GENETIC RISK FACTORS FOR CARPAL TUNNEL SYNDROME

By

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This thesis is dedicated to my parents, Jan and Zelda, and my sister, Antoinette, for their love, support and encouragement to pursue my dreams!

TABLE OF CONTENTS

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ABBREVIATIONS

ACAN	The gene encoding aggrecan
ACL	Anterior cruciate ligament
ADAM	A disintegrin and metalloproteinase
ADAMTS	A disintegrin and metalloproteinase with thrombospondin motifs
AL	Action limit
ANOVA	One-way analysis of variance
AOR	Adjusted odds ratio
AT	Achilles tendinopathy
BGN	The gene encoding biglycan
BMI	Body mass index
bp	Base pairs
CASP8	Caspase-8
CASP8	The gene encoding caspase-8
CI	Confidence interval
COL5A1	The gene encoding the α1 chain of type V collagen
COL5A2	The gene encoding the α2 chain of type V collagen
CON	Control group
CS	Cross sectional
CTS	Carpal tunnel syndrome
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dGTP	Deoxyguanosine thriphosphate
DNA	Deoxyribonucleic acid
dTTP	Deoxythymidine triphosphate
DUE	Distal upper extremity

ECM	Extracellular matrix
EDS	Ehlers-Danlos syndrome
EDTA	Ethylenediaminetetraacetic acid
EEG	Electroencephalography
FACITs	Fibril-associated collagens with interrupted triple helices
GAG	Glycosaminoglycan
GDF5	The gene encoding growth differentiation factor-5
HAL	Hand activity level
HDL	High-density lipoprotein
HF-LR	High frequency, low repetition
HR	Hazard ratio
HRT	Hormone replacement therapy
HWE	Hardy-Weinberg Equilibrium
IL	Interleukin
IL-1β	The gene encoding interleukin-1beta
IL-6	The gene encoding interleukin-6
IL-6R	The gene encoding interleuking-6 receptor
IRR	Incidence rate ratio
LD	Linkage disequilibrium
LDL	Low-density lipoprotein
LF-HR	Low frequency, high repetition
LF-LR	Low frequency, low repetition
MM5	median sensory peak latency of >0.5s larger than the ulnar sensory peak latency
MM8	median sensory peak latency of >0.8s larger than the ulnar sensory peak latency
MMP	Matrix metalloproteinase

MMP1	The gene encoding Matrix metalloproteinase-1
MMP3	The gene encoding Matrix metalloproteinase-3
MMP10	The gene encoding Matrix metalloproteinase-10
MMP12	The gene encoding Matrix metalloproteinase-12
ms	millisecond
MS	Musculoskeletal
MSD	Musculoskeletal disorder
NCBI	National Centre for Biotechnology Information
OA	Osteoarthritis
OR	Odds Ratio
PAGE	Polyacrylamide gel electrophoresis
PC	Prospective cohort
PCR	Polymerase chain reaction
PPT	Primary posterior tibial
PR	Prevalence ratio
PSMD9	The gene encoding Proteasome modulator 9
RA	Rheumatoid arthritis
RC	Retrospective cohort
RFLP	Restriction Fragment Length Polymorphism
RPE	Rate of perceived exertion
RR	Relative risk
SI	Strain index
SNP(s)	Single Nucleotide Polymorphism(s)
SSCT	Subsynovial connective tissue
STREGA	Strengthening the Reporting of Genetic Association Studies
TIMP	Tissue inhibitor of metalloproteinases
TIMP2	The gene encoding tissue inhibitor of metalloproteinases-2

TLV	Threshold limit value
TNC	The gene encoding tenascin-C
TWA	Time weighted average
UK	United Kingdom
UTR	Untranslated region
USA	United States of America
VEGFA	Vascular endothelial growth factor
VEGFA	The gene encoding Vascular endothelial growth factor
WBC	White blood cells

LIST OF FIGURES FROM THIS THESIS

LIST OF TABLES FROM THIS THESIS

ABSTRACT

BACKGROUND & AIMS

Carpal tunnel syndrome (CTS) is a common occupational injury that is caused by an increase in pressure within the carpal tunnel structure which, in turn, causes compression of the median nerve. Although several factors are believed to be associated with increased risk of CTS, the direct causes of this injury remain unknown and it is generally accepted that CTS, with the exception of acutely caused CTS, is a multifactorial condition. Although it is generally accepted that an increase in pressure within the carpal tunnel structure, which contains nine flexor tendons, causes compression of the median nerve, the involvement of these tendons and other connective tissue structures in the aetiology of CTS cannot be excluded. In support of this, pathology of these connective structures have been proposed as being comorbid conditions or a precursor of CTS, cause CTS and/or can lead to an increase in carpal tunnel pressure.

Several studies have suggested that specific non-occupational risk factors, such as anatomical, systemic and chronic factors as well as mostly repetition- and force-related occupational risk factors are associated with CTS. Although genetic influences in the aetiology of CTS have been proposed, this area has received little attention. Common DNA sequence variants on the other hand have previously been reported to associate with common exercise-associated tendon, such as chronic Achilles tendinopathy, and ligament injuries.

The aim of this thesis was to determine whether common DNA sequence variants within several genes that have been associated or implicated in the aetiology of exercise-related musculoskeletal soft tissue injuries, are associated with altered risk of CTS by using a genetic association case-control study approach. The specific objectives of this study were:

- To determine whether four variants within the matrix metalloproteinase genes (*MMPs*), *MMP10* rs486055 (C/T), *MMP1* rs1799750 (1G/2G), *MMP3* rs679620 (A/G) and *MMP12* rs2276109 (A/G), clustered on chromosome 11q22 are associated with altered risk of CTS (Chapter 4).
- To determine whether three variants within the *COL5A1* gene, rs13946 (C/T), rs12722 (C/T) and rs71746744 (-/AGGG) are associated with altered risk of CTS (Chapter 5).
- To determine whether variants within two proteoglycan genes, *BGN* rs1126499 (C/T) and *ACAN* rs1516797 (G/T), are associated with altered risk of CTS (Chapter 6).
- To determine whether variants within the cell signalling pathway, *IL-1β* rs16944 (C/T), *IL-6* rs1800795 (C/G), *IL-6R* rs2228145 (A/C), *CASP8* rs3834129 (I/D) and *VEGFA* rs699947 (C/A), are associated with altered risk of CTS (Chapter 7).

A secondary aim of this research was to determine whether there are any interactions between the different variants that are associated with altered risk of CTS. Inferred haplotypes were therefore constructed from the appropriate variants.

METHODS

This study was hypothesis-driven, following a case-control, genetic association study design. A total of 103 self-reported Coloured participants (94 female and 9 male), with a history of bi- or unilateral carpal tunnel release surgery (CTS), were recruited for this study from various Occupational Health clinics in the Western Cape region of South Africa. In addition, 150 apparently healthy, unrelated, self-reported Coloured participants (133 female and 17 male) without any reported history of CTS symptoms or surgery were recruited as control (CON) participants. The CON and CTS participants were matched for the type of occupation and years of exposure for wrist activity. This study sample investigated is an admixed population, representative of the indigenous populations of South Africa and thereby is predicted to

potentially contain more genetic variation compared to the South African populations of Caucasian ancestry. For this reason, this admixed population can be and has been used previously to refine genetic loci in their potential associations with multifactorial conditions. Participants were genotyped for the following variants: *MMP10* rs486055 (C/T), *MMP1* rs1799750 (1G/2G), *MMP3* rs679620 (A/G), *MMP12* rs2276109 (A/G) (Chapter 4), *COL5A1* rs13946 (C/T), *COL5A1* rs12722 (C/T), *COL5A1* rs71746744 (-/AGGG) (Chapter 5), *BGN* rs1126499, *ACAN* rs1516797 (Chapter 6), *IL-1β* rs16944 (C/T), *IL-6* rs1800795 (C/G), *IL-6R* rs2228145 (A/C), *CASP8* rs3834129 (I/D) and *VEGFA* rs699947 (C/A) (Chapter 7), by means of restriction fragment length polymorphism (RFLP) or real-time PCR. Inferred haplotypes were also constructed of appropriate variant combinations.

RESULTS

The main findings of this study were that several DNA sequence variants were independently associated with altered risk of CTS. In Chapter 5, the TT genotype of *COL5A1* rs13946 was significantly over-represented in the CON (69.3%) compared to the CTS (50.6%) group (p=0.007, OR=0.45, 95% CI=0.26-0.79, Sensitivity=50.6%, Specificity=30.7%). When the rs12722 variant was genotyped, two adjacent polymorphisms, rs146776422 (C/T) /rs55748801 (G/A) (W/M where W=CG), were genotyped simultaneously. The WW+CC genotype combination (41.7% p=0.008, OR=0.45, 95% CI=0.26-0.80) and WW+CT (40.3%, p=0.009, OR=2.0, 95% CI=1.2-3.4) genotypes were significantly over- and under-represented in the CON group, respectively, when compared to the CTS group (24.5% WW+CC, 59.2% WW+CT). In Chapter 6 the CC genotype of *BGN* rs1126499 was also over-represented in the CON group (CC vs CT + TT, p-0.0498, OR=0.55, 95% CI=0.30-0.99). Finally, the AA genotype of *IL-6R* rs2228145 was over-represented in the CON group (AA vs AC + CC, p=0.003, OR=0.41, 95% CI 0.22-0.75) in Chapter 7.

Various inferred haplotypes and pseudo-haplotypes were also found to be associated with altered risk of CTS.

No independent associations were identified with CTS for the *MMP10* rs486055 (C/T), *MMP1* rs1799750 (1G/2G), *MMP3* rs679620 (A/G), *MMP12* rs2276109 (A/G), *COL5A1* rs71746744 (-/AGGG), *ACAN* rs1516797 (G/T), *IL-1β* rs16944 (C/T), *IL-6* rs1800795 (C/G), *CASP8* rs3834129 (I/D) and *VEGFA* rs699947 (C/A) variants.

DISCUSSION & CONCLUSION

The first main novel findings of this thesis were that variants within (1) the 3'-UTR of *COL5A1*, (2) *BGN* and (3) *IL-6R* genes were independently associated with CTS within a self-reported Coloured South African population. Specifically, the *COL5A1* TT genotype of rs13946 (C/T) and the combined WW+CC genotypes of rs146776422/rs55748801 (W/M) and rs12722 (C/T) were significantly over-represented in the asymptomatic control participants. The upstream *COL5A1* 3'-UTR rs71746744 (−/AGGG) variant was however not independently associated with CTS. Similarly, the *BGN* CC genotype of rs1126499 (C/T) as well as the *IL-6R* AA genotype of rs2228145 (A/C) was significantly over-represented in the control participants. Variants within *MMP* genes, the *ACAN* gene as well as *IL-1β*, *IL-6*, *CASP8* and *VEGFA* genes were not independently associated with CTS. The second main finding of this thesis was that the independently associated *COL5A1*, *BGN* and *IL-6R* variants also interacted to modulate the risk of CTS.

The association of the *COL5A1* gene variants, within the 3'-UTR, have been extensively investigated in musculoskeletal soft tissue injuries, chronic Achilles tendinopathy and anterior cruciate ligament (ACL) injuries in Caucasian populations. This was the first study to investigate these variants in a Coloured population and occupational setting. The findings of this thesis is in agreement with previous findings with the combined WW+CC genotypes of

rs146776422/rs55748801 and rs12722 was associated being associated with reduced risk of CTS in a South African Coloured population. Interestingly, the rs13946 variant, that was not associated with chronic Achilles tendinopathy or ACL injury, was associated with altered risk of CTS with the TT genotype being associated with reduced risk. In contrast, the rs71746744 variant, previously associated with chronic Achilles tendinopathy, was not associated with CTS. This indicates that the region within the *COL5A1* 3'-UTR involved in the aetiology of CTS is slightly different from that of other soft tissue injuries

Similar to Type V collagen, biglycan (encoded by *BGN*) is also an important regulator of fibrillogenesis and these two matrix building blocks interact to regulate collagen fibrillogenesis which allows it to maintain the structure of the ECM. The findings in chapter 6, where the CC genotype of the *BGN* rs1126499 variant played a protective role against CTS, are in agreement with previously published results, confirming that the C-allele of rs1126499 contributes to a protective role of *BGN* for some musculoskeletal soft tissue injuries. The rs1126499 variant also interacted with *COL5A1* variants to significantly modify the risk of CTS. Interestingly, the *ACAN* rs1516797 variant, previously reported to alter the risk of ACL injuries, was not associated with risk of CTS.

The expression of structural proteins, such a type V collagen and MMPs, are regulated by signalling cascades in response to stimuli such as repetitive mechanical loading, which is often mentioned as a risk factor for CTS and other occupational and recreational overuse injuries. Previous studies have also shown altered expression of several inflammatory, apoptosis and angiogenesis genes in these injuries. Therefore, variants within five genes (*IL-β*, *IL-6*, *IL-6R*, *CASP8* and *VEGFA*) involved in this proposed cell signalling pathway were also investigated for their potential association with CTS risk. Only the interleukin-6 receptor rs2228145 (*IL-6R*) gene variant was independently associated with CTS. However, various

interactions between other cytokine gene variants (*IL-1β* and *IL-6*) were observed and *IL-6R* also interacted with *COL5A1* to modify the risk of CTS.

Although none of the matrix metalloproteinase (MMP) gene variants associated independently with CTS, a previous interaction between *MMP3* rs679620 and *COL5A1* rs12722 was found in chronic Achilles tendinopathy as well as ACL injuries in female participants. Therefore the same investigation was undertaken in the current study, revealing a similar interaction as was previously reported in ACL ruptures, but not in chronic Achilles tendinopathy.

In conclusion, this thesis investigated the association of genes that have previous been associated with chronic Achilles tendinopathy and/or anterior cruciate ligament ruptures with CTS. Various variants were, for the first time, shown to independently associate with altered risk for CTS and various gene-gene interactions were also observed, highlighting the complexity of this multifactorial injury. These novel findings therefore highlight the possible important role that genetic factors, and by implication pathology of the flexor tendons and other connective tissue structures within the carpal tunnel, have, at least in part, in the aetiology in CTS.

CHAPTER 1: RISK FACTORS FOR CARPAL TUNNEL SYNDROME

1.1 INTRODUCTION & SCOPE OF THE THESIS

Carpal tunnel syndrome (CTS) is considered to be one of the most problematic and common work-related injuries; accounting for up to 90% of all entrapment neuropathies of the upper limb[1–4].

This, and other occupational injuries, has a large negative financial impact within the industrial sector in terms of loss of time from work and actual cost of the injury (treatment, surgery, rehabilitation etc.). More specifically, a total of US $310 million was spent, between 1990 and 1994, on workman's compensation claims as a result of CTS in the United States alone[2]. This included not only medical costs, but also disability and job modification costs[2]. Furthermore, this injury also has a major negative impact on the individual suffering from the injury. This includes not only pain of the injury itself, but also loss of time from work, inability to perform certain tasks and eventually, not being able to work at all. Although common worldwide, idiopathic CTS is rarely seen in Black South African populations[5,6]. Even though there is no published data to support this, there seems to be a high prevalence of CTS amongst Coloured, blue-collar workers in the Western Cape (Dr H de Wet, personal communication), the population group investigated in this thesis. South African populations who self-identify as Coloured have a complex history of ancestrally derived admixture. This ethnic group is ancestrally derived from admixtures of one or more of the indigenous African populations (Khoe- and San-speaking or Bantu-speaking), immigrants from Western Europe, or slave labourers from West Africa, Indonesia, Madagascar, Java, India and Malaysia. The term "Coloured" in South Africa is therefore a name that encompasses a wide range of people who are unique to this country[7].

It is well accepted that CTS is a multifactorial condition, with several intrinsic and extrinsic risk factors playing a role in its aetiology[8]. These risk factors will be discussed briefly in section 1.5 of this chapter and systematically reviewed in subsequent chapters (Chapters 2 and 3) of this thesis. Although no specific studies have investigated the association of genetic variants with CTS, investigators have nevertheless suggested that there is, at least in part, a genetic component to this injury[8]. The primary objective of this thesis was therefore to identify specific genetic elements that could potentially predispose an individual to CTS.

Although the exact mechanisms of CTS are poorly understood, it is generally accepted that an increase in the pressure within the carpal tunnel structure causes compression of the median nerve and the associated symptoms[9,10]. Together with the median nerve the carpal tunnel also contains nine flexor tendons and peritendinous subsynovial connective tissue (SSCT)[11]. Although, at the structural level, research has focused on the median nerve, the involvement of these tendons and the SSCT in the aetiology of CTS cannot be excluded. In support of this, tendinopathy and tenosynovitis have both been mentioned as being comorbid conditions or a precursor of CTS[12]. Furthermore, flexor tenosynovitis can lead to an increase in carpal tunnel pressure[3] and it has also been suggested that fibrosis of the SSCT could be a cause of CTS[13].

With this in mind, the specific candidate genes investigated in this thesis were selected based on their structural and biological function within tendons. In addition they have all previously been associated with tendon and/or ligament overuse injuries as a result of participation in physical activity (Section 1.6). The hypothesis tested in this thesis is that common genetic polymorphisms previously shown to associate with exercise-associated tendon/ligament overuse injuries could also be associated with occupational overuse injuries that potentially involve tendons and other connective tissue structures. A genetic association, case-control study design following a candidate gene approach was used to

determine whether specific DNA sequence variants are associated with the risk of CTS (Chapters 4 to 7).

In preparation for the systematic reviews (Chapters 2 and 3), genetic association experimental chapters (Chapters 4 to 7) and the final discussion chapter (Chapter 8), the following sections will provide a brief overview of the gross anatomy of the carpal tunnel (Section 1.2), the mechanisms of CTS injuries (Section 1.3), the epidemiology of CTS injuries (Section 1.4), the risk factors for CTS injuries (Section 1.5) and the previously identified genetic risk factors of tendon overuse injuries in sport (Section 1.6).

1.2 GROSS ANATOMY OF THE CARPAL TUNNEL

CTS is the resulting condition of the compression of the median nerve which runs through the carpal tunnel in the anterior portion of the wrist. This tunnel contains the median nerve, nine tendons which are surrounded by synovial sheaths and is bordered by the rigid carpal bones and intercarpal ligaments on the medial, lateral and posterior sides and the flexor retinaculum on the anterior side (Figure 1.1)[14,15].

The median nerve supplies sensory and motor innervation to the thumb, index, middle and radial half of the ring finger[14]. The nine tendons, one flexor pollicis longus tendon, four flexor digitorum superficialis tendons and four flexor digitorum profundus tendons, are attached to their respective muscles to form musculo-tendinous units responsible for the movement of the digits. Finally, the flexor retinaculum is a fibrous band that attaches to the tubercle of the scaphoid and ridge of the trapezium on the radial side and to the pisiform and hook of the hamate on the ulnar side that forms the anterior border of the carpal tunnel structure. It provides mechanical protection of the contents of the carpal tunnel as well as having a minor role in the stabilisation of the carpal arch[16]. It has been proposed that the flexor retinaculum

consists of three different parts of which the transverse carpal ligament is only one part thereof[14,16], however the terms are often used synonymously.

