noted with an age-adjusted *p*-value in **bold**.

3.7. Bioinformatic Analyses

Analyses of gene set enrichment tools for *COMT* and *OPRM1* noted significant associations for both genes in several libraries (Figure S2). In the library for disease and drugs, the gene set was associated with several human diseases/conditions, including various pain conditions (Rare Disease GeneRIF and AutoRIF Gene lists). GeneMANIA analyses showed that *COMT* and *OPRM1* share secondary and tertiary gene–associated functional networks that includes the *AHCY*, *OPRD1*, *PENK* and *FGF2* genes (Table S7). Networks that include physical interactions, genetic interactions, co-expressed, predicted domains and pathway networks (Table S7).

4. Discussion

This study aimed to describe i) four *COMT* SNPs previously associated with chronic pain that form part of the central haploblock, and ii) to characterize the frequencies of the clinically relevant SNPs in the SA BCS cohort of mixed ancestry. The findings of this study shows that *COMT* polymorphisms are associated with the risk of chronic shoulder pain and disability. Further, that the association was observed in a unique South African cohort of mixed ancestry BCS. The findings revealed that a specific region between *COMT* rs6269 A > G–rs4680 G > A was implicated in the prevalence of chronic pain and disability. Furthermore, supporting evidence is provided implicating the potential role of gene–gene interactions, specifically between *COMT-OPRM1* SNPs and chronic pain and disability within the SA BCS cohort. Interestingly, the study highlights distinct frequency differ-

ences for the *COMT* central haploblock in the SA population compared to the global population. Thus, the clinical relevance needs to be further explored in the context of effective pain management in this unique population.

Evaluation of the nongenetic risk factors noted significant differences between the groups for the participants age, and nodal involvement. Younger participants reported greater pain, disability and combined (pain and disability) scores, and had fewer nodes involved [30]. As earlier reported, the results are in alignment with previous literature for age, however the association for nodal involvement requires further scrutiny [3,30,41].

Genotype analysis of the functional *COMT* SNP rs4680 G > A, showed A/A genotype carriers had an increase in risk for pain by 3.23, and combined (pain and disability) by 3.81. Similarly, allelic analysis of the rs4680 A allele showed an increase in risk for pain by 1.58 and combined (pain and disability) by 1.71. These findings agree with the published studies indicating that rs4680 A allele is associated with increased pain and that the A allele correlates with decreased *COMT* enzyme activity [42,43]. Furthermore, the levels of *COMT* activity have been linked to the regulation of neurotransmitters in the pain modulation pathway, including the opioid system [24]. Several inconsistencies have been reported for this genetic locus, as noted by Baumbauer et al. [44]. These conflicting findings may be an indication of differences in both the study design and characterization of pain. Specifically referring to the differentiation between chronic and persistent pain conditions. The classification of chronic pain in our cohort of BCS with upper limb sequelae falls within the spectrum of musculoskeletal conditions [45].

Analyses of the central haploblock of *COMT* highlighted marked frequency differences between the SA BCS cohort and the reported global populations. We hypothesize that this is reflective of the significant variations in the minor allele frequencies for rs6269 A > G and rs4633 C > T across the different populations. Emphasizing the importance of profiling the genetic structure of unique populations, as in the case of the SA mixed ancestry cohort. Evaluation of the LD structure between the SNP pairs further emphasized the LD decay in the cohort investigated. This observation is not surprising, as it was previously described [46]. Specific haplotypes of the *COMT* haploblock was significantly associated with pain and combined (pain and disability), specifically the haplotype pairs rs6269A > G-rs4680 G > A. The observed G-G allele pair showed a decrease in risk by 0.67 for pain. Whereas the alternate A-A allele pair showed an increase in risk of 2.09 for pain and, 2.18 for combined (pain and disability). These allele pairs reflect the high enzyme *COMT* activity associated with the G allele [47]**.** The study design was limited by sample size and therefore we could not evaluate the full haploblock containing *COMT* rs6269 A > G, rs4633 C > T, rs4818 C > G, and rs4680 G > A.

Bioinformatic analysis have shown that *COMT* and *OPRM1* do not directly interact with each other. However, both play pivotal functions within a broad network of shared partners towards modulating the descending pain pathway. GeneMANIA analysis showed the *AHCY*, *FGF2*, *OPRD1* and *PENK* genes connect *COMT* and *OPRM1* (Figure 5) [48]. The adenosyl homocysteinase enzyme *(AHCY)* and fibroblast growth factor 2 (*FGF2)* genes are responsible regulating methyltransferase (e.g., *COMT* activity), and fibroblasts activity, respectively [49,50]. Opioid receptor delta one *(OPRD1)* are related to- and can form a heterodimer with *OPRM1,* and proenkephalin (*PENK)* encodes the neuropeptide enkephalin, a strong agonist for the μ-opioid receptor [51,52]. Both *AHCY* and *FGF2* share functional associated networks with *PENK.* While a genetic interaction was inferred for *AHCY-PENK* (radiation hybrid panels), *FGF2-PENK* are co-expressed within tumorous specimens (gene expression microarrays) [53,54]. Evidence extracted from the gene set enrichment showed the genes function within the same biological compartments and expressed within the same tissues following epigenomic profiling [55]. *COMT* and *OPRM1* share target compatibility for a predicted microRNA (miRNA) interaction with mir-16-5p, micro molecules that are important for controlling gene expression [56]. Both genes were

associated with morphine and dopamine drug signatures, which supports the pharmacodynamic roles associated with *COMT* and *OPRM1* [57]. Furthermore, the genes are associated with pain and other conditions (Figure S1).