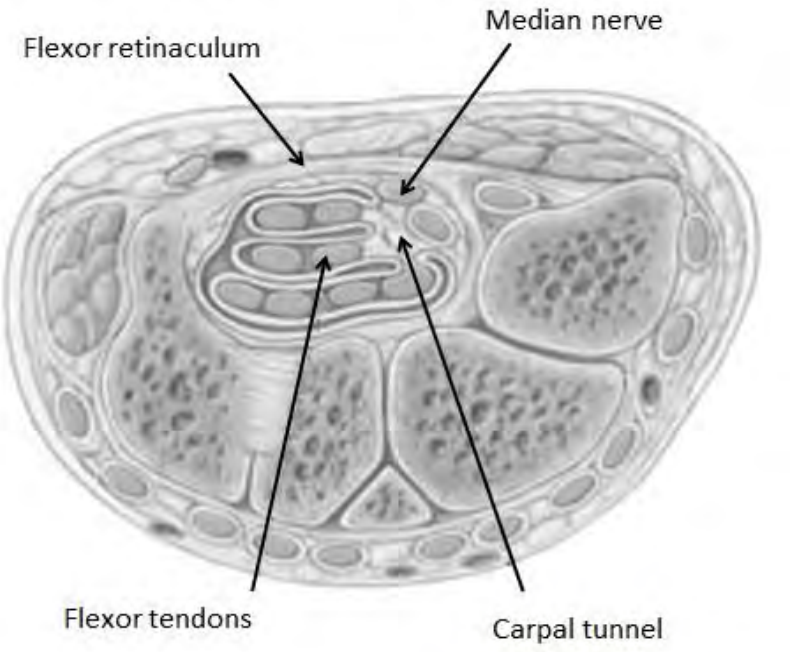

Figure 1.1 A schematic diagram of a cross-section of the wrist illustrating the location of the median nerve, the flexors tendons and the flexor retinaculum within the carpal tunnel structure. Figure adapted from Katz & Simmons, 2002[15]

1.3 MECHANISM OF INJURY

Although the mechanisms of injury are poorly understood, it is generally accepted that median nerve compression, resulting in CTS, is caused by either an increase in the contents of the carpal tunnel or a decrease in the size of the tunnel. There are four proposed categories of injury namely (i) acute, (ii) chronic, (iii) overuse and (iv) idiopathic. However, it is important to keep in mind that there is often overlap between these different categories and that these just serve as a basic guideline for CTS injuries. Different terms are often used in the literature to describe these four categories. For the purpose of this thesis, however, the following categories will be used.

1.3.1 Acute

This is the only mechanism of CTS injury where an actual inciting event takes place. An acute cause of CTS is most often the result of a rapid increase in the pressure within the carpal tunnel leading to a sudden increase in pressure on the median nerve, resulting in the typical CTS symptoms of pain, numbness and paraesthesia in the affected digits. This acute injury can be caused by a fracture of the radius, dislocation as well as direct insult to the area, such as burns or injections[1].

1.3.2 Chronic

CTS, as a result of a chronic injury/insult, typically has a longer onset and can be due to many different conditions, including causes localised to the carpal tunnel area (local causes), causes localised to the region in which the carpal tunnel is located (regional causes) and finally causes affecting the whole body (systemic causes)[1]. The potential causes are outlined in Table 1.1. These causes all compromise the space, either directly or indirectly, within the carpal tunnel which causes increased pressure on the median nerve.

1.3.3 Overuse

Similar to chronic causes, overuse as mechanism of CTS injury has a gradual onset but is different due to the fact that overuse is an extrinsic mechanism whereas chronic causes are intrinsic, local, regional or systemic, in nature. Overuse of the hands/wrists as a mechanism for CTS injury is highly debated[1,8] but generally believed to play a role in the aetiology of this condition when other potential causes have been excluded. Extreme range of motion, specifically flexion and extension of the wrist, especially when performed in conjunction with weight-bearing activity, increase the pressure within the carpal tunnel, significantly[3]. Increased pressure within the carpal tunnel therefore leads to increased pressure on the median nerve and the flexor tendons.

Table 1.1 Chronic causes of Carpal tunnel syndrome

Local causes
• Inflammatory
• Tumours
• Anatomical anomalies
Regional causes
• Osteoarthritis
•Rheumatoid arthritis
• Amyloidosis
• Gout
Systemic causes
• Diabetes
• Obesity
• Pregnancy
• Hypothyroidism
• Menopause
• Long-term haemodialysis

Table adapted from Aroori & Spence, 2008[1].

1.3.4 Idiopathic

Idiopathic CTS, also referred to as spontaneous CTS[3], is the onset of CTS symptoms with no apparent cause. Idiopathic CTS specifically emphasizes the lack of a systemic cause of injury (as described under Chronic injuries) and refers to spontaneous CTS in an otherwise healthy hand/arm[8].

1.4 EPIDEMIOLOGY OF CTS

The incidence of CTS, being the number of new injuries per unit of time, is not often reported but the few studies that do report incidence, show large differences between different countries. No information about the incidence of CTS in South Africa is available, but in a recent study, Atroshi et al., (2011) described the incidence of physician reported CTS in

females in Sweden to be 324 per 100 000 person years compared to that in the US, being 542 per 100 000 person years. Similarly, the incidence of CTS in Swedish males was 125 compared to 303 in the US, per 100 000 person years[17]. Similarly, a study described the incidence in the general population of a restricted area of Italy to be 506 per 100 000 person years in females and 139 in males[18].

The prevalence of CTS, being the amount of injuries present in the population at a fixed time-point, in the general population is generally reported to be between 1 and 5 %[17,18] depending on the criteria used to define the condition. However the prevalence of CTS has been reported to be as high as 61% in occupations involving repetitive use of the upper limbs[19].

From these figures it is clear that the incidence and prevalence can vary greatly between different circumstances, specifically geographical location and sex, highlighting these as potential risk factors for CTS. These factors will be discussed in the following section and in more detail in the following chapter.

1.5 RISK FACTORS FOR CTS

CTS is generally accepted to be a multifactorial condition and the factors that are proposed to alter the risk of developing this condition can be divided into two categories that will be discussed below. Although these risk factors will be extensively reviewed in Chapters 2 and 3, they are briefly summarised in the following sections.

1.5.1 Non-occupational

There are several non-occupational risk factors, also known as intrinsic risk factors, which are proposed to increase the risk of CTS. Age, sex and BMI are some of the most important risk factors mentioned in the literature, with females[1,20] older than 30 years[1,20] and those with

a higher BMI[20,21] being at higher risk of developing CTS. The size of the wrist, referred to as wrist ratio (wrist depth divided by wrist width) is also believed to influence CTS risk[22].

The presence of several medical conditions including diabetes, rheumatoid arthritis, osteoarthritis and thyroid disorders is also thought to increase the risk of CTS[1,8]. Furthermore, factors that can alter fluid balance in the body, such as pregnancy or menopause[1,23] are also believed to increase the risk of CTS. CTS symptoms caused by pregnancy will often disappear after pregnancy, but in some cases further treatment is required[23].

Although the studies are limited, several authors have suggested that genetic or familial factors[8–10] are potential intrinsic risk factors. These and other intrinsic, or non-occupational risk factors, will evaluated and discussed in Chapter 2.

Although not traditionally believed to play a large role in the aetiology, psychosocial factors have been investigated for its potential role in the development of CTS. Specifically, individuals with poor psychological well-being with little job control are believed to be at increased risk for developing CTS[26]. These psychosocial risk factors will be evaluated and discussed in Chapter 3.

1.5.2 Occupational

Important factors that are proposed to alter the risk of CTS are occupational of nature, also known as extrinsic risk factors. Occupational factors that are generally believed to increase the risk of CTS include keyboard/computer use[27], repetitive motion of the hands/arms[3], weight-bearing activity[3] and the hands/arms being exposed to vibration[28]. These occupational factors that could potentially play a role in the modification of CTS risk will be discussed and evaluated in Chapter 3.

1.6 GENETIC RISK FACTORS FOR TENDON AND LIGAMENT INJURIES

1.6.1 Overview

Overuse injuries are commonly seen in sporting activities with tendon injuries contributing between 30 and 50% of all sporting injuries with this injury rate being dominated by Achilles tendon injuries[29]. In addition, participation in sporting activities, in particular those that involve a sudden deceleration or change in direction, may place an individual at greater risk of anterior cruciate ligament (ACL) rupture[30]. Although the incidence is low in the general population[31,32], these acute injuries are considered one of the most severe injuries sustained in a sporting population[33].

As with CTS, the mechanisms of overuse and acute exercise-associated musculoskeletal soft tissue injuries are also poorly understood. However several extrinsic and intrinsic risk factors have been identified for these injuries, with a genetic component being an important intrinsic risk factor[34]. Previous research has identified several DNA sequence variants within genes that are responsible for the structural and biological maintenance and function of tendons and ligaments, to be associated with chronic Achilles tendinopathy[35] and ACL ruptures[36]. Although the focus of this thesis is the proposed involvement of the tendon structures in CTS, researchers have previously identified similarities and differences with respect to the association of genetic risk factors with chronic Achilles tendinopathy and ACL ruptures[34]. As outlined in the following sections, the association of DNA sequence variants with both these injuries will therefore be considered in this thesis. A detailed overview of the DNA sequence variants that have been reported to associate with altered risk of chronic Achilles tendinopathy and/or ACL ruptures is however beyond the scope of this thesis and has been extensively reviewed[34–37]. Only the DNA sequence variants included in the studies of this thesis will therefore be described in the following sections and chapters.

1.6.2 The Collagen Fibril

Both tendons and ligaments are collagenous structures, which have a similar composition and hierarchical structure[34]. Although two-thirds of these tissues consist of water, the basic structural unit of both tissues is the collagen fibril (Figure 1.2). The fibril consists predominately of type I collagen with other collagens, such as type V collagen, and non-collagen, such as the proteoglycans, macromolecules also being an important structural component of the fibril. The collagen fibril and other structural components of tendons and ligaments are synthesised and maintained by the fibroblast cells, known as tenocytes in tendons, which are embedded between the collagen fibrils within the tendon and ligament. Although the fibroblasts are a minor structural component of connective tissues, like tendons and ligaments, they are able to respond to mechanical loading and other stimuli[34,38]. The cells will usually respond appropriately to these stimuli, resulting in adaptation or healing of the tissue. It has however been proposed that “abnormal” stimuli, such as excessive unaccustomed mechanical loading of the tissue, can result in a response by the fibroblasts that may lead to maladaptation of the tissue, eventually leading to injury[39]

An overview of the collagen family and the structure of the collagen fibril is also beyond the scope of this introduction and has been recently reviewed[40]. Type V collagen, which is a focus of this thesis plays a vital role in collagen fibril formation[41]. The major isoform is a heterotrimer, consisting of two α1 and one α2 chains. These chains are encoded by the *COL5A1* and *COL5A2* genes respectively[42]. Rare mutations within these genes cause Ehlers-Danlos syndrome (EDS), a severe Mendelian connective tissue disorder[43]. In addition common DNA sequence variants within the 3’-untranslated region (UTR) of the *COL5A1* gene have been associated with chronic Achilles tendinopathy as well as ACL ruptures in females[44–46]. The role of *COL5A1* in severe connective tissue disorders, common musculoskeletal soft tissue injuries and other phenotypes will be discussed in more detail in the introduction of chapter 5.

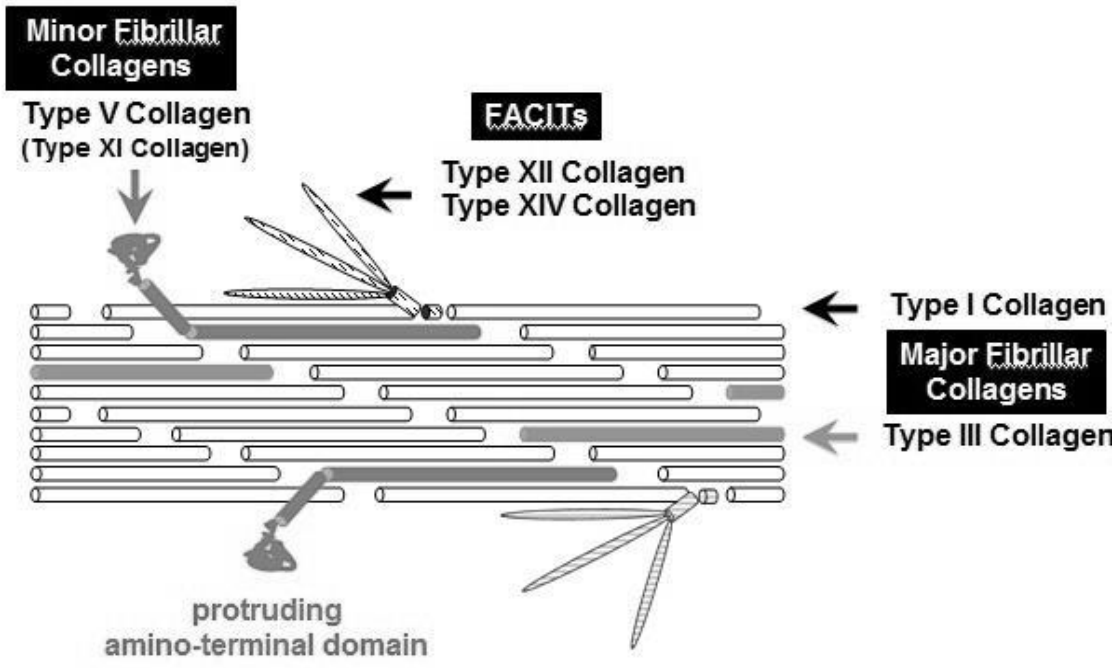

Figure 1.2 Schematic diagram of the collagen fibril, the basic unit of tendons and ligaments. This collagen fibril consists predominately of Type I collagen with other collagens, such as Type III and type V collagen also being important structural components of the fibril. Types XII and XIV collagen, associated with the surface of the fibril are known as Fibril-associated collagens with interrupted triple helices (FACITs). Other structural components of the fibril, such as other collagen types, proteoglycans and glycoproteins are not shown. Figure adapted from Collins & Raleigh (2009)[34].

Similar to the collagens, the proteoglycans, such as aggrecan and biglycan, are important structural components of tendons and other connective tissues[47]. More specifically, aggrecan is responsible for many of the physical properties of the fibrocartilage regions in tendons[48–51], whereas biglycan, the other proteoglycan investigated in this thesis, is an important regulator of tendon development, aging and healing[52]. Biglycan also plays a vital role in fibrillogenesis[48–51]. Rare mutations within the aggrecan (*ACAN*) gene cause either dominant familial osteochondritis dissecans (a joint disorder) or recessive skeletal dysplasia[53,54], while common variants have previously been associated with various multifactorial conditions[55,56]. Inferred haplotypes constructed from variants within the biglycan (*BGN*) gene are associated with ACL ruptures in females[56]. Since *BGN* is located on chromosome X[56] and female sex is considered to be a risk factor for CTS (section 1.5.1),

the association of *BGN* variants with CTS was investigated in chapter 6 of this thesis. The structure of proteoglycans and its functions in tendons and tendinopathy has previously been reviewed[57] and the role of *ACAN* and *BGN* in connective tissue disorders and musculoskeletal soft tissue injuries will be discussed in more detail in the introduction of chapter 6.

Similarly, variants within the genes that play an important role in the remodelling of the ECM, such as matrix metalloproteinases (MMPs), cytokines and growth factors, have also been associated with AT and/or ACL injuries[58–60]. This remodelling is dependent on the balance between tissue degradation, among other proteins, a family of 25 MMP endopeptidases, and their antagonists, the tissue inhibitors of metalloproteinases (TIMPs)[61–63]. Specifically, with respect to variants within the *MMP3* gene, the GG, CC and AA genotypes of rs679620, rs591058 and rs650108, respectively, were all independently associated with increased risk of chronic Achilles tendinopathy[64]. Furthermore, the *MMP3* rs679620 and *COL5A1* rs12722 variants have also been reported to interact to modify the risk of chronic Achilles tendinopathy[64]. Although four functional variants within *MMP10* (rs486055 C/T), *MMP1* (rs1799750 1G/2G), *MMP3* (rs679620 G/A) and *MMP12* (rs2276109 A/G), which clustered together on chromosome 11q22, are not independently associated with risk of ACL ruptures, these variants interacted to modify the risk of non-contact ACL ruptures within males and females[59]. The role of the MMPs in musculoskeletal tissue disorders and CTS will be discussed in Chapter 4.

The expression of structural proteins, such a type V collagen and MMPs, are regulated by signaling cascades in response to stimuli such as repetitive mechanical loading. Previous studies have shown that altered expression of several genes that encode for pro-inflammatory cytokines, such as interleukin-1β and interleukin-6, as well as Caspase-8 or Vascular endothelial growth factor (VEGF), involved in apoptosis and angiogenesis, in

tendon and ligament injuries[60,65,66]. Although variants within the *IL-1β* (rs16944) and *IL-6* (rs1800795) were not independently associated, inferred haplotypes constructed from these and other interleukin variants together with *COL5A1* rs12722 have been reported to modify the risk of chronic Achilles tendinopathy[60]. This highlights that a pathway-based approach may be more informative to fully elucidate the role of genetic risk factors in multifactorial conditions, such as tendinoapthy[60]. In addition, the *CASP8* rs3834129 variant has been reported to independently associate with altered risk of Achilles tendinopathy[65] whilst the *VEGFA* rs699947 variant has been reported to independently associate with altered risk of ACL rupture[66]. The association of interleukin, *CASP8* and *VEGFA* gene variants with CTS will be investigated in chapter 7.

The main aim of this thesis was therefore to determine whether DNA sequence variants within matrix metalloproteinase (*MMP10*, *MMP1*, *MMP3* and *MMP12*), collagen (*COL5A1*), proteoglycan (*ACAN* and *BGN*) and regulatory genes (*IL-1β, IL-6*, *CASP8* and *VEGFA*), which have previously been associated with exercise-associated injuries, are also associated with a common occupational overuse injury, namely, CTS. As discussed in chapter 7, the association of the *IL-6R* (rs2228145) gene, which encodes for the IL-6 receptor and hasn't previously been associated with exercise-associated musculoskeletal soft tissue injuries, with CTS was also investigated.

CHAPTER 2: NON-OCCUPTIONAL RISK FACTORS FOR CARPAL TUNNEL SYNDROME

2.1 INTRODUCTION

As discussed in the previous chapter carpal tunnel syndrome (CTS) is a common neuropathy accounting for up to 90% of all entrapment neuropathies of the upper limb. Although the exact aetiology of CTS is not fully understood, researchers have suggested that, in addition to the repetitive use of the upper limbs, several other non-occupational risk factors are also associated with CTS. Since there is, to our knowledge, no recent systematic review of these risk factors in the scientific literature, the objective of this chapter of the thesis is therefore to critically assess the published evidence on non-occupational risk factors of CTS.

2.2 METHODS

2.2.1 Search strategy

Three electronic databases, PubMed, Web of Knowledge (including Biological abstracts, Medline and Web of Science) and Springerlink, were searched using the keywords “CARPAL TUNNEL SYNDROME RISK” or “CARPAL TUNNEL SYNDROME RISK FACTORS”. The database search was performed for all articles published online until January 2014. Review articles were initially included in order to include their reference lists. A three-step method was followed to identify the articles that were included in this review. Titles, abstracts and full texts were screened. Articles were excluded at each step if they met the exclusion criteria as outlined in Table 2.1.

Table 2.1 A three-step method was followed to identify the articles that were included in the systematic review of risk factors associated with carpal tunnel syndrome (CTS). Titles, abstracts and full texts were screened and excluded at each step if they met the exclusion criteria.

Exclusion criteria	
1	Unrelated to the topic
2	Commentaries, book chapters, letters, editorials, conference proceedings, case reports, conference, abstracts or non-peer reviewed articles
3	Studies examining hand/upper limb injuries without reference to CTS/median nerve.
4	Studies of other medical/systemic conditions (eg diabetes, amyloidosis) without specific reference to CTS
5	Considered only self-reported CTS
6	Animal or cadaver studies

All the references within the included articles were also reviewed using the same criteria to identify any additional articles not identified during the initial screening process of the databases. All the identified articles were appraised according to the inclusion criteria listed in Table 2.2.