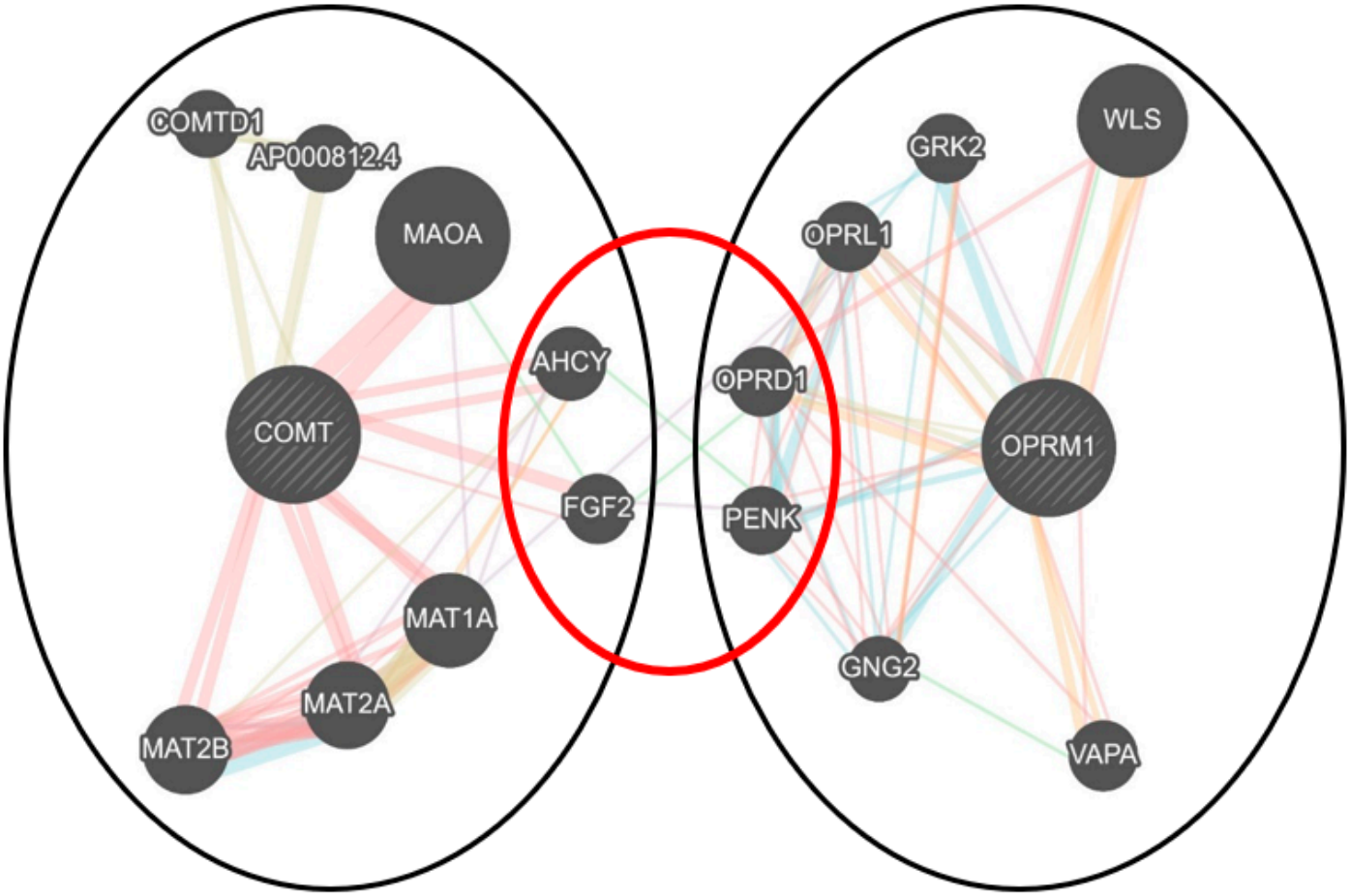


Figure 5. GeneMANIA network and gene–gene interaction analysis for the *COMT* and *OPRM1* genes. Venn diagram depicting the shared network pathways and secondary gene-associated networks that indirectly associates *COMT* and *OPRM1*. Indicated by color is physical interactions (pink), genetic interactions (dark green), co-localization (dark blue), co-expressed (purple), predicted (orange), pathway (light blue) and shared protein domain (light green) network for 15 genes.

We, therefore, conducted a proxy for gene–gene interaction by analyzing allele combinations between the *COMT* (rs4680 G > A) and *OPRM1* (rs1799971 A > G, and rs540825 T > A) SNPs. A few specific allele–allele combinations between *COMT* and *OPRM1* polymorphisms were shown to be associated with risk for reporting pain and combined (pain and disability). The most significant interaction noted was for the *COMT* (rs4680 G > A)–*OPRM1* (rs1799971 A > G) allele–allele combination. Analysis showed carriers with the A-A allele–allele pair had an increase in risk of 1.35 for pain and 1.42 for combined (pain and disability). Whereas the alternate G-G allele–allele combination pair had a decrease in risk for pain by 0.23. While our study did not measure opioid requirements, we did measure reported pain scores. Our findings contrasted with previous studies described in populations of European descent, which measured opioid requirements and rescue [31,58]. Our findings provide preliminary evidence to support a future study to investigate opioid administration and usage. This will allow for the exploration of these allele–pairs within the context of opioid use and pain management in this unique SA cohort.

The study could only detect effects with odds ratios of 1.5, with the current study sample powered at <80% [30]. One instrument i.e., the SPADI index was employed to

measure pain and disability symptoms related to musculoskeletal pathologies. Furthermore, following the hypothesis approach, we evaluated *COMT* and *OPRM1* SNPs that have been previously implicated in pain modulation. No correction was done for multiple testing given that more than two SNPs were evaluated (familywise error rate) accompanied by underpowered sample size. For gene–gene interactions, logistic regression analysis was applied, and allele frequencies of >3% were used to describe the interactions between *COMT* and *OPRM1*. However, given the extreme variances in data points generated for multi-locus genotype combinations, allele–allele frequency detection may be challenging [59]. Additionally, ethnicity in this cohort was self-reported. Future reports will include larger sample sizes to increase power and evaluate the association of pain and pain genes concerning pain treatment protocols. This may also allow for the consideration of other clinically relevant confounders within the analyses.

5. Conclusions

This study described the role of *COMT* polymorphisms in chronic shoulder pain and disability in BCS in a unique SA population. We report an association between polymorphisms of *COMT* with chronic pain and disability. The gene–gene interaction analysis highlighted significant and novel correlations between the *COMT-OPRM1* allele–allele combinations and pain and combined (pain and disability), which contrasts to previous literature. This contrasting finding therefore highlights the value of exploring genes and various gene–gene combinations in diverse population cohorts towards improving personalized pain protocols.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/genes14010009/s1, Figure S1: The genomic organization of the *COMT* gene. A) The chromosomal location 22q11.21 and region of interest (ROI) containing the *COMT* gene. B) A schematic diagram of the *COMT* gene, illustration the genomic organization and locations of the four snps, rs6269 A>G, rs4633 C>T, rs4818 C>G and rs4680 G>A. C) Linkage disequilibrium (LD) plot showing the $|D'|$ x 100 values for *COMT* SNP pairwise analysis for the SA BCS cohort. $|D'|$ values > 0.9 indicating strong LD. Figure S2: Gene set Enrichment analyses for the *COMT* and *OPRM1* genes from Enrichr (maayanlab.cloud). Bar length and color brightness represents the degree of significance attached to both genes relative to the term. The longer and brighter the shade of red, the more significant the association is between the gene set and the term. Significance is the adjusted p value ($p<0.005$) using the Benjamini-Hochberg method. Table S1: Clinical parameters evaluated in this study. Table S2: Clinical characteristics between pain, disability and the combined (pain and disability) categories. Table S3: Breast cancer treatment characteristics between pain, disability, and combined (pain and disability) categories. Table S4: Genotype effects of the COMT (rs6269 A>G, rs4633 C>T, rs4818 C>G and rs4680 G>A) on the quantitative clinical variables recorded for participants. Table S5: Genotype effects of the COMT rs6269 A>G and rs4633 C>T) polymorphism on categorical clinical variables recorded for participants. Table S6: Genotype effects of the COMT rs4818 C>G and rs4680 G>A) polymorphism on categorical clinical variables recorded for participants. Table S7: The list of genes that form part of the functionally associated network for the *COMT* and *OPRM1* genes, obtained by GeneMANIA.