Table 2.2 The identified articles were included in the systematic review of risk factors associated with carpel tunnel syndrome (CTS) if they met the following inclusion criteria.

Inclusion criteria	
1	The article must include original data
2	The article must be published in English
3	The article must include a minimum of one potential risk factor for CTS
4	Medically called/diagnosed (probable or operated CTS)
5	The article must include a point or risk estimate (eg, OR), with the 95% CI indicated

2.2.2 Data extraction

Study design, study population and the results of each identified article was reviewed in the appraisal step. Studies reporting risk estimates were identified. These included relative risk (RR), odds ratio (OR), incidence rate ratio (IRR) and hazard ratio (HR). These risk estimates are routinely used as measures of injury risk[67]. In order to avoid Type I and II errors made by rounding, the upper and lower 95% confidence interval cut-off values to indicate decreased and increased risk was set at 0.9 and 1.1, respectively. Studies reporting a p-value were included only if accompanied by a risk estimate since p-values are considered a measure of statistical significance but has limited value in the interpretation and estimation of risk. P-values were, however, reported if provided.

2.2.3 Level of evidence and certainty

Each article was reviewed using two established methods: 1) level of evidence and 2) level of certainty. Level of evidence, a ranking system for research articles, was determined by using previously described definitions[36,68,69]. High-quality prospective cohort studies are considered level I; retrospective studies and lesser-quality prospective studies are level II, these include prospective studies with small sample sizes or weaker risk estimates (eg OR instead of IRR); case-control studies are level III; case series are level IV; and expert opinions are level V[36,68]. Only articles with a level of evidence of I, II or III were included in this review.

The level of certainty, namely low, moderate and high level of certainty, for each risk factor was calculated by analysing all the included published studies for each risk factor. This classification system was based on previously published definitions by the US Preventative Services Task Force[70]. The levels of certainty were defined as follows: 1) high certainty is "The available evidence includes consistent results from level I studies. These studies provide a good estimate of risk and are unlikely to be strongly affected by future studies."[70], 2) moderate certainty is "The available evidence includes sufficient evidence to determine

that there is risk associated with the injury, but confidence in the estimate is constrained by factors such as the sample size and quality of studies, as well as inconsistency of findings across individual studies. As more information becomes available, the magnitude of risk could change or even alter the conclusion"[70] and 3) low certainty is "The available evidence is insufficient to assess risk. Evidence is insufficient because of the limited number or size of studies and inconsistency of findings across individual studies. More information may allow an estimation of risk"[70].

In various sections, different variables were grouped under one umbrella risk factor. Although this is not ideal, this was done for simplicity purposes to avoid having an excessive amount single risk factors investigated only by one study. Furthermore, groupings in this systematic review was made on the risk factor's effect on risk i.e. increased, decreased or no effect on risk. Although there are several methods of grouping risk factors, this method was chosen to increase the understanding of the effect of a particular risk factor on risk and to prevent confusion.

2.3 RESULTS & DISCUSSION

The article selection process is outlined in Figure 2.1. Initially 2492 unique titles (duplicates excluded) were identified from the three electronic databases. Based on the titles alone, 1355 articles were excluded and an additional 586 articles were excluded when the abstracts were also reviewed. Seventy-eight articles were finally included in this study from the initial database searches after the full texts of 551 articles were reviewed. When all the references within the articles that fit the inclusion criteria were also analysed using the same exclusion and exclusion criteria, an additional 4 articles were identified and included in the systematic review. Forty-one, non-occupational risk factors were identified from the 70 full text articles include in this review. The risk factors were divided into the following categories

(1) biological, (2) behavioural and social, (3) medication, (4) medical conditions and injuries and (5) other.

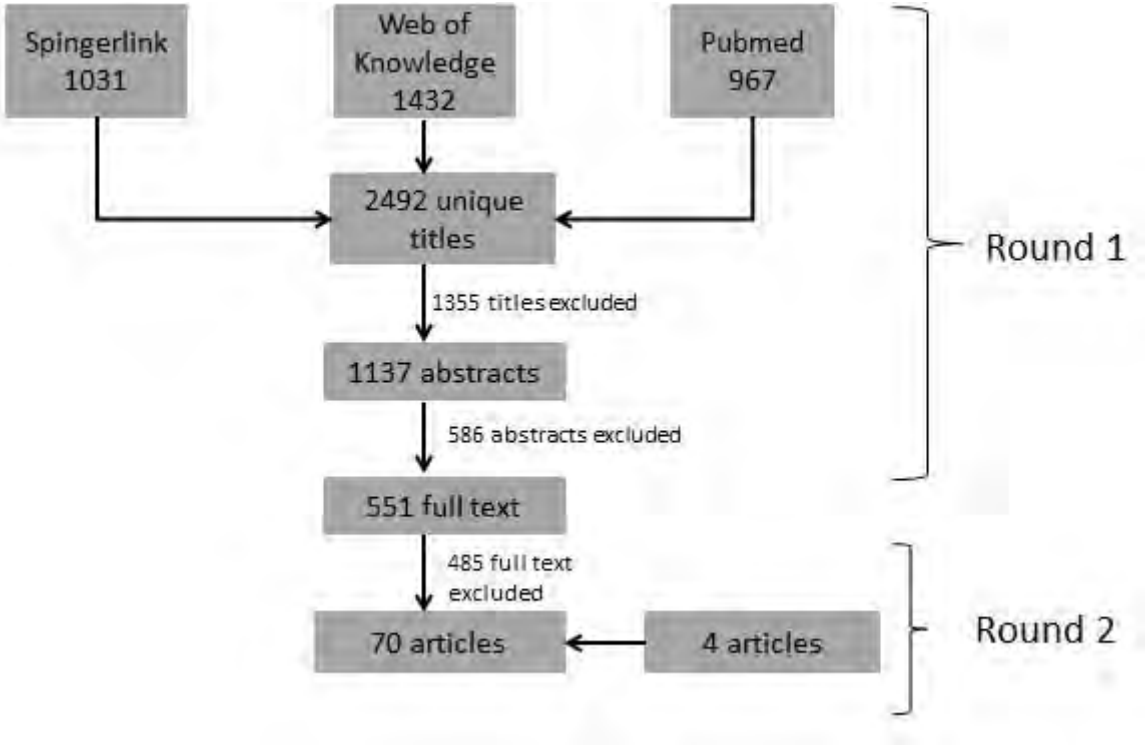

Figure 2.1 Outline of the literature search procedure. In round 1, a total of 2492 unique titles (duplicates excluded) were identified using the electronic database searched. After the three-step method, the number of articles were reduces to 1137 abstracts and then 551 full text articles. A total of 66 articles were included. When all the references of the articles that fit the inclusion criteria were analysed, using the same exclusion and exclusion criteria and three-step method (as in round one), an additional 4 articles were identified and included in the systematic review. A final selection of 70 articles was therefore included in this review.

2.3.1 Biological

The following section will review the role of eight intrinsic biological, namely ethnicity, age, sex, height, weight, BMI (or obesity), hand/wrist structure/dimension and genetic factors as risk factors for CTS.

2.3.1.1 Ethnicity

Six studies have examined ethnicity as an intrinsic risk factor for CTS (Table 2.3). Three cross sectional studies have suggested that ethnicity is a risk factor[71–73], each defining different ethnic groups in their investigations. However three prospective cohort (Level I) studies found ethnicity to have no effect on the risk of CTS[74–76]. Since ethniticity has only been identified as a risk factor in cross sectional studies (Level III), which have not been confirmed in prospective studies, it was assigned a low level of certainty.

Table 2.3 Summary of all the studies that have investigated ethnicity as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Tanaka et al. 1997[71]	CS	General population	145/29929	Non-white AOR: 16.69 (95% CI 5.22 - 53.32)	III
Tanaka et al. 1995[72]	CS	Working population	675/176525	White vs Non-white Multivariate OR: 4.20 (95%CI 1.78 - 9.92)	III
Sapuan et al. 2012[73]	CS	Pregnant maternal health clinic patients	82/251	Malay vs non-Malay in 3rd trimester of pregnancy: OR 2.262, 95% CI 1.10–4.46; p = 0.024	III
No effect on risk					
Harris-Adamson et al. 2013[74]	PC	Industrial workers	204/3311	Hispanic IRR: 0.64 (95% CI 0.35 - 1.08) African-american: IRR: 0.66 (95% CI 0.41 - 1.02) Asian: IRR 0.99 (95% CI 0.44 - 1.94) Other: IRR 1.07 (95% CI 0.42 - 2.27)	I
Gorsche et al., 1999[75]	PC	Industrial workers	140/525	Non-white (asian mix) RR: 1.19 (95% CI 0.63 - 2.23)	II
Nathan et al. 2002[76]	PC	Industrial workers	34/222	White vs Non-white: OR 1.25 (95% CI 0.34 - 4.53); Multivariate OR 1.11 (95% CI 0.25 - 4.89), p=0.890	II

CS, cross sectional; PC, prospective cohort; AOR, adjusted odds ratio; OR, odds ratio; CI, confidence interval; IRR, incidence rate ratio; RR, risk ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

2.3.1.2 Age

Older age, especially in females, is often reported as an intrinsic risk factor for CTS in the literature[77]. Of the 31 studies that examined age as a modifiable risk factor for CTS (Table 2.4), 16 found an age-related increase in risk[24,74,76,78–90], two found an age-related decrease in risk[91,92], while 27 reported no association with CTS risk[24,71,72,75,76,78,79,89,90,93–110].

Four prospective and one retrospective studies investigating industrial workers found an increase in CTS risk in higher age groups[74,76,78,79,86]. Three of these studies investigated various age groups and found that workers older than 20, 30, 40 and 50 years[74,76,78,86], respectively, are at increased risk of CTS. Interestingly, Harris-Adamson et al. (2013), Violante et al. (2007) and Wolf et al. (2009) found that risk increases with an increase in age[74,78,86]. In contrast, Frost et al. (1998) found that workers between 35 and 49 years are at increased risk, but that being older than 50 years has no effect on CTS risk[79].

The majority of the prospective (n=7) and retrospective (n=2) studies investigating industrial workers have however reported that age or age groups are not associated with risk of CTS[75,76,78,79,93–96,108]. The single prospective and single retrospective studies that reported decreased risk of CTS in age groups older than 60 years as well as older than 70 years of age, considered only breast cancer patients and did not investigate workers or the working population specifically[91,92]. The relevance of these findings, in a working population, should therefore be investigated.

Although all different age groups were investigated together in this review and revealed contradictory information, a more in-depth investigation on the different age groups could potentially report different results and is thus warranted. Future research in the form of prospective studies should aim to investigate different age groups instead of considering age as a whole.

As summarised in table 2.4 numerous level III studies have also reported that age either increases or has no effect on the risk for CTS. Therefore, because of the contradicting evidence of the high quality (levels I and II), as well as the level III, studies age was assigned a low level of certainty.

Table 2.4 Summary of all the studies that have investigated age as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	≥ 30 and <40 years: IRR 2.20 (95% CI 1.38 - 3.57), Multivariate HR 2.12 (95% CI 1.34 - 3.34), p<0.01 ≥40 and <50 years: IRR 3.10 (95% CI 2.01 - 4.88), Multivariate HR 2.84 (95% CI 1.85 - 4.37), p<0.01 ≥50 years: IRR 3.55 (95% CI 2.26 - 5.66), Multivariate HR 3.04 (95% CI 1.96 - 4.71), p<0.01	I
Violante et al., 2007[78]	PC	Working population	153/1939	41-45 years: Univariate OR 2.5 (95% CI 1.4 - 4.4), Multivariate OR 2.2 (95% CI 1.2-4.1) >50 years: Univariate OR 3.0 (95% CI 1.7 - 5.4), Multivariate OR 1.7 (95% CI 0.9 - 3.3)	II
Nathan et al., 2002[76]	PC	Industrial workers	34/222	≥50 years: Univariate OR 6.58 (95% CI 2.08 - 20.84), p=0.001, Multivariate OR 15.88 (95% CI 3.03 - 83.40), p=0.001	II
Wolf et al., 2009[86]	PC	Military population	48957 [5]	20–24 years: Adjusted IRR 2.05 (95% CI 1.93 - 2.17) 25-29 years: Adjusted IRR 3.19 (95% CI 3.00 - 3.39) 30-35 years: Adjusted IRR 4.36 (95% CI 4.09 - 4.65) 36-39 years: Adjusted IRR 7.19 (95% CI 6.75 - 7.67) ≥40 years: Adjusted IRR 11.63 (95% CI 10.09 - 12.41)	II
Frost et al., 1998[79]	RC	Active workers (slaughterhouse & chemical)	81/1060	35-49 years Either hand: AOR 2.3 (95% CI 1.15 - 4.63); Dominant hand: AOR 3.24 (95% CI 1.15 - 9.15); Non-dominant hand: AOR 1.82 (95% CI 0.80 - 4.15)	II
Tseng et al., 2012[80]	CC	General population	15802/31604	20-39 years: Univariate OR 6.69 (95% CI 6.13 - 7.29) Multivariate OR 6.25 (95% CI 5.72 - 6.83) 40-59 years: Univariate OR 13.4 (95% CI 12.3 - 14.6) Multivariate OR 11.4 (95% 10.5 - 12.5) ≥60 years: Univariate OR 9.33 (95% CI 8.46 - 10.3) Multivariate OR 7.52 (95% CI 6.79 - 8.33)	III
Armstrong et al., 2008[81]	CS	Newly hired manual workers	131/940	Per 10 year increase: Logistic regression OR 1.55 (95% CI 1.31 - 1.86)	III

Table 2.4 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Morgenstern et al., 1991[82]	CS	Female grocery checkers	127/931	25-34 years: OR 3.79 (95% CI 1.69 - 8.52) p=0.001 35-44 years: OR 8.08 (95% CI 3.55 - 18.4) p<0.001 >44 years: OR 8.70 (95% CI 3.70 - 20.5) p<0.001	III
Becker et al., 2002[83]	CC	Hospital patients	791/981	41-60 years: OR 1.91 (95% CI 1.58 - 2.31), Multiple regression OR 1.35, p<0.001	III
Rosecrance et al., 2002[84]	CS	Construction workers	91/1024	p<0.001 when comparing workers with and without CTS >31 years: AOR 4.12 (95% CI 2.10 – 8.08)	III
Atroshi et al., 2007[85]	CS	General population	97/1906	≥40 years: Adjusted PR 2.48 (95% CI 1.33 - 4.60), p=0.004	III
Werner et al., 1997[87]	CS	Active workers (industrial & clerical sites)	158/348	10 year difference MM5[1]: AOR 3.3 (95% CI 3.3 - 3.4) MM8[2]: AOR 3.5 (95% CI 3.4 - 3.5)	III
Jianmongkol et al,. 2005[88]	CS	Fishnet factory employees	96/566	≤30 years: OR 2.07 (95% CI 1.40 - 3.07), p=0.000	III
Bland, 2005[89]	CS	EEG Patients	2408/1747	Age quintile 3: Multivariate OR 5.29 (95% CI 1.79 - 15.66), p<0.005 Age quintile 4: Multivariate OR 7.42 (95% CI 2.34 - 23.50), p<0.001 Age quintile 5: Multivariate OR 38.3 (95% CI 12.1 - 121.29), p<0.001	III
Hakim et al., 2002[24]	CC	Female-female twin pairs	520/3154	46-50 years: OR 1.99 (95% CI 1.42 - 2.79) AOR (for age and BMI) 2.01 (95% CI 1.44 - 2.81)	III
Mondelli et al., 2006[90]	CS	Female floor cleaners	70/75	Age quartile 4: Univariate OR 3.82 (95% CI 1.43 - 10.22), Multivariate OR 1.69 (95% CI 0.50 - 5.75)	III
Decreased risk					
Sestak et al., 2009[91]	PC	Postmenopausal women received adjuvant therapy for breast cancer	103/6083	61-70 years: Univariate OR 0.48 (95% CI 0.30 - 0.76), p=0.002; Multivariate OR 0.53 (95% CI 0.33 - 0.85), p=0.008 >70 years: Univariate OR 0.51 (95% CI 0.31 - 0.84), p=0.009; Multivariate OR 0.58 (95% CI 0.33 - 1.00), p=0.05	II
Mieog et al., 2012[92]	RC	Female breast cancer patients	79/4578	61-70 years OR 0.49 (99% CI 0.25 - 0.95), p=0.006, AOR 0.47 (95% CI 0.23 - 0.94), p=0.005 >70 years: OR 0.33 (99% CI 0.12 - 0.89), p=0.004, AOR 0.36 (99% CI 0.12 - 1.02), p=0.012	II
No effect on risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	≤44.3 years: Univariate HR 1.102 (95% CI 1.03 - 1.18), p=0.008; Multivariate HR 1.076 (95% CI 0.99 - 1.17), p=0.09 >44.3 years: Univariate HR 0.955 (95% CI 0.89 - 1.03), p=0.02; Multivariate HR 0.959 (95% CI 0.89 - 1.04), p=0.10 *Several multivariate analyses – similar values	I

Table 2.4 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Bonfiglioli et al., 2013[94]	PC	Industrial and service worker groups	84/2837	Univariate IRR 1.08 (9% CI 1.06 - 1.10) Multivariate IRR 1.06 (95% CI 1.05 - 1.08)	I
Violante et al., 2007[78]	PC	Working population	153/1939	31-35 years: Univariate OR 1.2 (95% CI 0.7 - 2.2), Multivariate OR 1.1 (95% CI 0.6 - 2.1) 36-40 years: Univariate OR 1.6 (95% CI 0.9 - 2.8), Multivariate OR 1.4 (95% CI 0.8 - 2.6)	II
Nathan et al., 2002[76]	PC	Industrial workers	34/222	30-39 years: Univariate OR 2.5 (95% CI 0.93 - 6.77), p=0.070, Multivariate OR 2.19 (95% CI 0.70 - 6.88), p=0.170 40-49 years: Univariate OR 1.14 (95% CI 0.31 - 4.27), p=0.840, Multivariate OR 1.13 (95% CI 0.25 - 5.04)	II
Gorsche et al., 1999[75]	PC	Industrial workers	140/525	10 year increments: RR 1.304 (95% CI 0.921 - 1.847), Adjusted RR 1.025 (95% CI 0.988 - 1.063)	II
Cartwright et al., 2013[95]	PC	Latinos in Manual labour occupations	36/173	AOR 1.00 (95% CI 0.95 - 1.05),p=0.969	II
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	40-49 years: OR 0.7 (95% CI 0.2 - 2.0) ≥50 years: OR 2.1 (95% CI 0.5 - 8.2)	II
Leclerc et al., 2001[108]	PC	Workers in Manual labour occupations (repetitive work)	131/467	Female 30-39 years: OR 1.55 (95% CI 0.47 - 5.11) ≥40 years: OR 1.09 (95% CI 0.33 - 3.56) Male 30-39 years: OR 1.05 (95% CI 0.34 - 3.25) ≥40 years: OR 0.46 (95% CI 0.10 - 2.04)	II
Frost et al., 1998[79]	RC	Active workers (slaughterhouse & chemical)	81/1060	≥50 years Either hand: AOR 1.15 (95% CI 0.45 - 2.95), Dominant hand: AOR 1.37 (95% CI 0.35 - 5.41) Non-dominant hand: AOR 1.37 (95% CI 0.47 - 4.04)	II
Tanaka et al., 1995[72]	CS	Working population	675/176525	Per year increment: Multivariate OR 1.03 (95% CI 1.021.04)	III
Silverstein et al., 2009[97]	CS	Manufacturing & Healthcare workers	63/657	AOR 1.07 (95% CI 1.04 - 1.10)	III
Rigouin et al., 2013[98]	CS	Working population	156/3554	1 year increments Men: OR 1.05 (95% CI 1.02 - 1.08), p=0.001 Women: OR 1.07 (95% CI 1.04 - 1.10), p<0.001	III
Maghsoudipour et al., 2008[99]	CS	Industrial workers	47/348	Multivariate OR 1.08 (95% CI 0.99 - 1.18), p=0.05	III
Bonfiglioli et al., 2007[100]	CS	Female supermarket cashiers	26/324	Multivariate OR 1.11 (95% CI 1.04 - 1.18)	III
Raman et al., 2012[101]	CS	Office workers	88/382	-40 years: OR 1.9 (95% CI 1.1 - 3.2) 41-50 years: OR 1.0 (95% CI 0.5 - 2.2) >50 years: OR 1.1 (95% CI 0.3 - 3.9)	III