Author Contributions: Conceptualization, F.F., D.S. and A.V.S.; methodology, F.F., D.S. and A.V.S.; software, F.F.; validation, F.F., A.V.S. and D.S.; formal analysis, F.F., A.V.S. and D.S.; investigation, F.F., A.V.S. and D.S.; resources, F.F., A.V.S. and D.S.; data curation, F.F., A.V.S. and D.S.; writing—original draft preparation, F.F.; writing—review and editing, F.F., A.V.S. and D.S.; visualization, F.F., A.V.S. and D.S.; supervision, D.S. and A.V.S.; project administration, D.S. and A.V.S.; funding acquisition, D.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the University of Cape Town (WUN scholarship and Foundation Contingency award) and the National Research Foundations (Grant number: 102470), South Africa. The opinions and conclusions summarized in the study, are those of the author/s and do not necessarily reflect the opinions of the funders.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of the Human Research Ethics Committee, University of Cape Town (HREC REF: 650/2016, 125/2017).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable

Acknowledgments: The authors would like to thank (1) all participants in this study, (2) the nursing and administrative staff of the CRC, and (3) T.S Mafu for their participation and assistance in the recruitment process and study data capturing.

Conflicts of Interest: The authors declare no conflicts of interest surrounding the research conducted in this study. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Hidding, J.T.; Beurskens, C.H.; van der Wees, P.J.; van Laarhoven, H.W.; Nijhuis-van der Sanden, M.W. Treatment related impairments in arm and shoulder in patients with breast cancer: A systematic review. *PLoS ONE* **2014**, *9*, e96748. https://doi.org/10.1371/journal.pone.0096748.
2. Shamley, D.; Srinaganathan, R.; Oskrochi, R.; Lascurain-Aguirrebena, I.; Sugden, E. Three-dimensional scapulothoracic motion following treatment for breast cancer. *Breast Cancer Res. Treat.* **2009**, *118*, 315–322. https://doi.org/10.1007/s10549-008-0240-x.
3. Wang, L.; Guyatt, G.H.; Kennedy, S.A.; Romerosa, B.; Kwon, H.Y.; Kaushal, A.; Chang, Y.; Craigie, S.; de Almeida, C.P.B.; Couban, R.J.; et al. Predictors of persistent pain after breast cancer surgery: A systematic review and meta-analysis of observational studies. *Can. Med Assoc. J.* **2016**, *188*, E352–E361. https://doi.org/10.1503/cmaj.151276.
4. Salz, T.; Lavery, J.A.; Lipitz-Snyderman, A.N.; Boudreau, D.M.; Moryl, N.; Gillespie, E.F.; Korenstein, D. Trends in Opioid Use Among Older Survivors of Colorectal, Lung, and Breast Cancers. *J. Clin. Oncol.* **2019**, *37*, 1001–1011. https://doi.org/10.1200/JCO.18.00938.
5. Knisely, M.R.; Conley, Y.P.; Smoot, B.; Paul, S.M.; Levine, J.D.; Miaskowski, C. Associations Between Catecholaminergic and Serotonergic Genes and Persistent Arm Pain Severity Following Breast Cancer Surgery. *J. Pain* **2019**, *20*, 1100–1111. https://doi.org/10.1016/j.jpain.2019.03.008.
6. Andersen, K.G.; Duriaud, H.M.; Jensen, H.E.; Kroman, N.; Kehlet, H. Predictive factors for the development of persistent pain after breast cancer surgery. *Pain* **2015**, *156*, 2413–2422. https://doi.org/10.1097/j.pain.0000000000000298.
7. Dimova, V.; Lotsch, J.; Huhne, K.; Winterpacht, A.; Heesen, M.; Parthum, A.; Weber, P.G.; Carbon, R.; Griessinger, N.; Sittl, R.; et al. Association of genetic and psychological factors with persistent pain after cosmetic thoracic surgery. *J. Pain Res.* **2015**, *8*, 829–844. https://doi.org/10.2147/JPR.S90434.
8. Wang, F.Y.; Wang, P.; Zhao, D.F.; Gonzalez, F.J.; Fan, Y.F.; Xia, Y.L.; Ge, G.B.; Yang, L. Analytical methodologies for sensing catechol-O-methyltransferase activity and their applications. *J. Pharm. Anal.* **2021**, *11*, 15–27. https://doi.org/10.1016/j.jpha.2020.03.012.
9. Bates, G.W.; Edman, C.D.; Porter, J.C.; MacDonald, P.C. Catechol-O-methyltransferase activity in erythrocytes of pregnant women. *Am. J. Obstet. Gynecol.* **1978**, *131*, 555–557. https://doi.org/10.1016/0002-9378(78)90118-7.
10. Paravati, S.; Rosani, A.; Warrington, S.J. Physiology, Catecholamines. In *StatPearls*; StatPearls Publishing: Treasure Island, FL, USA, 2022.
11. Garcha, A.S.; Cohen, D.L. Catecholamine excess: Pseudopheochromocytoma and beyond. *Adv. Chronic Kidney Dis.* **2015**, *22*, 218–223. https://doi.org/10.1053/j.ackd.2014.11.002.
12. Diatchenko, L.; Slade, G.D.; Nackley, A.G.; Bhalang, K.; Sigurdsson, A.; Belfer, I.; Goldman, D.; Xu, K.; Shabalina, S.A.; Shagin, D.; et al. Genetic basis for individual variations in pain perception and the development of a chronic pain condition. *Hum. Mol. Genet.* **2005**, *14*, 135–143. https://doi.org/10.1093/hmg/ddi013.
13. Schlereth, T.; Birklein, F. The sympathetic nervous system and pain. *Neuromolecular. Med.* **2008**, *10*, 141–147. https://doi.org/10.1007/s12017-007-8018-6.
14. Bjorland, S.; Moen, A.; Schistad, E.; Gjerstad, J.; Roe, C. Genes associated with persistent lumbar radicular pain; a systematic review. *BMC Musculoskelet. Disord.* **2016**, *17*, 500. https://doi.org/10.1186/s12891-016-1356-5.
15. Nackley, A.G.; Shabalina, S.A.; Tchivileva, I.E.; Satterfield, K.; Korchynskyi, O.; Makarov, S.S.; Maixner, W.; Diatchenko, L. Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. *Science* **2006**, *314*, 1930–1933. https://doi.org/10.1126/science.1131262.
16. Machius, M.; Declerck, N.; Huber, R.; Wiegand, G. Kinetic stabilization of Bacillus licheniformis alpha-amylase through introduction of hydrophobic residues at the surface. *J. Biol. Chem.* **2003**, *278*, 11546–11553. https://doi.org/10.1074/jbc.M212618200.
17. Schmack, K.; Rossler, H.; Sekutowicz, M.; Brandl, E.J.; Muller, D.J.; Petrovic, P.; Sterzer, P. Linking unfounded beliefs to genetic dopamine availability. *Front. Hum. Neurosci.* **2015**, *9*, 521. https://doi.org/10.3389/fnhum.2015.00521.
18. Khalil, H.; Sereika, S.M.; Dai, F.; Alexander, S.; Conley, Y.; Gruen, G.; Meng, L.; Siska, P.; Tarkin, I.; Henker, R. OPRM1 and COMT Gene-Gene Interaction Is Associated With Postoperative Pain and Opioid Consumption After Orthopedic Trauma. *Biol. Res. Nurs.* **2017**, *19*, 170–179. https://doi.org/10.1177/1099800416680474.

19. Sadhasivam, S.; Chidambaran, V.; Olbrecht, V.A.; Esslinger, H.R.; Zhang, K.; Zhang, X.; Martin, L.J. Genetics of pain perception, COMT and postoperative pain management in children. *Pharmacogenomics* **2014**, *15*, 277–284. https://doi.org/10.2217/pgs.13.248.
20. Zhang, Y.; Belfer, I.; Nouraie, M.; Zeng, Q.; Goel, R.; Chu, Y.; Krasiy, I.; Krishnamurti, L. Association of genetic variation in COMT gene with pain related to sickle cell disease in patients from the walk-PHaSST study. *J. Pain Res.* **2018**, *11*, 537–543. https://doi.org/10.2147/JPR.S149958.
21. Mukherjee, N.; Kidd, K.K.; Pakstis, A.J.; Speed, W.C.; Li, H.; Tarnok, Z.; Barta, C.; Kajuna, S.L.; Kidd, J.R. The complex global pattern of genetic variation and linkage disequilibrium at catechol-O-methyltransferase. *Mol. Psychiatry* **2010**, *15*, 216–225. https://doi.org/10.1038/mp.2008.64.
22. de Wit, E.; Delport, W.; Rugamika, C.E.; Meintjes, A.; Moller, M.; van Helden, P.D.; Seoighe, C.; Hoal, E.G. Genome-wide analysis of the structure of the South African Coloured Population in the Western Cape. *Hum. Genet.* **2010**, *128*, 145–153. https://doi.org/10.1007/s00439-010-0836-1.
23. Ikediobi, O.; Aouizerat, B.; Xiao, Y.; Gandhi, M.; Gebhardt, S.; Warnich, L. Analysis of pharmacogenetic traits in two distinct South African populations. *Hum. Genom.* **2011**, *5*, 265–282. https://doi.org/10.1186/1479-7364-5-4-265.
24. Kowarik, M.C.; Einhauser, J.; Jochim, B.; Buttner, A.; Tolle, T.R.; Riemenschneider, M.; Platzer, S.; Berthele, A. Impact of the COMT Val(108/158)Met polymorphism on the mu-opioid receptor system in the human brain: Mu-opioid receptor, met-enkephalin and beta-endorphin expression. *Neurosci. Lett.* **2012**, *506*, 214–219. https://doi.org/10.1016/j.neulet.2011.11.008.
25. Yao, P.; Ding, Y.Y.; Wang, Z.B.; Ma, J.M.; Hong, T.; Pan, S.N. Effect of gene polymorphism of COMT and OPRM1 on the preoperative pain sensitivity in patients with cancer. *Int. J. Clin. Exp. Med.* **2015**, *8*, 10036–10039.
26. Corder, G.; Castro, D.C.; Bruchas, M.R.; Scherrer, G. Endogenous and Exogenous Opioids in Pain. *Annu. Rev. Neurosci.* **2018**, *41*, 453–473. https://doi.org/10.1146/annurev-neuro-080317-061522.
27. Ren, Z.Y.; Xu, X.Q.; Bao, Y.P.; He, J.; Shi, L.; Deng, J.H.; Gao, X.J.; Tang, H.L.; Wang, Y.M.; Lu, L. The impact of genetic variation on sensitivity to opioid analgesics in patients with postoperative pain: A systematic review and meta-analysis. *Pain Physician* **2015**, *18*, 131–152.
28. Hwang, I.C.; Park, J.Y.; Myung, S.K.; Ahn, H.Y.; Fukuda, K.; Liao, Q. OPRM1 A118G gene variant and postoperative opioid requirement: A systematic review and meta-analysis. *Anesthesiology* **2014**, *121*, 825–834. https://doi.org/10.1097/ALN.0000000000000405.
29. Saiz-Rodriguez, M.; Ochoa, D.; Herrador, C.; Belmonte, C.; Roman, M.; Alday, E.; Koller, D.; Zubiaur, P.; Mejia, G.; Hernandez-Martinez, M.; et al. Polymorphisms associated with fentanyl pharmacokinetics, pharmacodynamics and adverse effects. *Basic Clin. Pharmacol. Toxicol.* **2019**, *124*, 321–329. https://doi.org/10.1111/bcpt.13141.