Table 2.4 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Cartwright et al., 2012[102]	CS	Latinos in Manual labour occupations	215/298 Possible 34/479 Definite	Increasing age: AOR 1.04 (95% CI 1.02 - 1.06), p<0.001, Multivariate AOR 1.04, p=0.008	III
Conlon & Rempel, 2005[103]	CS	Engineers	25/177	Univariate OR: 1.07 (95% CI 1.02 - 1.13), p=0.004	III
Anton et al., 2002[104]	CS	Dental hygienists	8/81	OR 1.23 (95% CI 1.08 - 1.39), AOR 1.38 (95% CI 1.09 - 1.74)	III
Bland 2005[89]	CS	EEG Patients	2408/1747	Age per year: Univariate OR 1.031 (95% CI 1.027 - 1.036), p<0.001 Age quintile 2: Multivariate OR 1.52 (95% CI 0.53 - 4.39), p=0.430	III
Hakim et al., 2002[24]	CC	Female-female twin pairs	520/3154	51-55 years: OR 1.26 (95% CI 0.90 - 1.78) AOR (for age and BMI) 1.30 (95% CI 0.92 - 1.83) 56-59 years: OR 1.29 (95% CI 0.90 - 1.86) AOR (for age and BMI) 1.33 (95% CI 0.92 - 1.92) ≥60 years: OR 1.24 (95% CI 0.91 - 1.68) AOR (for age and BMI) 1.28 (95% CI 0.94 - 1.75)	III
Latko et al., 1999[105]	CS	Workers at manufacturing facility	19/333	OR 1.02 (95% CI 0.97 - 1.06)	III
Eleftheriou et al., 2012[106]	CS	Computer workers	A: 51/410[3] B: 167/294[4]	Case def A: ≥45: Univariate RR 1.38 (95% CI 0.81 - 2.35) Logistic Regression OR 1.16 (95% CI 0.53 - 2.55) Case def B: ≥45: Univariate RR 1.31 (95% CI 1.02 - 1.68), p=0.003 Logistic Regression OR 1.48 (95% CI 0.90 - 2.43), p=0.117	III
Chiang et al., 1993[107]	CS	Fish-processing workers	30/177	OR 1.0 (95% CI 0.9 - 1.1) Women: OR 0.9 (95% CI 0.9 - 1.0)	III
Tanaka et al. 1997[71]	CS	General population	145/29929	≥40 years: AOR 1.2 (95% CI 0.81 - 1.77)	III
Mondelli et al., 2006[90]	CS	Female floor cleaners	70/75	Age quartile 2: Univariate OR 1.65 (95% CI 0.66 - 4.11); Mulivariate OR 1.32 (95% CI 0.44 - 4.00) Age quartile 3: Univariate OR 2.53 (95% CI 0.94 - 6.79); Multivariate OR 1.5 (95% CI 0.45 - 4.96)	III
Leclerc et al., 1998[109]	CS	Workers in Manual labour occupations (repetitive work)	151/1059	Model 1 30-39 years: OR 1.21 (95% CI 0.69 - 2.14) 40-49 years: OR 1.55 (95% CI 0.89 - 2.70) ≥50 years: OR 1.22 (95% CI 0.63 - 2.39) Model 2: 30-39 years: OR 1.85 (95% CI 0.91 - 3.76) 40-49 years: OR 2.11 (95% CI 1.05 - 4.22 ≥50 years: OR 1.71 (95% CI 0.77 - 3.82) Model 3: 30-39 years: OR 1.93 (95% CI 0.95 - 3.95) 40-49 years: OR 2.20 (95% CI 1.09 - 4.44) ≥50 years: OR 1.71 (95% CI 0.76 - 3.83)	III
Solomon et al., 1999[110]	CC	Members of medical aid programs	626/3618	45-64 years: OR 1.2 (95% CI 1.0 - 1.5), AOR 1.1 (95% CI 0.8 - 1.6) ≥65 years: OR 0.9 (95% CI 0.8 - 1.1), AOR 1.0 (95% CI 0.7 - 1.3)	III

From previous page:
PC, prospective cohort; RR, risk ratio; RC, retrospective cohort; CC, case control; CS, cross sectional; AOR, adjusted odds ratio OR; odds ratio; CI, confidence interval; IRR, incidence rate ratio; HR, hazard ratio;
[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.
[1] MM5: median sensory peak latency of > 0 5 ms longer than the ulnar sensory peak latency
[2] MM8: median sensory peak latency of > 0 5 ms longer than the ulnar sensory peak latency
[3] Case definition A: Participants with personal medical history of CTS or surgery due to CTS
[4] Case definition B: Case definition A and participants identified through clinical examination (score ≥12)
[5] The total number of participants was not reported

2.3.1.3 Sex

Female sex is the second biological factor frequently reported to increase risk of CTS[77]. Of the 33 studies investigating sex as risk factor (Table 2.5), 19 found female sex to be associated with increased risk[71,72,74,76,78,80,81,83,86,88,94,99,101,106,107,110–113] whereas 14 found no effect of sex on risk of CTS[75,79,85,89,93,95–97,102,103,105,109,113,114].

Female sex was reported to cause an increase in CTS risk in five prospective and one retrospective studies[74,76,78,86,94,111] with a combined number of 288 CTS cases in a combined study population of 6148 in the two level I prospective studies[74,94] (Figure 2.2 A and B). Another 13 level III studies have also reported that female sex increases CTS risk[71,72,80,81,83,88,99,101,106,107,110,112,113] (Figure 2.2 A and B). Although, five prospective and one retrospective studies have found female sex has no effect on risk of CTS[75,79,93,96,114], only one high quality prospective (level I) study reported 35 CTS cases in a study population of 501[93]. Another eight level III studies found sex to have no effect on CTS risk[85,89,97,102,103,105,109,113].

Although there is contradictory evidence from prospective studies in the literature, one high quality study with a large sample size found female sex to increase the risk of CTS. The prospective studies, however, that reported that sex did not modify CTS were of a poorer

quality with smaller sample sizes. Sex was, therefore, assigned a moderate level of certainty. Future prospective studies with larger sample sizes could change this conclusion.

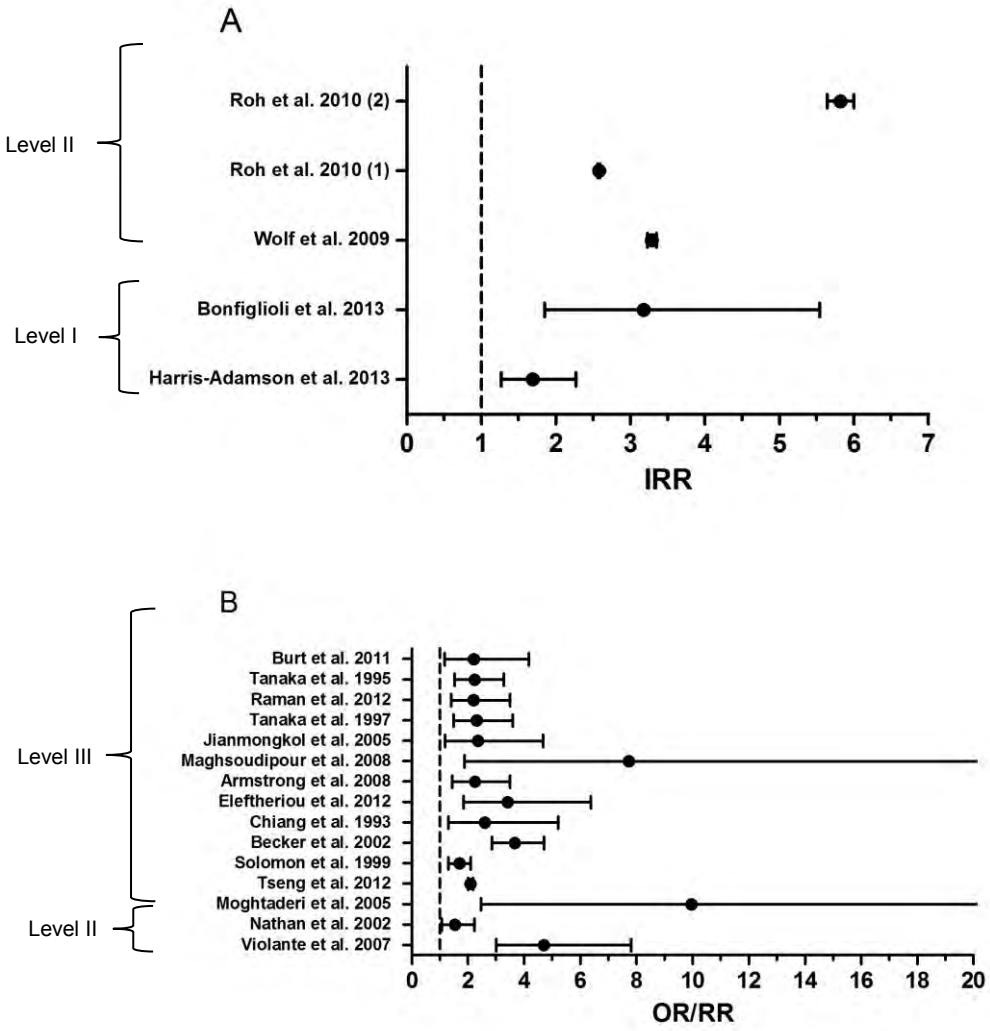

Figure 2.2 Forest plot of the studies that found sex to be associated with increased risk in (A) studies reporting incidence rate ratio (IRR) and (B) studies reporting odds ratio (OR) or relative risk (RR) with the 95% Confidence intervals (CI) indicated. In the Maghsoudipour et al. (2008) and Moghtaderi et al. (2005) studies, the upper limit of the CI was cut at 20 units. The cut-off value of "1" is indicated.

Table 2.5 Summary of all the studies that have reported sex to increase risk for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	Female: IRR 1.69 (95% CI 1.27 - 2.27) Multivariate HR 1.30 (95% CI 0.98 - 1.72), p=0.070	I
Bonfiglioli et al., 2013[94]	PC	Industrial and service worker groups	84/2837	Female: Univariate IRR 3.18 (95% CI 1.85 - 5.54), Multivariate IRR 2.85 (95% CI 1.51 - 5.37)	I
Wolf et al., 2009[86]	PC	Military population	48957 [3]	Female: Adjusted IRR 3.29 (95% CI 3.23 - 3.35)	II
Violante et al., 2007[78]	PC	Working population	153/1939	Female: Univariate OR 4.7 (95% CI 3.0 - 7.8) Multivariate OR 4.0 (95% CI 2.3 - 6.7)	II
Nathan et al., 2002[76]	PC	Industrial workers	34/222	Female: Univariate OR 1.53 (95% CI 1.06 - 2.23), Multivariate OR 5.11 (95% CI 1.68 - 15.53), p=0.02	II
Roh et al., 2010[111]	RC	Members of the Korean Health Insurance Review Agency	Diagnosed: 538711 Surgically treated: 31148	(1) Diagnosed CTS, female: IRR 2.58 (95% CI 2.56 - 2.59) (2) Surgically treated CTS, female: IRR 5.82 (5.64 - 6.00)	II
Moghtaderi et al., 2005[112]	CC	Hospital patients and relatives	128/109	Female: OR 9.95 (95% CI 2.46 - 40.17), p=0.001	III
Tseng et al., 2012[80]	CC	General population	15802/31604	Female: Univariate OR 2.09 (95% CI 2.01 - 2.17) Male: Multivariate OR 2.13 (95% CI 2.04 - 2.22)	III
Solomon et al., 1999[110]	CC	Members of medical aid programs	626/3618	Female: OR 1.7 (95% CI 1.3 - 2.1) AOR 1.6 (95% CI 1.3 - 2.0)	III
Becker et al., 2002[83]	CC	Hospital patients	791/981	Female: OR 3.66 (95% CI 2.84 - 4.71), Multiple regression OR 1.87, p<0.001	III
Chiang et al., 1993[107]	CS	Fish-processing workers	30/177	Female: OR 2.6 (95% CI 1.3 - 5.2)	III
Eleftheriou et al., 2012[106]	CS	Computer workers	A: 51/4101 B: 167/2942	Case def B: Female: Univariate RR 3.41 (95% CI 1.83 - 6.37), p<0.001, Logistic regression RR 4.08 (95% CI 1.51 - 11.04), p=0.005	III
Armstrong et al., 2008[81]	CS	Newly hired manual workers	131/940	Female: Logistic regression OR 2.24 (95% CI 1.43 - 3.50)	III
Maghsoudi pour et al., 2008[99]	CS	Industrial workers	47/348	Male vs Female: Univariate OR 7.73 (95% CI 1.88 - 32.52), p=0.00, Multivariate OR 0.27 (95% CI 0.30 - 2.09), p=0.21	III
Jianmongk ol et al,. 2005[88]	CS	Fishnet factory employees	96/566	Female: OR 2.35 (95% CI 1.18 - 4.67), p=0.018	III

Table 2.5 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Tanaka et al. 1997[71]	CS	General population	145/29929	Female: AOR 2.31 (95% CI 1.48 - 3.60)	III
Raman et al., 2012[101]	CS	Office workers	88/382	Female: OR 2.2 (95% CI 1.4 - 3.5), AOR 4.7 (95% CI 2.1 - 10.3)	III
Tanaka et al., 1995[72]	CS	Working population	675/176525	Female: Multivariate OR 2.23 (95% CI 1.52 - 3.27)	III
Burt et al., 2011[113]	CS	Working population with low, medium or high level of hand activity	57/420	Female - Ratings based model : Multivariate OR 2.21 (95% CI 1.17 - 4.15)	III
No effect on risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	Female: Univariate HR 2.3 (95% CI 1.00 - 5.25), p=0.005	I
Gorsche et al., 1999[75]	PC	Industrial workers	140/525	Female: Relative Risk 1.90 (95% CI 0.99 - 3.66), p=0.055 Adjusted Relative Risk 1.88 (95% CI 0.97 - 3.65)	II
Werner et al., 2005[114]	PC	Assembly workers	20/169	Female:Male OR 4.12 (95% CI 0.89 - 19.10), p=0.07	II
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	Female: OR 0.8 (95% CI 0.3 - 2.0)	II
Cartwright et al., 2013[95]	PC	Latinos in Manual labour occupations	36/173	Male: AOR 1.24 (95% CI 0.58 - 2.67)	II
Frost et al., 1998[79]	RC	Active workers (slaughterhouse & chemical)	81/1060	Female Either hand: AOR 1.44 (95% CI 0.65 - 3.19), Dominant hand: AOR 1.13 (95% CI 0.36 - 3.57), Non-dominant hand: AOR 2.20 (95% CI 0.91 - 5.30)	II
Latko et al., 1999[105]	CS	Workers at manufacturing facitlity	19/333	Female vs male: OR 0.86 (95% CI 0.32 - 2.31)	III
Silverstein et al., 2009[97]	CS	Manufacturing & Healthcare workers	63/657	AOR 1.74 (95% CI 0.99 - 3.05)	III
Atroshi et al., 2007[85]	CS	General population	97/1906	Female - Adjusted PR 1.54 (95% CI 0.94 - 2.52), p=0.084	III
Burt et al., 2011[113]	CS	Working population with low, medium or high level of hand activity	57/420	HAL TLV Model Female: Multivariate OR 1.77 (95% CI 0.99 - 3.17)	III
Cartwright et al., 2012[102]	CS	Latinos in Manual labour occupations	215/298 Possible 34/479 Definite	Female: AOR 1.03 (95% CI 0.74 - 1.43) Multivariate AOR 1.09, p=0.705	III
Bland, 2005[89]	CS	EEG Patients	2408/1747	Female: Multivariate OR 1.11 (95% CI 0.96 - 1.27)	III

Table 2.5 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Conlon & Rempel, 2005[103]	CS	Engineers	25/177	Female: Univariate OR 1.30 (95% CI 0.46 - 3.70), p=0.624	III
Leclerc et al., 1998[109]	CS	Workers in Manual labour occupations (repetitive work)	151/1059	Female - Model 1: OR 1.58 (95% CI 0.89 - 2.83) Model 2: OR 1.49 (95% CI 0.73 - 304), Model 3: OR 1.65 (95% CI 0.80 - 3.42)	III

PC, prospective cohort; IRR, incidence rate ratio; CI, confidence interval; OR, odds ratio; RC, retrospective cohort; CC, case control; CS, cross sectional; AOR, adjusted odds ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

[1] Case definition A: Participants with personal medical history of CTS or surgery due to CTS

[2] Case definition B: Case definition A and participants identified through clinical examination (score ≥12)

[3] The total number of participants was not reported

2.3.1.4 Height

Three studies have evaluated height as an intrinsic risk factor for CTS (Table 2.6). All the studies to date have identified that tall stature decreases the risk of CTS in both men and women while short stature is not associated with risk. A single prospective study (level II) reported that a tall stature with either a short or long forearm leads to a decreased risk of CTS in both univariate and multivariate analyses [Tall stature, short forearm: Univariate OR 0.4 (95% CI 0.2 - 0.7), Multivariate OR 0.5 (95% 0.3 - 0.9); Tall stature, long forearm: Univariate OR 0.5 (95% CI 0.3 - 0.7), Multivariate OR 0.6 (0.4 - 0.9)][78]. A single case-control study also reported a decreased risk for CTS in men taller than 175 cm and women taller than 165 cm in both an univariate and multivariate analyses (women ≥165 cm and men ≥175cm: Univariate OR 0.4, 95% CI 0.3 - 0.7; Multivariate OR 0.5, 95% CI 0.3 - 0.8) [115] .

The same prospective study which reported that tall stature decreased risk of CTS reported that short sature was however not a risk factor for CTS[78]. A cross sectional study, which did

not supply any specific information on tall and short stature groups, reported that height was not a risk factor[116]. Due to the low number of studies available that considered height as a risk factor, it was assigned a low level of certainty.

Table 2.6 Summary of all the studies that have investigated height as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Decreased risk					
Violante et al. 2007[78]	PC	Working population	153/1939	Tall stature, short forearm: Univariate OR 0.4 (95% CI 0.2 - 0.7), Multivariate OR 0.5 (95% 0.3 - 0.9) Tall stature, long forearm: Univariate OR 0.5 (95% CI 0.3 - 0.7), Multivariate OR 0.6 (0.4 - 0.9)	II
Mattioli et al. 2009[115]	CC	General population	191/286	≥165 cm (women) & ≥175cm (men): Univariate OR 0.4 (95% CI 0.3 - 0.7), Multivariate OR 0.5 (95% CI 0.3 - 0.8)	III
No effect on risk					
Violante et al. 2007[78]	PC	Working population	153/1939	Short stature, long forearm: Univariate OR 0.6 (95% CI 0.4 - 1.0), Multivariate OR 0.7 (95% CI 0.4 - 1.1)	II
De Krom et al, 1990[116]	CC	General population	156/473	Risk Ratio: 0.96 (95% CI 0.93 - 0.98)	III

PC, prospective cohort; OR, odds ratio; CI, confidence interval; CC, case control

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases

2.3.1.5 Weight

Only four studies: one prospective[92], two case-control[116,117] and one cross-sectional[103] studies, have investigated weight as an isolated extrinsic risk factor for CTS (Table 2.7). None of these investigations found weight to be associated with CTS, due to these findings and the limited number of studies it was difficult to be conclusive and weight was therefore assigned a low level of certainty as a modifier of CTS risk.

Table 2.7 Summary of all the studies that have investigated weight as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
No effect on risk					
Mieog et al., 2012[92]	RC	Female breast cancer patients	79/4578	70-79kg: OR 1.57 (99% CI 0.71 - 3.46), p=0.14; AOR 1.75 (99% CI 0.77 - 3.96), p=0.077 ≥80: OR 2.17 (99% CI 1.01 - 4.63), 0.009; AOR 2.17 (99% CI 0.98 - 4.77), p=0.012	II
Ferry et al., 2000[117]	CC	Female survey responders	1264/1264	Weight gain: OR 1.37 (95% CI 0.99 - 1.89)	III
De Krom et al., 1990[116]	CC	General population	156/473	Risk Ratio 1.02 (95% CI 1.00 - 1.03)	III
Conlon & Rempel, 2005[103]	CS	Engineers	25/177	Univariate OR 1.03 (95% CI 1.00 - 1.05), p=0.027	III

RC, retrospective cohort; OR, odds ratio; AOR, adjusted odds ratio; CC, case control; CI, confidence interval; CS, cross sectional

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

2.3.1.6 BMI/Obesity

Together with female sex and increased age, increased BMI or obesity is often mentioned in the literature as a risk factor for CTS[77]. Out of the 53 studies that considered BMI or obesity as a risk factor (Table 2.8), 26 found an association with increased risk[71,74,80,81,83–85,87,96,100,101,104,109,112,115,117–127] of CTS while 36 reported no effect on risk[24,27,75,76,78,79,89,90,93–99,101–103,106,108,109,113–116,123,125–134]. Only one level III study found a decreased risk (odds ratio of 0.214, 95% confidence interval between 0.065 and 0.702) for CTS in orthopaedic patients with a BMI less than 18.5 [128].

Only one level I and two level II studies reported a BMI ≥30 to be associated with an increased risk of CTS[74,96,118]. Another 23 level III studies found an increased risk of CTS with a higher BMI or individuals who are overweight or obese[71,80,81,83–85,87,100,101,104,109,112,115,117,119–127]. In contrast, ten level I and II and 26 level III studies found an increased BMI to have no effect on CTS risk[24,27,75,76,78,79,89,90,93–99,101–103,106,108,109,113–116,123,125–134].

Although different BMI groups were investigated together in this review and revealed contradictory information, a more in-depth investigation on the BMI groups could potentially report different results and is thus warranted. Future research in the form of prospective studies should aim to investigate different BMI groups instead of considering age as a whole.

Even though obesity and being overweight is often mentioned as a risk factor in the literature and readily accepted as such, the conflicting evidence and large amount of studies that found no effect on risk with an increased BMI causes this risk factor to be assigned a low level of certainty.

Table 2.8 Summary of all the studies that have investigated BMI and/or obesity as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	≥30: IRR 1.89 (95% CI 1.42 - 2.52), Multivariate HR 1.67 (95% CI 1.26 - 2.21), p=0.00	I
Burt et al., 2013[118]	PC	Working population with low, medium or high level of hand activity	29/318	≥30 Model 1: Multivariate HR 3.19 (95% CI 1.28 - 7.98) Model 2: Multivariate HR 3.26 (95% CI 1.45 - 7.31)	II
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	>30: OR 4.8 (95% CI 1.2 - 19.4), p<0.05 Logistic regression: OR 4.4 (95% CI 1.1 - 17.1), p=0.33	II
Moghtaderi et al., 2005[112]	CC	Hospital patients and relatives	128/109	Increased BMI: OR 1.75 (95% CI 1.50 - 2.04), p=0.000	III
Tseng et al., 2012[80]	CC	General population	15802/31604	Obese: Univariate OR 3.33 (95% CI 2.67 - 4.15), Multivariate OR 2.30 (95% CI 1.82 - 2.91)	III
Becker et al., 2002[83]	CC	Hospital patients	791/981	>30: OR 2.90 (95% CI 2.25 - 3.73) Multiple regression OR 1.85, p<0.001	III
Mattioli et al., 2009[115]	CC	General population	191/286	≥30 : Univariate OR 3.4 (95% CI 1.9 - 6.1), Multivariate OR 3.3 (95% CI 1.6 - 6.6)	III
Coggon et al., 2013[119]	CC	General population	475/799	≥25 and <30: AOR 1.6 (95% CI 1.2 - 2.2); Multivariate OR 1.6 (95% CI 1.1 - 2.1) ≥30: AOR 2.3 (95% CI 1.7 - 3.1); Multivariate OR 2.1 (95% CI 1.6 - 2.9)	III
Stallings et al., 1997[122]	CC	Hospital patients	300	Obese: OR 3.92 (95% CI 2.65 - 5.79), p<0.001 MH OR 3.75 (95% CI 3.18 - 4.45), p<0.001	III

Table 2.8 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Geoghegan et al., 2004[125]	CC	General practice patients	3391/13564	25.1-30: Univariate OR 1.66 (95% CI 1.48 - 1.86), Multivariate OR 1.63 (95% CI 1.45 - 1.84); Operated CTS: AOR 1.79 (95% CI 1.41 - 2.27) 30-40: Univariate OR 2.28 (95% CI 1.80 - 3.70), Multivariate OR 2.06 (95% CI 1.79 - 2.38); Operated CTS: AOR 2.74 (95% CI 2.07 - 3.63) >40: Univariate OR 2.58 (95% CI 1.80 - 3.70), Multivariate OR 2.22 (95% CI 1.53 - 3.21); Operated CTS: AOR 2.60 (95% CI 1.27 - 5.30)	III
Awada et al., 1998[126]	CC	Hospital patients	100/100	Obesity (women): OR 2.17 (95% CI 1.41 - 4.15) Obesity (total): OR 2.32 (95% CI 1.35 - 3.98)	III
Ferry et al., 2000[117]	CC	Female survey responders	1264/1264	Obese: OR 2.01 (95% CI 1.58 - 2.55)	III
Yagev et al., 2007[120]	CC	Hospital patients	127/102	High BMI: Multivariate OR 1.14 (95% CI 1.06 - 1.23), p=0.001	III
Wieslander et al., 1989[127]	CC	Hospital patients	38/76	Group 2 10% above reference weight: OR 3.4 (95% CI 1.2- 9.8), p=0.02	III
Atroshi et al., 2007[85]	CS	General population	97/1906	Overweight: Adjusted PR 2.17 (95% CI 1.32 - 3.56), p=0.002	III
Armstrong et al., 2008[81]	CS	Newly hired manual workers	131/940	Per 5 point increase: Logistic regression OR 1.32 (95% CI 1.15 - 1.52)	III
Tanaka et al. 1997[71]	CS	General population	145/29929	≥25: AOR 2.00 (95% CI 1.33 - 3.02)	III
Leclerc et al., 1998[109]	CS	Workers in Manual labour occupations (repetitive work)	151/1059	Model 1, ≥27 to <31: OR 2.16 (95% CI 1.35 - 3.45), Model 2, ≥27: OR 2.16 (1.41 - 3.29) Model 3, ≥27: OR 2.23 (95% CI 1.46 - 3.40)	III
Rosecrance et al., 2002[84]	CS	Construction workers	91/1024	>28.6 (compared to <23.5) AOR 4.9 (95% CI 2.40 - 10.02)	III
Bonfiglioli et al., 2007[100]	CS	Female supermarket cashiers	26/324	≥30: Multivariate OR 4.97 (95% CI 1.56 - 15.84), p=0.007	III
Forst et al., 2007[121]	CS	Spine surgeons	107/264	≥30 : Multivariate OR 2.04 (95% CI 1.11 - 3.76)	III
Shiri et al., 2011[123]	CS	General population	79/6175	Overweight: AOR 2.0 (95% CI 1.1 - 3.7) Obese: AOR 2.8 (95% CI 1.3 - 5.8)	III
Hou et al., 2007[124]	CS	Male visual terminal display workers	13/327	≥28: Multivariate OR 4.1 (95% CI 1.2 - 14.4), p=0.029	III
Werner et al., 1997[87]	CS	Active workers (industrial & clerical sites)	158/348	Obese MM5: OR 4.0 (95% CI 2.4 - 6.4) MM8: OR 7.4 (95% CI 3.3 - 16.6) 10 point difference MM5: OR 3.4 (95% CI 3.3 - 3.5) MM8: OR 3.5 (95% CI 3.4 - 3.6)	III
Anton et al., 2002[104]	CS	Dental hygienists	8/81	OR 1.43 (95% CI 1.17 - 1.75), AOR 1.58 (95% CI 1.18 - 2.12)	III
Raman et al., 2012[101]	CS	Office workers	88/382	Obese (vs normal): OR 2.2 (95% CI 1.2 - 4.1), AOR 3.7 (95% CI 1.5 - 9.6)	III

Table 2.8 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Decreased risk					
Fung et al., 2007[128]	CC	Orthopaedic patients	166/111	<18.5: OR 0.214 (95% CI 0.065 - 0.702)	III
No effect on risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	Per unit increase: Univariate HR 1.073 (95% CI 1.03 - 1.12), p=0.003	I
Bonfiglioli et al., 2013[94]	PC	Industrial and service worker groups	84/2837	Univariate IRR 1.10 (9% CI 1.07 - 1.13) Multivariate IRR 1.09 (95% CI 1.04 - 1.14)	I
Frost et al., 1998[79]	RC	Active workers (slaughterhouse & chemical)	81/1060	25-30 Either hand: AOR 1.00 (95% CI 0.53 - 1.91) Dominant hand: AOR 1.48 (95% CI 0.61 - 3.60) Non-dominant hand: AOR 0.75 (95% CI 0.34 - 1.66) ≥30 Either hand: AOR 1.42 (95% CI 0.55 - 3.67) Dominant hand: AOR 2.13 (95% CI 0.63 - 7.12) Non-dominant hand: AOR 0.63 (95% CI 0.14 - 2.81)	II
Cartwright et al., 2013[95]	PC	Latinos in Manual labour occupations	36/173	AOR 1.02 (95% CI 0.94 - 1.10), p=0.713	II
Gorsche et al., 1999[75]	PC	Industrial workers	140/525	RR 1.006 (95% CI 0.921 - 1.099), p=0.888 Adjusted RR 1.016 (95% CI 0.922 - 1.119), p=0.748	II
Roquelaure et al,. 2001[96]	PC	Blue-collar workers	34/100	>27: OR 2.2 (95% CI 0.6 - 8.5)	II
Gell et al., 2005[134]	PC	Industrial & clerical workers	29	>27: OR 2.29 (95% CI 0.9 - 5.9)	II
Werner et al., 2005[114]	PC	Assembly workers	20/169	Per 5-point increase: OR 2.47 (95% CI 1.03 - 5.93), p=0.04	II
Violante et al., 2007[78]	PC	Working population	153/1939	Overweight, slim wrist (vs normal weight, slim wrist): OR 1.0 (95% CI 0.5 - 2.2), AOR 1.5 (95% CI 0.7 - 3.4) Normal weight, robust wrist: OR 1.1 (95% CI 0.7 - 1.7), AOR 1.1 (95% CI 0.7 - 1.7) Overweight, robust wrist: 1.3 (95% CI 0.9 - 2.0), AOR 1.4 (95% CI 0.9 - 2.2)	II
Nathan et al., 2002[76]	PC	Industrial workers	34/222	21.59-23.67 (Quintile 2): Univariate OR 2.52 (95% CI 0.62 - 10.35), p=0.200; Multivariate OR 3.01 (95% CI 0.65 - 13.80), p=0.15 23.68-25.85 (Quintile 3): Univariate OR 2.37 (95% CI 0.56 - 10.04), p=0.240; Multivariate OR 2.40 (95% CI 0.51 - 11.24), p=0.26 25.86 - 28.23 (Quintile 4): Univariate OR 3.55 (95% CI 0.91 - 13.96), p=0.07; Multivariate OR 4.45 (95% CI 0.98 - 20.15) ≥28.24 (Quintile 5): Univariate OR 4.02 (95% CI 1.02 - 15.87), p=0.040; Multivariate OR 6.39 (95% CI 1.39 - 29.34), p=0.02	II

Table 2.8 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Leclerc et al., 2001[108]	PC	Workers in Manual labour occupations (repetitive work)	131/467	Female, increased BMI: OR 2.38 (95% CI 1.04 - 5.47)	II
Fung et al., 2007[128]	CC	Orthopaedic patients	166/111	23-25: OR 0.901 (95% CI 0.475 - 1.710) 25-30: OR 1.815 (95% CI 0.925 - 3.559) >30: OR 0.811 (95% CI 0.340 - 1.931)	III
Nordstrom et al., 1997[129]	CC	General population	206/211	Normal: AOR 0.63 (95% CI 0.28 - 1.45) Overweight: AOR 1.29 (95% CI 0.53 - 3.14) Obese: AOR 1.86 (95% CI 0.72 - 4.80), Overall p<0.001 Increasing BMI (working population): Multivariate OR 1.06 (95% CI 1.02 - 1.11)	III
Mattioli et al., 2009[115]	CC	General population	191/286	25-29: Univariate OR 1.6 (95% CI 1.0 - 2.4), Multivariate OR 1.4 (95% CI 0.9 - 2.4)	III
McCabe et al., 2011[130]	CC	Hospital patients	68/138	28.1 vs 32.8: OR 1.11 (95% CI 1.00 - 1.23), p=0.048	III
Geoghegan et al., 2004[125]	CC	General practice patients	3391/13564	<18.5: Univariate OR 0.69 (95% CI 0.44 - 1.09), Multivariate OR 0.64 (95% CI 0.40 - 1.01); Operated CTS: AOR 0.74 (95% CI 0.28 - 1.94)	III
Boz et al., 2004[131]	CC	Hospital patients & relatives/friends	194/194	Female: OR 1.120 (95% CI 1.048 - 1.198), p=0.001 Male: OR 1.257 (95% CI 1.073 - 1.471), p=0.005	III
Maghsoudipour et al., 2008[99]	CS	Industrial workers	47/348	Multivariate OR 0.94 (95% CI 0.86 - 1.04), p=0.27	III
Leclerc et al., 1998[109]	CS	Workers in Manual labour occupations (repetitive work)	151/1059	≥31 - Model 1: OR 1.91 (95% CI 1.09 - 3.37)	III
Burt et al., 2011[113]	CS	Working population with low, medium or high level of hand activity	57/420	Quantitative model ≥30 versus <30 if exerts/min cat=1: OR 0.77 (95% CI 0.24 - 2.48) ≥30 versus <30 if exerts/min cat=2: OR 1.60 (95% CI 0.52 - 5.00) ≥30 versus <30 if exerts/min cat=3: OR 2.26 (95% CI 1.01 - 5.10) Ratings-based model BMI 18-61: OR 1.07 (95% CI 1.03 - 1.11)	III
Bland, 2005[89]	CS	EEG Patients	2408/1747	Univariate OR 1.052 (95% CI 1.039 - 1.065), p<0.001	III
Shiri et al., 2011[123]	CS	General population	79/6175	Underweight: AOR 1.9 (95% CI 0.2 - 15.1)	III

Table 2.8 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Cartwright et al., 2012[102]	CS	Latinos in Manual labour occupations	215/298 Possible 34/479 Definite	Increasing BMI: AOR 1.08 (95% CI 1.05 - 1.12), p<0.001; Multivariate AOR 1.04, p=0.0842	III
Kouyoumdjian et al., 2002[132]	CC	Hospital patients	210/320	Per unit increase: OR 1.11 (95% CI 1.05 - 1.16), p<0.001	III
Rigouin et al., 2013[98]	CS	Working population	156/3554	Per unit increase Male: OR 1.10 (95% CI 1.02 - 1.17), p=0.009 Female: OR 1.04 (95% CI 0.99 - 1.09), p=0.108	III
Raman et al., 2012[101]	CS	Office workers	88/382	Overweight (vs normal): OR 1.0 (95% CI 0.5 - 1.7), AOR 0.8 (95% CI 0.3 - 1.9)	III
Eleftheriou et al., 2012[106]	CS	Computer workers	A: 51/410[1] B: 167/294[2]	Case def A, ≥25: Univariate RR 1.22 (95% CI 0.72 - 2.06) Case def B, ≥25: Univariate RR 1.07 (95% CI 0.75 - 1.52)	III
Wieslander et al., 1989[127]	CC	Hospital patients	38/76	Group 1 - 10% above reference weight: OR 2.0 (95% CI 0.8 - 4.8)	III
Hakim et al., 2002[24]	CC	Female-female twin pairs	520/3154	21.1-23: OR 0.94 (95% CI 0.71 - 1.25), AOR 0.91 (95% CI 0.69 - 1.22) 23.1-25: OR 0.91 (95% CI 0.66 - 1.24), AOR 0.89 (95% CI 0.65 - 1.23) 25.1-28: OR 0.86 (95% CI 0.60 - 1.22), AOR 0.84 (95% CI 0.59 - 1.21) ≥28.1: OR 0.85 (95% CI 0.58 - 1.24), AOR 0.85 (95% CI 0.57 - 1.23)	III
Ali et al., 2006[27]	CS	Computer workers	85/563	≥25: OR 1.0 (95% CI 0.6 - 1.6), p=1.000	III
Mondelli et al., 2006[90]	CS	Female floor cleaners	70/75	>25: OR 1.79 (95% CI 0.85 - 3.78), Multivariate OR 1.3 (95% CI 0.68 - 4.44)	III
Awada et al., 1998[126]	CC	Hospital patients	100/100	Obesity (men): OR 1.44 (95% CI 0.51 - 4.06)	III
De Krom et al., 1990[116]	CC	General population	156/473	Risk Ratio 1.09 (95% CI 1.04 - 1.14)	III
Babski-Reeves & Crumpton-Young, 2003[133]	CS	Workers at a fish processing facility	33/73 hands	Logistic regression OR 1.02 (95% CI 1.00 - 1.03), p=0.025	III

CS, cross sectional; PC, prospective cohort; RC, retrospective cohort; AOR, adjusted odds ratio; CI, confidence interval; OR, odds ratio; PR, prevalence ratio; IRR, incidence rate ratio; RR, relative risk

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

[1] Case definition A: Participants with personal medical history of CTS or surgery due to CTS

[2] Case definition B: Case definition A and participants identified through clinical examination (score ≥12)

2.3.1.7 Hand/Wrist structure/dimension

Only seven level III studies have considered hand/wrist structure or dimensions as an intrinsic risk factor for CTS (Table 2.9). Two of these found an increase in risk with a difference in hand/wrist structure, specifically a wrist index (wrist depth/wrist width) of greater than 0.7[81] or an increase in digit index (digit 3 length x 100/hand length) or shape index (hand width x 100/hand length)[131]. Four studies found no effect on risk[105,112,131,132]. Only one study found that an increased wrist circumference led to a decreased risk of developing CTS[112].