30. Firfirey, F.; September, A.V.; Shamley, D. ABCB1 and OPRM1 single-nucleotide polymorphisms collectively modulate chronic shoulder pain and dysfunction in South African breast cancer survivors. *Pharmacogenomics* **2022**, *23*, 513–530. https://doi.org/10.2217/pgs-2022-0020.
31. Reyes-Gibby, C.C.; Shete, S.; Rakvag, T.; Bhat, S.V.; Skorpen, F.; Bruera, E.; Kaasa, S.; Klepstad, P. Exploring joint effects of genes and the clinical efficacy of morphine for cancer pain: OPRM1 and COMT gene. *Pain* **2007**, *130*, 25–30. https://doi.org/10.1016/j.pain.2006.10.023.
32. Little, J.; Higgins, J.P.; Ioannidis, J.P.; Moher, D.; Gagnon, F.; Von Elm, E.; Khoury, M.J.; Cohen, B.; Davey-Smith, G.; Grimshaw, J. STrengthening the REporting of Genetic Association Studies (STREGA)—an extension of the STROBE statement. *Genet. Epidemiol. Off. Publ. Int. Genet. Epidemiol. Soc.* **2009**, *33*, 581–598.
33. Tengrup, I.; Tennvall-Nittby, L.; Christiansson, I.; Laurin, M. Arm morbidity after breast-conserving therapy for breast cancer. *Acta Oncol.* **2000**, *39*, 393–397. https://doi.org/10.1080/028418600750013177.
34. MacDermid, J.C.; Solomon, P.; Prkachin, K. The Shoulder Pain and Disability Index demonstrates factor, construct and longitudinal validity. *BMC Musculoskelet. Disord.* **2006**, *7*, 12. https://doi.org/10.1186/1471-2474-7-12.
35. Gauderman, W.; Morrison, J. QUANTO 1.1: A Computer Program for Power and Sample Size Calculations for Genetic-epidemiology Studies, Version 1.2.4. 2006. Available online: http://hydra.usc.edu/gxe (accessed on 22 November 2022).
36. Dell. Inc. Dell Statistica (Data Analysis Software System) [Computer Program]. Version 13, 2016. Available online: www.statsoft.com (accessed on 30 April 2021).
37. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2013; ISBN 3-900051-07-0.
38. Warnes, G.; Gorjanc, wcfG.; Leisch, F.; Man, M. _genetics: Population Genetics_. R package version 1.3.8.1.3, 2021. Available online: https://CRAN.R-project.org/package=genetics (accessed on 22 November 2022).
39. González, J.; Armengol, L.; Guinó, E.; Solé, X.; Moreno, V. SNPassoc: SNPs-based whole genome association studies. *R Package Version* **2014**, *1*, 2–9.
40. Sinnwell, J.P.; Schaid, D. *Statistical Methods for Haplotypes When Linkage Phase Is Ambiguous*; Mayo Clinic Division of Health Sciences Research: Rochester, MN, USA, 2011.
41. Doong, S.H.; Dhruva, A.; Dunn, L.B.; West, C.; Paul, S.M.; Cooper, B.A.; Elboim, C.; Abrams, G.; Merriman, J.D.; Langford, D.J.; et al. Associations between cytokine genes and a symptom cluster of pain, fatigue, sleep disturbance, and depression in patients prior to breast cancer surgery. *Biol. Res. Nurs.* **2015**, *17*, 237–247. https://doi.org/10.1177/1099800414550394.
42. Henker, R.A.; Lewis, A.; Dai, F.; Lariviere, W.R.; Meng, L.; Gruen, G.S.; Sereika, S.M.; Pape, H.; Tarkin, I.S.; Gowda, I. The associations between OPRM 1 and COMT genotypes and postoperative pain, opioid use, and opioid-induced sedation. *Biol. Res. Nurs.* **2013**, *15*, 309–317.

43. Martinez-Jauand, M.; Sitges, C.; Rodriguez, V.; Picornell, A.; Ramon, M.; Buskila, D.; Montoya, P. Pain sensitivity in fibromyalgia is associated with catechol-O-methyltransferase (COMT) gene. *Eur. J. Pain* **2013**, *17*, 16–27. https://doi.org/10.1002/j.1532-2149.2012.00153.x.
44. Baumbauer, K.M.; Ramesh, D.; Perry, M.; Carney, K.B.; Julian, T.; Glidden, N.; Dorsey, S.G.; Starkweather, A.R.; Young, E.E. Contribution of COMT and BDNF Genotype and Expression to the Risk of Transition From Acute to Chronic Low Back Pain. *Clin. J. Pain* **2020**, *36*, 430–439. https://doi.org/10.1097/AJP.0000000000000819.
45. Shamley, D. A Cross-Disciplinary Look at Shoulder Pain and Dysfunction after Treatment for Breast Cancer. *Int. J. Cancer Clin. Res.* **2015**, *2*. https://doi.org/10.23937/2378-3419/2/1/1009.
46. Chimusa, E.R.; Meintjies, A.; Tchanga, M.; Mulder, N.; Seoighe, C.; Soodyall, H.; Ramesar, R. A genomic portrait of haplotype diversity and signatures of selection in indigenous southern African populations. *PLoS Genet.* **2015**, *11*, e1005052. https://doi.org/10.1371/journal.pgen.1005052.
47. Chen, J.; Lipska, B.K.; Halim, N.; Ma, Q.D.; Matsumoto, M.; Melhem, S.; Kolachana, B.S.; Hyde, T.M.; Herman, M.M.; Apud, J.; et al. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): Effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am. J. Hum. Genet.* **2004**, *75*, 807–821. https://doi.org/10.1086/425589.
48. Warde-Farley, D.; Donaldson, S.L.; Comes, O.; Zuberi, K.; Badrawi, R.; Chao, P.; Franz, M.; Grouios, C.; Kazi, F.; Lopes, C.T.; et al. The GeneMANIA prediction server: Biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res.* **2010**, *38*, W214–W220. https://doi.org/10.1093/nar/gkq537.
49. Vizan, P.; Di Croce, L.; Aranda, S. Functional and Pathological Roles of AHCY. *Front. Cell Dev. Biol.* **2021**, *9*, 654344. https://doi.org/10.3389/fcell.2021.654344.
50. Mori, S.; Hatori, N.; Kawaguchi, N.; Hamada, Y.; Shih, T.C.; Wu, C.Y.; Lam, K.S.; Matsuura, N.; Yamamoto, H.; Takada, Y.K.; et al. The integrin-binding defective FGF2 mutants potently suppress FGF2 signalling and angiogenesis. *Biosci. Rep.* **2017**, *37*, BSR20170173. https://doi.org/10.1042/BSR20170173.
51. Wu, B.; Hand, W.; Alexov, E. Opioid Addiction and Opioid Receptor Dimerization: Structural Modeling of the OPRD1 and OPRM1 Heterodimer and Its Signaling Pathways. *Int. J. Mol. Sci.* **2021**, *22*, 10290. https://doi.org/10.3390/ijms221910290.
52. Gonzalez-Nunez, V.; González, A.J.; Barreto-Valer, K.; Rodríguez, R.E. In Vivo Regulation of the μ Opioid Receptor: Role of the Endogenous Opioid Agents. *Mol. Med.* **2013**, *19*, 7–17. https://doi.org/10.2119/molmed.2012.00318.
53. Lin, A.; Wang, R.T.; Ahn, S.; Park, C.C.; Smith, D.J. A genome-wide map of human genetic interactions inferred from radiation hybrid genotypes. *Genome Res.* **2010**, *20*, 1122–1132. https://doi.org/10.1101/gr.104216.109.
54. Dobbin, K.K.; Beer, D.G.; Meyerson, M.; Yeatman, T.J.; Gerald, W.L.; Jacobson, J.W.; Conley, B.; Buetow, K.H.; Heiskanen, M.; Simon, R.M.; et al. Interlaboratory comparability study of cancer gene expression analysis using oligonucleotide microarrays. *Clin. Cancer Res.* **2005**, *11*, 565–572.
55. Xie, Z.; Bailey, A.; Kuleshov, M.V.; Clarke, D.J.B.; Evangelista, J.E.; Jenkins, S.L.; Lachmann, A.; Wojciechowicz, M.L.; Kropiwnicki, E.; Jagodnik, K.M.; et al. Gene Set Knowledge Discovery with Enrichr. *Curr. Protoc.* **2021**, *1*, e90. https://doi.org/10.1002/cpz1.90.
56. Li, L.; Jia, J.; Liu, X.; Yang, S.; Ye, S.; Yang, W.; Zhang, Y. MicroRNA-16-5p Controls Development of Osteoarthritis by Targeting SMAD3 in Chondrocytes. *Curr. Pharm. Des.* **2015**, *21*, 5160–5167. https://doi.org/10.2174/1381612821666150909094712.
57. Patel, J.N.; Hamadeh, I.S. Pharmacogenomics-guided opioid management. *BMJ Support. Palliat. Care* **2020**, *10*, 374–378. https://doi.org/10.1136/bmjspcare-2020-002589.
58. Matic, M.; Jongen, J.L.; Elens, L.; de Wildt, S.N.; Tibboel, D.; Sillevis Smitt, P.A.; van Schaik, R.H. Advanced cancer pain: The search for genetic factors correlated with interindividual variability in opioid requirement. *Pharmacogenomics* **2017**, *18*, 1133–1142. https://doi.org/10.2217/pgs-2017-0060.
59. Gilbert-Diamond, D.; Moore, J.H. Analysis of gene-gene interactions. *Curr. Protoc. Hum. Genet.* **2011**, *70*, 14. https://doi.org/10.1002/0471142905.hg0114s70.