Many authors have argued that the structure of the wrist, in particular any parameter that will result in the narrowing of the carpal tunnel which reduces the available space for the median and flexor tendons, will increase the risk of CTS[77]. However, there is a lack of high quality studies providing evidence for this hypothesis. In a case control study wrist circumference was found to predict a decreased risk of CTS whereas wrist ratio (wrist depth divided by wrist width) had no effect on risk[112].

Considering the low quality and conflicting results of the studies, it is clear that more research in the form of high quality, prospective studies need to be performed to get a better idea of whether the effect of hand/wrist shape and dimensions has an influence the risk of developing CTS. Because of the low quality and small number of studies in this category, wrist/hand structure or dimensions were assigned a low level of certainty.

Table 2.9 Summary of all the studies that have investigated hand/wrist structure/dimension as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Armstrong et al., 2008[81]	CS	Newly hired manual workers	131/940	Wrist index ≥0.7: Logistic regression OR 2.53 (95% CI 1.70 - 3.78)	III
Boz et al., 2004[131]	CC	Hospital patients & relatives/friends	194/194	Female Digit index (Per 1 unit increase): OR 1.375 (95% CI 1.164 - 1.624), p<0.001 Shape index: OR 1.362 (95% CI 1.207 - 1.537), p<0.001	III
Decreased risk					
Moghtaderi et al., 2005[112]	CC	Hospital patients and relatives	128/109	Wrist circumference: OR 0.819 (95% CI 0.763 - 0.877), p=0.000	III
No effect on risk					
Latko et al., 1999[105]	CS	Workers at manufacturing facitlity	19/333	Wrist ratio ≥0.73: OR 2.53 (95% CI 0.97 - 6.57)	III
Moghtaderi et al., 2005[112]	CC	Hospital patients and relatives	128/109	Wrist ratio: OR 1.12 (95% CI 1.03 - 1.21), p=0.008	III
Kouyoumdjian et al., 2002[132]	CC	Hospital patients	210/320	Per 0.01 unit increase: OR 1.11 (95% CI 1.07 - 1.16), p<0.001	III
Boz et al., 2004[131]	CC	Hospital patients & relatives/friends	194/194	Female Wrist index (per 0.01 unit increase): OR 1.157 (95% CI 1.099 - 1.219), p<0.001 Hand length/body height ratio (per 1 unit increase): OR 1.246 (95% CI 0.650 - 2.287), p=0.508 Male Wrist index: OR 1.257 (95% CI 1.073 - 1.471), p=0.266 Shape index: OR 1.041 (95% CI 0.878 - 1.233), p=0.646 Digit index: OR 1.177 (95% CI 0.880 - 1.574), p=0.272 Hand length/body height ratio: OR 1.069 (95% CI 0.381 - 2.998), p=0.99	III

CS, cross sectional; OR, odds ratio; CI, confidence interval

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

2.3.1.8 Familial CTS

Although a family history and genetic factors are often mentioned as risk factors[135], only eight studies have considered family history as a potential modifier of risk for developing CTS (Table 2.10). One case-control study that investigated 520 female twin pairs found a decreased risk of CTS with regards to a genetic component or heritability[24].

Only one level II study found familial factors to influence CTS risk, the number of siblings with this condition influenced the risk to develop CTS significantly[136]. Three other level III studies also found an increase in risk if a family member suffers from CTS[100,115,129]. Two prospective studies, however, found a positive family history to have no effect on risk of CTS[78,93]. Similarly, two level III studies also found no effect of family history on CTS risk[89,115].

Even though there are a few studies that investigated the effect of familial CTS, this does not give any information about potential specific genetic variants that could potentially be involved in the aetiology of CTS, yet 'genetic make-up' is often referred to as playing a vital role in this condition[8]. Considering the limited information available on this specific risk factor, it was assigned a low level of certainty. Future research should aim to investigate genetic elements rather than only familial predisposition.

Table 2.10 Summary of all the studies that have investigated familial factors as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Hemminki et al., 2007[136]	RC	Swedish database	2250/27436	One sibling with CTS: SIR 4.08 (95% CI 2.07 - 7.84) Both siblings with CTS: SIR 6.18 (95% CI 2.88 - 12.73)	II
Bonfiglioli et al., 2007[100]	CS	Female supermarket cashiers	26/324	Family history: Multivariate OR 3.60 (95% CI 1.20 - 10.75), p=0.22	III
Nordstrom et al., 1997[129]	CC	General population	206/211	Family history: AOR 2.09 (95% CI 1.28 - 3.41), p=0.003; Multivariate AOR 2.00 (95% CI 1.18 - 3.37), p=0.09	III
Mattioli et al., 2009[115]	CC	General population	191/286	Sibling: OR 8.1 (95% CI 2.3 - 29.2), Multivariate OR 6.6 (95% CI 1.5 - 29.4)	III
Decreased risk					
Hakim et al., 2002[24]	CC	Female-female twin pairs	520/3154	Heritability/Genetic component: OR 0.46 (95% CI 0.34 - 0.58) AOR (1): OR 0.46 (95% CI 0.32 - 0.59) AOR (2): OR 0.47 (95% CI 0.34 - 0.59)	III
No effect on risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	Family history: Univariate HR 2.1 (95% CI 1.04 - 4.35), p=0.04	I
Violante et al., 2007[78]	PC	Working population	153/1939	Family history: OR 1.4 (95% CI 0.9 - 2.4), Multivariate OR 1.2 (95% CI 0.7 - 2.0)	II
Mattioli et al., 2009[115]	CC	General population	191/286	Parent: OR 1.1 (95% CI 0.6 - 2.2), Multivariate OR 1.3 (95% CI 0.5 - 3.1)	III
Bland, 2005[89]	CS	EEG Patients	2408/1747	Family history: OR 1.257 (95% CI 1.038 - 1.523), p=0.019, Multivariate OR 1.11 (95% CI 0.91 - 1.34)	III

RC. Retrospective cohort; SIR, standardized incidence ratio; CS, cross sectional; OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio; PC, prospective cohort; HR, hazard ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

2.3.2 Behavioural and Social

The following section will review the role of six extrinsic behavioural and social factors, namely smoking, alcohol use, socio-demographic, geographical location, hobbies and exercise as risk factors for CTS.

2.3.2.1 Smoking

Out of the 20 studies that investigated the influence of smoking on the risk of developing CTS (Table 2.11), only one level III, cross-sectional study found an increased risk in both the unadjusted and adjusted analyses (OR 7.29 (95% CI 3.49 - 15.23), p<0.001, Multivariate OR 4.68 (95% CI 1.80 - 11.80), p=0.001)[99]. Two studies reported a decreased risk for smokers[89,119]. However, upon multivariate analyses, the effect of smoking in one of the two studies was lost[89].

Eighteen studies found no association between smoking and risk of CTS[27,71,74,76,78,79,91,93,96,101,103,106,115,117,119,123,125,127]. This included previous smokers[74,93,115,123], current smokers[74,93,96,103,115,127] and individuals who have smoked at some point in their lives, termed “ever smoked” or “ever smokers”[27,71,76,78,79,91,106,117,125]. The number of packs per year also made no difference to the risk of CTS[103,123]. Violante *et al*. found no effect of ever smoking on risk of CTS in their univariate analysis, but upon multivariate analysis they found an increase in risk[78], but because of the lack of association in the initial analyses, this result should be interpreted with caution.

The low level of evidence and small number of studies (n=3) that found an effect of smoking on CTS risk, together with the fact that multivariate analyses further decreased this to only two studies compared to the large amount of studies finding no evidence of smoking being a risk factor were considered contradictory. Smoking was therefore assigned a low level of certainty to be a risk factor of CTS.

Table 2.11 Summary of all the studies that have investigated smoking as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Maghsoudipour et al., 2008[99]	CS	Industrial workers	47/348	Univariate OR 7.29 (95% CI 3.49 - 15.23), p=0.00, Multivariate OR 4.68 (95% CI 1.80 - 11.80), p=0.001	III
Decreased risk					
Bland, 2005[89]	CS	EEG Patients	2408/1747	OR 0.549 (95% CI 0.477 - 0.632), p<0.001, Multivariate OR 1.11 (95% CI 0.94 - 1.29)	III
Coggon et al., 2013[119]	CC	General population	475/799	Current smoker: AOR 0.6 (95% CI 0.5 - 0.8); Multivariate OR 0.6 (95% CI 0.4 - 0.8)	III
No effect on risk					
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	Current smoker: IRR 1.09 (95% CI 0.78 - 1.51) Previous smoker: IRR 1.05 (95% CI 0.70 - 1.54)	I
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	Current smoker: Univariate HR 1.3 (95% CI 0.68 - 2.65) Previous smoker: Univariate HR 1.3 (95% CI 0.67 - 2.63)	I
Nathan et al., 2002[76]	PC	Industrial workers	34/222	Univariate OR 1.74 (95% CI 0.84 - 3.60), p=0.130, Multivariate OR 2.54 (95% CI 1.09 - 5.89), p=0.03	II
Violante et al., 2007[78]	PC	Working population	153/1939	Ever vs never: OR 1.3 (95% CI 0.9 - 1.8), Multivariate OR 1.7 (1.2 - 2.4)	II
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	Current smoker: OR 0.5 (95% CI 0.1 - 2.2)	II
Sestak et al., 2009[91]	PC	Postmenopausal women received adjuvant therapy for breast cancer	103/6083	Ever: Univariate OR 1.28 (95% CI 0.86 - 1.89), p=0.22	II
Frost et al., 1998[79]	RC	Active workers (slaughterhouse & chemical)	81/1060	Ever vs never, either hand: AOR 0.65 (95% CI 0.34 - 1.24); Dominant hand: AOR 0.47 (95% CI 0.21 - 1.08); Non-dominant hand: AOR 0.63 (95% CI 0.29 - 1.37)	II
Tanaka et al. 1997[71]	CS	General population	145/29929	Ever vs never: AOR 1.64 (95% CI 1.03 - 2.62)	III
Eleftheriou et al., 2012[106]	CS	Computer workers	A: 51/410[1] B: 167/294[2]	Case def A - Ever vs non: Univariate RR 1.74 (95% CI 1.02 - 2.93), p=0.003; Logistic regression OR 1.99 (95% CI 1.01 - 3.54), p=0.043 Case def B - Ever vs non: Univariate RR 1.22 (95% CI 0.96 - 1.55)	III
Conlon & Rempel, 2005[103]	CS	Engineers	25/177	Current smoker: Univariate OR 1.25 (95% CI 0.14 - 11.2), p=0.842 Pack-year: Univariate OR 1.00 (95% CI 0.89 - 1.12), p=0.971	III
Mattioli et al., 2009[115]	CC	General population	191/286	Current smoker: OR 1.1 (95% CI 0.7 - 1.7) Previous smoker: OR 0.7 (95% CI 0.4 - 1.1)	III
Geoghegan et al., 2004[125]	CC	General practice patients	3391/13564	Smoker: OR 1.02 (95% CI 0.92 - 1.12), Multivariate OR 1.03 (95% CI 0.93 - 1.13); Operated CTS: AOR 1.04 (95% CI 0.86 - 1.26)	III

Table 2.11 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Raman et al., 2012[101]	CS	Office workers	88/382	Non-smoker: OR 1.0 (95% CI 0.5 - 1.8)	III
Wieslander et al., 1989[127]	CC	Hospital patients	38/76	Current smoker: OR 1.5 (95% CI 0.7 - 3.5)	III
Ali et al., 2006[27]	CS	Computer workers	85/563	Smoker: OR 1.6 (95% CI 1.0 - 2.6), p=0.06	III
Ferry et al., 2000[117]	CC	Female survey responders	1264/1264	Smoker: OR 1.05 (95% CI 0.89 - 1.23)	III
Coggon et al., 2013[119]	CC	General population	475/799	Ex-smoker: AOR 1.1 (95% 0.8 - 1.4); Multivariate OR 1.1 (95% CI 0.8 - 1.4)	III
Shiri et al., 2011[123]	CS	General population	79/6175	Previous smoker: AOR 1.0 (95% CI 0.5 - 1.8); Occational smoker: AOR 2.4 (95% CI 0.9 - 6.3) Current smoker, <10 pack-years: AOR 1.4 (95% CI 0.5 - 3.7); 10-20 pack-years: OR 1.8 (95% CI 0.6 - 4.7); >20 pack-years: 1.5 (95% CI 0.6 - 3.7)	III

CS, cross sectional; OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio; PC, prospective cohort; IRR, incidence rate ratio; HR, hazard ratio; RC, retrospective cohort; CC, case control

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

[1] Case definition A: Participants with personal medical history of CTS or surgery due to CTS

[2] Case definition B: Case definition A and participants identified through clinical examination (score ≥12)

2.3.2.2 Alcohol use

Out of a total of five studies that investigated alcohol use as a possible risk factor for CTS (Table 2.12), only one cross-sectional study found an increase in risk with increased alcohol consumption[27]. The other four studies found no effect of various types of alcohol use (light, moderate, excessive) on CTS risk[78,93,115,123] and this factor was therefore assigned a low level of certainty.

Table 2.12 Summary of all the studies that have investigated alcohol use as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Ali et al., 2006[27]	CS	Computer workers	85/563	Increase - OR 1.7 (95% CI 1.1 - 2.8), p=0.04	III
No effect on risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	HR 1.3 (95% CI 0.66 - 2.55), p=0.45	I
Violante et al., 2007[78]	PC	Working population	153/1939	1-2 drinks p/w: OR 0.7 (95% CI 0.4 - 1.0), Multivariate OR 0.7 (95% CI 0.4 - 1.2) 2-4 drinks p/w: OR 0.1 (95% CI 0.0 - 0.5), Multivariate OR 0.2 (95% CI 0.1 - 1.0) 1-2 drinks p/d: OR 1.0 (95% CI 0.6 - 1.4), Multivariate OR 1.1 (95% CI 0.7 - 1.8) 3-4 drinks p/d: OR 0.3 (95% CI 0.1 - 0.7), Multivariate OR 0.6 (95% CI 0.2 - 1.4) >4 drinks p/d: OR 0.8 (95% CI 0.3 - 2.3), Multivariate OR 2.3 (95% CI 0.7 - 7.2)	II
Mattioli et al., 2009[115]	CC	General population	191/286	1-2 drinks p/w: OR 0.8 (95% CI 0.5 - 1.4), Multivariate OR 0.8 (95% CI 0.4 - 1.6) 3-6 drinks p/w: OR 0.5 (95% CI 0.2 - 1.1), Multivariate OR 0.4 (95% CI 0.1 - 1.1) 1-2 drinks p/d: OR 0.9 (95% CI 0.5 - 1.4), Multivariate OR 0.8 (95% CI 0.5 - 1.5) >3 drinks p/d: OR 0.6 (95% CI 0.3 - 1.1), Multivariate OR 0.7 (95% CI 0.3 - 1.6)	III
Shiri et al., 2011[123]	CS	General population	79/6175	Light: AOR 0.9 (95% CI 0.3 - 2.2) Moderate: AOR 0.8 (95% CI 0.3 - 2.0) Excessive: AOR 1.3 (95% CI 0.5 - 3.3)	III

CS, cross sectional; OR, odds ratio; CI, confidence interval; PC, prospective cohort; HR, hazard ratio; RC, retrospective cohort; CC, case control

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

2.3.2.3 Socio-demographic factors

Twelve studies investigated educational and social factors as possibly playing a role in the aetiology of CTS (Table 2.13). Only one level III study that investigated 15 802 participants found an increase in risk, OR 1.38 (95% CI 1.32 - 1.45), with a higher income level[80] but this effect was lost when a multivariate analysis was performed, Multivariate OR 1.15 (95% CI 1.09 - 1.22). On the other hand, two level III studies found a decreased risk of CTS with a higher education level[99,115]. According to the authors, a possible explanation of this protective effect could be that more educated workers only start manual labour at a later

stage in life[99], therefore decreasing the exposure of occupational activities that may contribute to development of CTS.

There was only one prospective study that considered educational level as risk factors and found it to have no effect on CTS risk[74]. Eight level III studies also found no effect on risk with an difference in education[71,100,104,120], income[71,129], urbanization[80], social class[117] or home/leisure activity[24].

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Considering the low number of studies considering education, income and other socio-economic variables, they were all grouped together under an umbrella of educational/social/income factors. These are all proxies for broad socio-occupational groupings[115] and future research should aim to rather investigate these broad groupings than several, smaller individual risk factors. Educational/social factors were, as a result, assigned a low level of certainty.

2.3.2.4 Geographical location

Geographical location as risk factor was considered in only two studies (Table 2.14) of which both level II found that living in the USA leads to increased risk compared to the UK, Southern Hemisphere and Hong Kong, which did not alter the risk of CTS[91,92].

Owing to the limited number of studies investigating this variable, geographical location was assigned a low level of certainty as a modifier of CTS risk.

Table 2.13 Summary of all the studies that have investigated socio-demographic factors as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Tseng et al., 2012[80]	CC	General population	15802/31604	Higher income: OR 1.38 (95% CI 1.32 - 1.45), Multivariate OR 1.15 (95% CI 1.09 - 1.22)	III
Decreased risk					
Mattioli et al., 2009[115]	CC	General population	191/286	High school diploma or above: OR 0.2 (95% CI 0.2 - 0.4)	III
Maghsoudipour et al., 2008[99]	CS	Industrial workers	47/348	Multivariate OR 0.19 (95% CI 0.05 - 0.66), p=0.009	III
No effect on risk					
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	High school graduate or above: IRR 0.66 (95% CI 0.46 - 0.99)	I
Bonfiglioli et al., 2007[100]	CS	Female supermarket cashiers	26/324	>8 years: Multivariate OR 2.15 (95% CI 0.75 - 6.17), p=0.155	III
Anton et al., 2002[104]	CS	Dental hygienists	8/81	Education (years): OR 1.22 (95% CI 0.71 - 2.10)	III
Tanaka et al. 1997[71]	CS	General population	145/29929	Education (>12 vs ≤12 years): OR 1.17 (95% CI 0.77 - 1.78) Income (≥$20 000 vs <$20 000): OR 1.51 (95% CI 0.95 - 2.41)	III
Ferry et al., 2000[117]	CC	Female survey responders	1264/1264	Social class (III vs I & II): OR 1.05 (95% CI 0.84 - 1.31) IV: OR 1.23 (95% CI 1.02 - 1.50)	III
Hakim et al., 2002[24]	CC	Female-female twin pairs	520/3154	Home (low vs high level): OR 1.13 (95% CI 0.83 - 1.64), AOR 1.21 (95% CI 0.95 - 1.55) Leisure (low vs high level): OR 1.04 (95% CI 0.70 - 1.55), AOR 1.00 (95% CI 0.80 - 1.26)	III
Tseng et al., 2012[80]	CC	General population	15802/31604	Urbanization - moderate: Univariate OR 0.97 (95% CI 0.90 - 1.04), Multivariate OR 1.03 (95% CI 0.95 - 1.12) High: Univariate OR 1.08 (95% CI 1.02 - 1.15), Multivariate OR 1.07 (95% CI 0.98 - 1.14)	III
Yagev et al., 2007[120]	CC	Hospital patients	127/102	Year: Multivariate OR 0.82 (95% CI 0.74 - 0.92), p=0.001	III
Nordstrom et al., 1997[129]	CC	General population	206/211	Income below poverty level: AOR 1.72 (95% CI 0.82 - 3.59), p=0.140	III

CC, case control; CS, cross sectional; OR, odds ratio; CI, confidence interval; PC, prospective cohort; IRR, incidence rate ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

Table 2.14 Summary of all the studies that have investigated geographical location as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Sestak et al., 2009[91]	PC	Postmenopausal women received adjuvant therapy for breast cancer	103/6083	North America: OR 2.20 (95% CI 1.35 - 3.58), p=0.002; Multivariate OR 1.98 (95% CI 1.18 - 3.33), p=0.01	II
Mieog et al., 2012[92]	RC	Female breast cancer patients	79/4578	USA: OR 2.54 (99% CI 1.10 - 5.90), p=0.0042; Multivariate OR 1.27 (99% CI 0.48 - 3.38), p0.53	II
No effect on risk					
Sestak et al., 2009[91]	PC	Postmenopausal women received adjuvant therapy for breast cancer	103/6083	UK: OR 1.27 (95% CI 0.75 - 2.15), p=0.38; Multivariate OR 1.26 (95% CI 0.72 - 2.20), p=0.42	II
Mieog et al., 2012[92]	RC	Female breast cancer patients	79/4578	UK: OR 1.64 (99% CI 1.64 (95% CI 0.73 - 3.70), p=0.12; Multivariate OR 1.03 (99% CI 0.43 - 2.48), p=0.92 Central and eastern Europe: OR 0.07 (99% CI 0.01 - 1.01), p=0.010; Multivariate OR 0.09 (99% CI 0.01 - 1.31), p=0.021 Southern hemisphere & Hong Kong: OR 1.98 (99% CI 0.68 - 5.79), p=0.10; Multivariate OR 2.18 (99% CI 0.70 - 6.78), p=0.76	II

PC, prospective cohort; RC, retrospective cohort; CC, case control; CS, cross sectional; OR, odds ratio; CI, confidence interval;

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

2.3.2.5 Hobbies

Four studies investigated different hobbies or recreational activities as possible modifier of CTS risk (Table 2.15). Only one prospective study found an increase in risk for knitting and gardening, both activities involving repetitive hand movement[93] whilst the same study found no effect on risk for computer work and maintenance hobbies. Only one other level III study found knitting to increase risk of CTS[100]. Another two prospective studies and one cross sectional study found no effect of hobbies in general on risk of CTS[74,76,90].

Considering the low number of studies finding any effect of hobbies on CTS, this risk factor was assigned a low level of certainty.

Table 2.15 Summary of all the studies that have investigated hobbies as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	*Knitting* HR 2.5 (95% CI 1.26 - 4.87), p=0.009 *Gardening* HR 3.4 (95% CI 1.47 - 7.70), p=0.004	I
Bonfiglioli et al., 2007[100]	CS	Female supermarket cashiers	26/324	*Knitting* OR 2.0 (95% CI 0.68 - 5.87), p=0.206	III
No effect on risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	*Computer* HR 0.7 (95% CI 0.36 - 1.41), p=0.33 *Maintenance* HR 0.6 (95% CI 0.23 - 1.50), p=0.26	I
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	*Non-occupational hand intensive activity* IRR 0.66 (95% CI 0.47 - 0.94); Multivariate HR 0.58 (95% CI 0.41 - 0.82), p=0.00	I
Mondelli et al., 2006[90]	CS	Female floor cleaners	70/75	*Other* Univariate OR 1.54 (95% CI 0.79 - 3.03); Multivariate OR 1.73 (95% CI 0.75 - 0.98)	III

PC, prospective cohort; HR, hazard ratio; IRR, incidence rate ratio; CC, case control; CS, cross sectional; OR, odds ratio; CI, confidence interval;

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

2.3.2.6 Exercise

Exercise, as a risk factor for developing CTS, was investigated by eight studies (Table 2.16). There were no prospective studies that found altered risk of CTS with exercise. However, three level III studies found a decrease in risk with exercise, which included sport participation, any physical activity as well as frequency of exercise[101,106,129]. In contrast, three prospective studies found no effect on risk in workers who exercised by means of walking[93] , general avocational physical activity[76] or aerobic, non-hand intensive activity, for more than 3 hours per week[74]. Similarly, four level III studies found no effect of various forms and amounts of exercise per week on the risk of CTS. This included frequency of sport/exercise

participation, aerobic activity, exercise defined as causing shortness of breath as well as any physical activity[101,103,106,123].

Considering the lack of good quality, prospective studies; exercise as a modifier for risk of CTS was assigned a low level of certainty.

Table 2.16 Summary of all the studies that have investigated exercise as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Decreased risk					
Nordstrom et al., 1997[129]	CC	General population	206/211	3 months, 3 hours a week sport participation: AOR 0.55 (95% CI 0.35 - 0.87), p=0.01; Multivariate AOR 0.57 (95% CI 0.35 - 0.95), p=0.031	III
Raman et al., 2012[101]	CS	Office workers	88/382	≥2 hr/wk: OR 0.3 (95% CI 0.2 - 0.7)	III
Eleftheriou et al., 2012[106]	CS	Computer workers	A: 51/410[1] B: 167/294[2]	Case def A: Univariate RR 0.40 (95% CI 0.19 - 0.84); Logistic regression OR 0.38 (95% CI 0.16 - 0.87), p=0.023	III
No effect on risk					
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	≥3 hr/wk: IRR 0.99 (95% CI 0.65 - 1.50); Multivariate HR 0.82 (95% CI 0.55 - 1.22), p=0.32	I
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	*Walking* HR 1.9 (95% CI 0.98 - 3.84), p=0.06	I
Nathan et al., 2002[76]	PC	Industrial workers	34/222	*Avocational activity* *Univariate OR 0.83 (95% CI 0.57 - 1.21), p=0.330; Multivariate OR 1.37 (95% CI 0.84 - 2.24), p=0.210*	II
Conlon & Rempel, 2005[103]	CS	Engineers	25/177	Exercise frequency: Univariate OR 1.23 (95% CI 0.74 - 2.05) Aerobic activity (hr/week): Univariate OR 1.09 (95% CI 0.93 - 1.27) Upper extremity activity - hobbie/exercise (hr/week): Univariate OR 1.01 (95% CI 0.98 - 1.05)	III
Shiri et al., 2011[123]	CS	General population	79/6175	2-3 times a week: AOR 1.1 (95% CI 0.6-1.9) ≥4 times a week: AOR 1.2 (95% CI 0.6 - 2.0)	III
Raman et al., 2012[101]	CS	Office workers	88/382	1 hr/wk: OR 0.8 (95% CI 0.5 - 1.5)	III
Eleftheriou et al., 2012[106]	CS	Computer workers	A: 51/410[1] B: 167/294[2]	Case def B: Univariate RR 0.72 (95% CI 0.54 - 0.96); Logisitic regression: OR 0.72 (95% CI 0.44 - 1.20), p=0.217	III

PC, prospective cohort; IRR, incidence rate ratio; RC, retrospective cohort; CC, case control; CS, cross sectional; OR, odds ratio; CI, confidence interval;

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.
[1] Case definition A: Participants with personal medical history of CTS or surgery due to CTS
[2] Case definition B: Case definition A and participants identified through clinical examination (score ≥12)

2.3.3 Medication

The following section will review the role of six specific treatment(s)/medication use, namely corticosteroids, chemotherapy, contraceptive, hormone replacement therapy, radiotherapy as well as other medical treatments, as risk factors for CTS.

2.3.3.1 Corticosteroid use

Although generally considered as a risk factor for injury[44], only two level III studies reported on the use of corticosteroids to alter CTS risk (Table 2.17). Both level III studies reported an increase risk with the use of this medication[110,125] but owing to the low number of studies providing evidence towards this, corticosteroid use as risk factor for CTS could only be assigned a low level of certainty.

Table 2.17 Summary of all the studies that have investigated corticosteroid use as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Solomon et al., 1999[110]	CC	Members of medical aid programs	626/3618	OR 1.9 (95% CI 1.4 - 2.4), AOR 1.6 (95% CI 1.2 - 2.1)	III
Geoghegan et al., 2004[125]	CC	General practice patients	3391/13564	Univariate OR 1.64 (95% CI 1.39 - 1.93); Multivariate OR 1.07 (95% CI 0.90 - 1.27) Operated CTS: AOR 0.98 (95% CI 0.71 - 1.37)	III

CC, case control; OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio
[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

2.3.3.2 Chemotherapy

Two level II studies investigated the effect of chemotherapy on risk of CTS (Table 2.18). Mieog *et al*. (2012) found this treatment to have no effect on risk[92] whereas Sestak *et al*. (2009) initially found an increase in risk, but upon multivariate analysis found no effect[91]. Chemotherapy as risk factor for CTS was therefore assigned a low level of certainly, considering the lack of evidence of it being a true risk factor.

Table 2.18 Summary of all the studies that have investigated chemotherapy as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Sestak et al., 2009[91]	PC	Postmenopausal women received adjuvant therapy for breast cancer	103/6083	Univariate OR 1.73 (95% CI 1.14 - 2.62), p=0.01; Multivariate OR 1.36 (95% CI 0.85 - 2.14), p=0.200	II
No effect on risk					
Mieog et al., 2012[92]	RC	Female breast cancer patients	79/4578	OR 1.01 (99% CI 0.53 - 1.93), p=0.96	II

PC, prospective cohort; RC, retrospective cohort; OR, odds ratio; CI, confidence interval

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

2.3.3.3 Contraceptive use

Of the seven studies investigating contraceptive use as a modifier in the risk of CTS (Table 2.19), only two level III studies found use of contraceptives to be associated an increased risk[107,117]. The other investigations found current use, use in the past and number of years of contraceptive use to have no effect on risk[82,90,96,116,117,125]. Contraceptive use as risk factor for CTS was therefore assigned a low level of certainty.

Table 2.19 Summary of all the studies that have investigated contraceptive use as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Ferry et al., 2000[117]	CC	Female survey responders	1264/1264	Oral contraceptive use - former: OR 1.45 (95% CI 1.18 - 1.78)	III
Chiang et al., 1993[107]	CS	Fish-processing workers	30/177	OR 2.0 (95% CI 1.2 - 5.4)	III
No effect on risk					
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	OR 1.0 (95% CI 0.3 - 2.8)	II
Geoghegan et al., 2004[125]	CC	General practice patients	3391/13564	Combined Oral Contraceptive Pill: Univariate OR 1.12 (95% CI 0.97 - 1.29), Multivariate OR 0.82 (95% CI 0.71 - 0.95) Operated CTS: AOR 0.83 (95% CI 0.59 - 1.17)	III
Ferry et al., 2000[117]	CC	Female survey responders	1264/1264	Oral contraceptive use - current: OR 0.87 (95% CI 0.74 - 1.03) Months of oral contraceptive use - 1-48: OR 1.30 (95% CI 0.99 - 1.70); 49-108: OR 1.14 (95% CI 0.99 - 1.31); Months of contraceptive use - >108: OR 0.98 (95% CI 0.87 - 1.11)	III
De Krom et al., 1990[116]	CC	General population	156/473	Use during the past 5 years 1-4 years: Risk Ratio 0.7 (95% CI 0.3 - 1.6); ≥5: Risk Ratio 2.0 (95% CI 0.8 - 5.2)	III
Mondelli et al., 2006[90]	CS	Female floor cleaners	70/75	OR 1.61 (95% CI 0.70 - 3.68), Multivariate OR 1.52 (95% CI 0.58 - 4.04)	III
Morgenstern et al., 1991[82]	CS	Female grocery checkers	127/931	Multivariate OR 0.84 (95% CI 0.46 - 1.56), p=0.590	III

CC, case control; OR, odds ratio; CI, confidence interval; PC, prospective cohort; AOR, adjusted odds ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

2.3.3.4 Hormone replacement therapy

Although the main aspect of this section is focused on hormone replacement therapy (HRT) as risk factor for CTS, several other hormonal conditions have been added for simplicity purposes.

Ten studies considered various forms of hormonal influences on the risk of developing CTS (Table 2.20). Six of these found an increased risk for CTS[91,92,110,116,117,125], of which two level II studies found an increased risk in participant currently or previously using HRT[91,92]. However, upon multiple regression or adjustment, the effect of the therapy disappeared in both studies. Of the studies that found hormonal factors to have no effect on risk, two prospective studies considered hormone receptor status, which is defined as the receptor status of estrogen and progesterone (i.e. positive or negative) and used in the diagnosis and treatment of breast cancer,[91] and hormone use[76] whereas one retrospective study investigated time since menopause and previous oophorectomy[92].

Four case control (level III) studies also found an increased risk of CTS with various hormonal influences[110,116,117,125]. Five level III studies further investigated various hormonal influences but found none to have a modifying effect on CTS [24,82,99,116,117].

The lack of studies with a high level of evidence lead to HRT and other hormonal factors being assigned a low level of certainty, however, future research should aim to investigate all the mentioned risk factors individually to assess their potential effect on CTS risk.

Table 2.20 Summary of all the studies that have investigated hormone replacement therapy and other hormonal factors as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Sestak et al., 2009[91]	PC	Postmenopausal women received adjuvant therapy for breast cancer	103/6083	HRT: Univariate OR 1.72 (95% CI 1.16 - 2.54), p=0.007; Multivariate OR 1.31 (95% CI 0.86 - 1.99), p=0.21	II
Mieog et al., 2012[92]	RC	Female breast cancer patients	79/4578	Previous use of HRT: OR 2.49 (99% CI 1.33 - 4.67), p<0.001; AOR 1.28 (99% CI 0.63 - 2.59), p=0.370	II
De Krom et al., 1990[116]	CC	General population	156/473	Hysterectomy with oophorectomy: Risk Ratio 2.0 (95% CI 1.1 - 3.6) Years since last menstrual period <1: Risk Ratio 6.5 (95% CI 1.9 - 21.8)	III
Ferry et al., 2000[117]	CC	Female survey responders	1264/1264	Any menstrual disorder: OR 1.36 (95% CI 1.11 - 1.66) Pre-menstrual tension: OR 1.53 (95% CI 1.17 - 2.00)	III
Geoghegan et al., 2004[125]	CC	General practice patients	3391/13564	HRT: Univariate OR 1.39 (95% CI 1.23 - 1.57), Multivariate OR 0.95 (95% CI 0.84 - 1.08) Operated CTS - HRT: OR 0.86 (95% CI 0.67 - 1.09) Thyroxine: Univariate OR 1.82 (95% CI 1.46 - 2.27), Multivariate OR 1.36 (95% CI 1.08 - 1.70); Operated CTS: AOR 1.78 (95% CI 1.19 - 2.65)	III
Solomon et al., 1999[110]	CC	Members of medical aid programs	626/3618	Estrogen replacement use: OR 2.0 (95% CI 1.2 - 3.6), AOR 1.8 (95% CI 1.0 - 3.2)	III
No effect on risk					
Sestak et al., 2009[91]	PC	Postmenopausal women received adjuvant therapy for breast cancer	103/6083	Hormone receptor status - negative: Univariate OR 0.86 (95% CI 0.42 - 1.78), p=0.69 Hormone receptor status - unknown: Univariate OR 0.46 (95% CI 0.17 - 1.26), p=0.13	II
Nathan et al., 2002[76]	PC	Industrial workers	34/222	Hormone use: Univariate OR 0.58 (95% CI 0.07 - 4.65), p=0.600; Multivariate OR 1.43 (95% CI 0.10 - 19.64), p=0.790	II
Mieog et al., 2012[92]	RC	Female breast cancer patients	79/4578	Time since menopause <5 years: OR 1.97 (99% CI 0.91 - 4.25), p=0.024; Time since menopause 5-9 years: OR 1.47 (99% CI 0.69 - 3.12), p=0.190 Previous oophorectomy: OR 1.08 (99% CI 0.38 - 3.02), p=0.850	II

Table 2.20 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Hakim et al., 2002[24]	CC	Female-female twin pairs	520/3154	Peri-menopausal: OR 1.46 (95% CI 1.06 - 2.00); AOR 1.53 (95% CI 1.01 - 2.32) Post-menopausal: OR 1.20 (95% CI 0.93 - 1.55); AOR 1.43 (95% CI 0.89 - 2.29); Hysterectomy: OR 1.33 (95% CI 1.06 - 1.65), OR (after accounting for menopause): 1.12 (95% CI 0.70 - 1.75); AOR 1.20 (95% CI 0.89 - 1.63) HRT: OR 0.87 (95% CI 0.67 - 1.13); AOR 0.85 (95% CI 0.62 - 1.16) Thyroxine replacement therapy: OR 1.15 (95% CI 0.78 - 1.69); AOR 1.13 (95% CI 0.72 - 1.78)	III
De Krom et al., 1990[116]	CC	General population	156/473	Age at menopause <45: Risk Ratio 1.3 (95% CI 0.5 - 3.6); 45-50: Risk Ratio 0.8 (95% CI 0.3 - 2.1) Age at menopause (women >59 years) <45: Risk Ratio 0.9 (95% CI 0.2 - 3.8) 45-50: Risk Ratio 1.1 (95% CI 0.3 - 3.6) Years since last menstrual period 2-5: Risk Ratio 2.4 (95% CI 0.8 - 7.5) Age at menarche <12: Risk Ratio 0.6 (95% CI 0.3 - 1.2) >15: Risk Ratio 0.8 (95% CI 0.4 - 1.6) Number of pregnancies 1-2: Risk Ratio 0.8 (95% CI 0.4 - 1.5) ≥3: Risk Ratio 1.0 (95% CI 0.5 - 1.9)	III
Ferry et al., 2000[117]	CC	Female survey responders	1264/1264	HRT: OR 1.39 (95% CI 0.90 - 2.08) Endogenous Hormonal factors 1: OR 1.27 (95% CI 0.58 - 2.80); 2: OR 1.18 (95% CI 0.93 - 1.50) ≥3: OR 1.10 (95% CI 0.91 - 1.33) Menopausal symptoms: OR 1.47 (95% CI 1.06 - 2.03)	III
Maghsoudipour et al., 2008[99]	CS	Industrial workers	47/348	Hormone disease: Univariate OR 0.92 (95% CI 0.11 - 7.55), p=0.94; Hormone drug: Univariate OR 0.42 (95% CI 0.05 - 3.25), p=0.39	III
Morgenstern et al., 1991[82]	CS	Female grocery checkers	127/931	Use of exogenous oestrogens: Multivariate OR 0.58 (95% CI 0.23 - 1.46), p=0.24 Currently pregnant: Multivariate OR 1.27 (95% CI 0.52 - 3.09), p=0.60	III

PC, prospective cohort; HRT, hormone replacement therapy; OR, odds ratio; CI, confidence interval; RC, retrospective cohort; CC, case control;; AOR, adjusted odds ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

2.3.3.5 Radiotherapy

The two cohort studies that investigated radiotherapy, found that this treatment has no effect on risk of CTS[91,92] (Table 2.21). Radiotherapy was therefore assigned a low level of certainty.

Table 2.21 Summary of all the studies that have investigated radiotherapy as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
No effect on risk					
Sestak et al., 2009[91]	PC	Postmenopausal women received adjuvant therapy for breast cancer	103/6083	Radiotherapy: Univariate O R 1.15 (95% CI 0.76 - 1.74), p=0.500	II
Mieog et al., 2012[92]	RC	Female breast cancer patients	79/4578	OR 2.33 (95% CI 1.08 - 5.03), p=0.005; AOR 1.67 (95% CI 0.60 - 4.66), p=0.200	II

PC, prospective cohort; OR, odds ratio; CI, confidence interval; RC, retrospective cohort

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

2.3.3.6 Other medication/treatment

Different types of medication or treatment, each assessed by a single study and not previously evaluated in section 2.3.3, is discussed below (Table 2.22). Five studies, of which two were level II, found an increase in CTS risk with various treatments, including Anastrozole and exemestane medication, medication for hypertension, insulin, metformin, sulphonyl and heamodialysis[91,92,103,110,125]. Two studies investigated diuretic use, but found it to have no effect on CTS risk[82,92].

As a result of insufficient research on these different types of medication/treatments as potential risk factors, a low certainty was assigned to each.

Table 2.22 Summary of all the studies that have investigated other medication/treatment as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Sestak et al., 2009[91]	PC	Postmenopausal women received adjuvant therapy for breast cancer	103/6083	Anastrozole vs Tamoxifen(ref): Univariate OR 3.55 (95% CI 2.22 - 5.65), p<0.001; Multivariate OR 3.55 (95% CI 2.22 - 5.66), p<0.001	II
Mieog et al., 2012[92]	RC	Female breast cancer patients	79/4578	Exemestane vs Tamoxifen (ref): OR 9.75 (99% CI 3.49 - 27.24), p<0.001; AOR 9.90 (99% CI 3.52 - 27.82), p<0.001	II
Conlon & Rempel, 2005[103]	CS	Engineers	25/177	Blood pressure medication: Univariate OR 4.08 (95% CI 1.24 - 13.4), p=0.020; Multivariate OR 5.26 (95% CI 1.28 - 2.18), p=0.022	III
Solomon et al., 1999[110]	CC	Members of medical aid programs	626/3618	Haemodialysis: OR 8.4 (95% CI 4.0 - 17.7), AOR 9.0 (95% CI 4.2 - 19.6)	III
Geoghegan et al., 2004[125]	CC	General practice patients	3391/13564	Insulin: Univariate OR 2.24 (95% CI 1.57 - 3.17), Multivariate OR 1.52 (95% CI 1.06 - 2.18) Metformin: Univariate OR 1.87 (95% CI 1.32 - 2.66), Multivariate OR 1.20 (95% CI 0.84 - 1.72) Sulphonyl: Univariate OR 2.18 (95% CI 1.61 - 2.95), Multivariate OR 1.45 (95% CI 1.07 - 1.97)	III
No effect on risk					
Mieog et al., 2012[92]	RC	Female breast cancer patients	79/4578	Diuretic use: OR 1.41 (95% CI 0.22 - 9.10), p=0.64	II
Morgenstern et al., 1991[82]	CS	Female grocery checkers	127/931	Diuretic use: Multivariate OR 2.66 (95% CI 1.00 - 7.04), p=0.050	III

PC, prospective cohort; OR, odds ratio; CI, confidence interval; RC, retrospective cohort; AOR, adjusted OR; CS, cross sectional; CC, case control

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

2.3.4 Medical conditions & Injuries

The following section will review the role of seven specific medical conditions and injuries, namely diabetes, thyroid disorders, rheumatoid arthritis, osteoarthritis, hypertension, gout, previous musculoskeletal disorders/injuries as well other factors, which included various medical conditions investigated only by one study, as risk factors for CTS.

2.3.4.1 Diabetes

Often mentioned to be a risk factor for CTS, diabetes was investigated in 16 studies for its role in influencing the risk of this condition (Table 2.23). Five studies, of which only one was a level II study, found it to be associated with increased risk[80,110,117,126,137], reporting between an 1.4 and 4.5-fold increase in risk (for male and female participants combined). On the other hand, a total of eleven studies, including two level I studies consisting of 35 and 204 participants, respectively, found no effect of diabetes on CTS risk[74,81,83,92,93,106,114–116,119,125].

Although diabetes was assigned a low level of certainty because of the conflicting evidence found, there is a promising trend towards this condition not influencing CTS risk and future research should investigate this further.

Table 2.23 Summary of all the studies that have investigated diabetes as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Wessel et al., 2013[137]	RC	Patients with trigger digits	84/216	Multivariate OR 1.876 (95% CI 1.1 - 3.3), p=0.026	II
Awada et al., 1998[126]	CC	Hospital patients	100/100	Total: OR 4.51 (95% CI 2.91 - 7.00); Men: OR 6.39 (95% CI 2.37 - 17.23); Women: OR 3.78 (95% CI 2.31 - 6.18)	III
Ferry et al., 2000[117]	CC	Female survey responders	1264/1264	OR 1.83 (95% CI 1.68 - 4.98)	III
Solomon et al., 1999[110]	CC	Members of medical aid programs	626/3618	OR 1.4 (95% CI 1.2 - 1.8), AOR 1.4 (95% CI 1.2 - 1.8), p<0.01	III

Table 2.23 Continued.

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Tseng et al., 2012[80]	CC	General population	15802/31604	Univariate OR 3.59 (95% CI 3.06 - 4.21), Multivariate OR 2.04 (95% CI 1.72 - 2.41)	III
No effect on risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	Univariate HR 2.0 (95% CI 0.49 - 8.49), p=0.33	I
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	IRR 1.08 (95% CI 0.43 - 2.26); Multivariate HR 0.64 (95% CI 0.30 - 1.40), p=0.27	I
Werner et al., 2005[114]	PC	Assembly workers	20/169	OR 6.55 (95% CI 1.08 - 39.59), p=0.04	II
Mieog et al., 2012[92]	RC	Female breast cancer patients	79/4578	OR 0.82 (95% CI 95% 0.27 - 2.48), p=0.65	II
Geoghegan et al., 2004[125]	CC	General practice patients	3391/13564	OR 1.83 (95% CI 0.68 - 4.98)	III
Armstrong et al., 2008[81]	CS	Newly hired manual workers	131/940	Logistic regression OR 2.71 (95% CI 1.02 - 7.15)	III
Mattioli et al., 2009[115]	CC	General population	191/286	Univariate OR2.3 (95% CI 0.8 - 6.7), Multivariate OR 2.6 (0.7 - 8.7)	III
Coggon et al., 2013[119]	CC	General population	475/799	AOR 1.1 (95% CI 0.7 - 1.7)	III
Eleftheriou et al., 2012[106]	CS	Computer workers	A: 51/410[1] B: 167/294[2]	Case def B - Univariate RR 1.26 (95% CI 0.65 - 2.44)	III
De Krom et al., 1990[116]	CC	General population	156/473	Risk Ratio 0.6 (95% CI 0.2 - 2.0) During pregnancy: RR 1.6 (95% CI 0.7 - 3.7)	III
Becker et al., 2002[83]	CC	Hospital patients	791/981	OR 1.82 (95% CI 1.08 - 3.06); Multivariate OR 1.49, p=0.012	III

RC, retrospective cohort; OR, odds ratio; CI, confidence interval; CC, case control; AOR, adjusted OR; CS, cross sectional; PC, prospective cohort; IRR, incidence rate ratio; HR, hazard ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

[1] Case definition A: Participants with personal medical history of CTS or surgery due to CTS

[2] Case definition B: Case definition A and participants identified through clinical examination (score ≥12)

2.3.4.2 Thyroid disorders

Eight studies investigated thyroid disorders (including hypo- and hyperthyroidism) as risk factor for CTS (Table 2.24). Only two level III studies found an increased risk of CTS in

participants suffering from hypothyroidism[80,110] whereas six studies found thyroid disorders to have no effect[74,92,93,106,115,116].

Table 2.24 Summary of all the studies that have investigated thyroid disorders as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Solomon et al., 1999[110]	CC	Members of medical aid programs	626/3618	Hypothyroidism: OR 1.9 (95% CI 1.2 - 3.1), AOR 1.7 (95% CI 1.1 - 2.8)	III
Tseng et al., 2012[80]	CC	General population	15802/31604	Hypothyroidism: Univariate OR 2.62 (95% CI 2.22 - 3.10), Multivariate OR 1.38 (95% CI 1.16 - 1.65)	III
No effect on risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	Thyroid problem: Univariate HR 2.0 (95% CI 0.61 - 6.55), p=0.25	I
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	Thyroid disease: HR 1.81 (95% CI 1.01 - 3.01); Multivariate HR 1.24 (95% CI 0.72 - 2.12), p=0.44	I
Mieog et al., 2012[92]	RC	Female breast cancer patients	79/4578	Hypothyroidism: OR 1.56 (95% CI 0.59 - 4.14), p=0.24	II
Mattioli et al., 2009[115]	CC	General population	191/286	Thyroid disorder: Univariate OR 1.3 (95% CI 0.7 - 2.2)	III
Eleftheriou et al., 2012[106]	CS	Computer workers	A: 51/410[1] B: 167/294[2]	Case def A - Hypothyroidism: Univariate RR 1.58 (95% CI 0.76 - 3.29) Case def B - Hypothyroidism: Univariate RR 0.91 (95% CI 0.58 - 1.42) Case def B - Hyperthyroidism: Univariate RR 0.78 (95% CI 0.33 - 1.80)	III
De Krom et al., 1990[116]	CC	General population	156/473	Thyroid disease (women): Risk Ratio 1.0 (95% CI 0.4 - 2.6)	III

CC, case control; OR, odds ratio; CI, confidence interval; PC, prospective cohort; HR, hazard ratio RC, retrospective cohort; RR, relative risk

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

[1] Case definition A: Participants with personal medical history of CTS or surgery due to CTS

[2] Case definition B: Case definition A and participants identified through clinical examination (score ≥12)

Since hypothyroidism is often associated with significant weight gain, it is possible that an increase in BMI (as seen in section 1.3.6) is responsible for an increase in risk of CTS rather

than the thyroid disorder itself. Further research should investigate this possibility. As a consequence of the small number of investigations on this risk factor, it was assigned a low level of certainty.

2.3.4.3 Rheumatoid arthritis

Rheumatoid arthritis (RA), an autoimmune disorder characterized by joint inflammation[138], is also increasingly mentioned as a risk factor for CTS. Nine studies investigated RA as a modifier of CTS risk (Table 2.25) of which five, including one level I study, found participants with RA to be at increased risk of CTS[80,93,110,115,125]. In contrast, four studies, also including one level I study that investigated 204 industrial workers, found RA to have no effect on CTS risk[74,106,117,119]. Considering the conflicting evidence presented to determine whether RA is in fact a true risk factor for CTS, it was assigned a low level of certainty.

2.3.4.4 Osteoarthritis

Similar to RA (section 2.3.4.3), osteoarthritis (OA) is also often mentioned as potential risk factor in the aetiology of CTS. A total of six studies investigated OA for risk of this condition (Table 2.26). Only two level III studies found an increased risk of CTS in participants with OA[117,125]. Ferry *et al*. investigated various forms of OA and found that OA of the spine is associated with increased risk of CTS whereas participants who did not specify the type of OA or the type of arthritis they were suffering from, had no difference in their risk of developing CTS[117]. Besides this finding, four other studies also found no effect of OA on CTS risk[92,93,113,119]. Considering the contradicting evidence and low number of good quality studies, a low level of certainty was assigned to OA as risk factor for CTS.

Table 2.25 Summary of all the studies that have investigated rheumatoid arthritis as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	Univariate HR 3.8 (95% CI 1.47 - 9.79), p=0.006	I
Solomon et al., 1999[110]	CC	Members of medical aid programs	626/3618	Inflammatory arthritis: OR 3.1 (95% CI 2.4 - 4.1), AOR 3.1 (95% CI 2.2 - 4.2), p<0.01	III
Geoghegan et al., 2004[125]	CC	General practice patients	3391/13564	Univariate OR 3.31 (95% CI 2.34 - 4.67); Multivariate OR 2.23 (95% CI 1.57 - 3.17); Operated CTS: AOR 2.48 (95% CI 1.25 - 4.94)	III
Tseng et al., 2012[80]	CC	General population	15802/31604	Univariate OR 3.65 (95% CI 3.22 - 4.13), Multivariate OR 2.21 (95% CI 1.94 - 2.52)	III
Mattioli et al., 2009[115]	CC	General population	191/286	Univariate OR 2.6 (95% CI 1.4 - 4.9), Multivariate OR 2.2 (95% CI 1.0 - 4.6)	III
No effect on risk					
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	IRR 1.66 (95% CI 0.60 - 3.69); Multivariate HR 1.13 (95% CI 0.50 - 2.57), p=0.77	I
Ferry et al., 2000[117]	CC	Female survey responders	1264/1264	OR 1.31 (95% CI 0.63 - 2.70)	III
Coggon et al., 2013[119]	CC	General population	475/799	AOR 1.3 (95% CI 0.8 - 2.3)	III
Eleftheriou et al., 2012[106]	CS	Computer workers	A: 51/410[1] B: 167/294[2]	Case def A - Univariate RR 2.32 (95% CI 0.67 - 7.89) Case def B - Univariate RR 1.38 (95% CI 0.68 - 2.80)	III

PC, prospective cohort; HR, hazard ratio; CI, confidence interval; CC, case control; OR, odds ratio; AOR, adjusted odds ratio; IRR, incidence rate ratio; RC, retrospective cohort; RR, relative risk

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

[1] Case definition A: Participants with personal medical history of CTS or surgery due to CTS

[2] Case definition B: Case definition A and participants identified through clinical examination (score ≥12)

Table 2.26 Summary of all the studies that have investigated osteoarthritis as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Geoghegan et al., 2004[125]	CC	General practice patients	3391/13564	Univariate OR 2.62 (95% CI 2.29 - 2.99), Multivariate OR 1.89 (95% CI 1.65 - 2.17) Operated CTS: AOR 1.92 (95% CI 1.50 - 2.47)	III
Ferry et al., 2000[117]	CC	Female survey responders	1264/1264	OA - spine: OR 2.16 (95% CI 1.52 - 3.06)	III
No effect on risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	Univariate HR 2.2 (95% CI 0.92 - 5.33), p=0.08	I
Mieog et al., 2012[92]	RC	Female breast cancer patients	79/4578	OR 1.93 (95% CI 0.89 - 4.19), p=0.029	II
Ferry et al., 2000[117]	CC	Female survey responders	1264/1264	OA - unspecified: OR 1.23 (95% CI 0.82 - 1.85) Unspecified arthritis: OR 1.49 (95% CI 1.02 - 2.17)	III
Burt et al., 2011[113]	CS	Working population with low, medium or high level of hand activity	57/420	Arthritis[1]: OR 2.03 (95% CI 1.02 - 4.04)	III
Coggon et al., 2013[119]	CC	General population	475/799	AOR 1.0 (95% CI 0.8 - 1.4)	III

CC, case control; OR, odds ratio; AOR, adjusted odds ratio; CI, confidence interval; PC, prospective cohort; HR, hazard ratio; RC, retrospective cohort

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

[1] Type of arthritis not specified

2.3.4.5 Hypertension

Although not commonly thought to be associated with altered risk of CTS, four studies investigated hypertension as playing a possible role in CTS risk (Table 2.27). Not surprisingly, only one of these studies found participants of the general population suffering from hypertension to be at increased risk[80] whereas three studies, including one prospective study, found it to have no effect[93,113,117]. Considering the lack of sufficient evidence to support hypertension as a risk factor for CTS, it was assigned a low level of certainty.

Table 2.27 Summary of all the studies that have investigated hypertension as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Tseng et al., 2012[80]	CC	General population	15802/31604	Univariate OR 2.93 (95% CI 2.57 - 3.35), Multivariate OR 1.84 (95% CI 1.60 - 2.11)	III
No effect on risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	Univariate HR 1.2 (95% CI 0.57 - 2.47), p=0.65	I
Ferry et al., 2000[117]	CC	Female survey responders	1264/1264	OR 1.12 (95% CI 0.84 - 1.49)	III
Burt et al., 2011[113]	CS	Working population with low, medium or high level of hand activity	57/420	OR 1.89 (95% CI 1.01 - 3.53)	III

CC, case control; OR, odds ratio; CI, confidence interval; PC, prospective cohort; HR, hazard ratio; CS, cross sectional

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

2.3.4.6 Gout

Only two studies investigated gout as potentially modifying the risk of CTS (Table 2.28). One, a case-control study, reported gout to be associated with increased risk of CTS in members of the general population[80] whilst a prospective study, reported it to have no effect on CTS risk in industrial workers[74]. Subsequently, gout as a modifier of CTS risk was also assigned a low level of certainty.

Table 2.28 Summary of all the studies that have investigated gout as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Tseng et al., 2012[80]	CC	General population	15802/31604	Univariate OR 3.21 (95% CI 2.79 - 3.70), Multivariate OR 2.29 (95% CI 1.97 - 2.67)	III
No effect on risk					
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	IRR 2.23 (95% CI 0.81 - 4.94); Multivariate HR 1.57 (95% CI 0.72 - 3.44), p=0.26	I

CC, case control; OR, odds ratio; CI, confidence interval; PC, prospective cohort; IRR, incidence rate ratio; HR, hazard ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

2.3.4.7 Previous MSD/injury

A common intrinsic risk factor for soft tissue injury is a previous injury[34] and previous musculoskeletal disorder (MSD) or injury was investigated for their effect on CTS risk in 14 studies (Table 2.29). Several different MSD and injuries were combined in this section for simplicity purposes and should ideally, in future investigations, be investigated individually. Nine investigations found an increased risk of CTS with previous MSD/injury[74,79,92,93,101,115,117,125,137] whereas nine found previous injury to have no effect on future risk of CTS[79,81,82,93,114–117,129].

Two prospective studies found previous injury and distal upper extremity musculoskeletal disorder (DUE MSD) to increase the risk of developing CTS between 2 and 4.8-fold[74,93]. The same study by Garg *et al*. found that, although DUE MSD increases the risk of CTS, previous wrist fracture has no effect on future risk[93].

Several types of wrist injury of MSD were investigated by various level II and III studies. Interestingly, both studies that investigated the condition “trigger finger” found that it leads to

an increased risk of CTS[115,137]. Furthermore, neck pain, brachial neuralgia and shoulder tendonitis did not indicate increased risk for CTS[81,117] but arm fracture, epicondylitis (tennis elbow), limb or joint pain was an indication for CTS[117].Owing to the limited number of studies and range of different types of MSD and/or injury, this risk factor was assigned a low level of certainty.

Table 2.29 Summary of all the studies that have investigated previous musculoskeletal disorder/injury as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	Previous injury: IRR 2.13 (95% CI 1.39 - 3.18), Multivariate HR 1.58 (95% CI 1.05 - 2.37), p=0.03	I
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	DUE MSD 1-2: Univariate HR 2.9 (95% CI 1.45 - 5.83), p=0.003 ≥3: Univariate HR 4.8 (95% CI 1.39 - 16.37), p=0.01	I
Mieog et al., 2012[92]	RC	Female breast cancer patients	79/4578	Previous MS injury: OR 2.60 (99% CI 1.41 - 4.78), p<0.0001; AOR 2.17 (99% CI 1.10 - 4.25), p=0.0031	II
Frost et al., 1998[79]	RC	Active workers (slaughterhouse & chemical)	81/1060	Wrist trauma Either hand: AOR 3.87 (95% CI 1.74 - 8.60); Dominant hand: AOR 5.14 (95% CI 1.40 - 18.97)	II
Wessel et al., 2013[137]	RC	Patients with tigger digits	84/216	Multiple trigger digits: Multivariate OR 3.618 (95% CI 2.1 - 6.3), p<0.001	II
Ferry et al., 2000[117]	CC	Female survey responders	1264/1264	Any MSD: OR 2.38 (95% CI 1.98 - 2.87) Arm fracture: OR 2.70 (95% CI 1.31 - 5.58), AOR 2.50 (95% CI 1.15 - 5.45) Tennis Elbow: OR 2.00 (95% CI 1.59 - 2.51), AOR 1.88 (95% CI 1.48 - 2.39) Limb pain: OR 1.68 (95% CI 1.30 - 2.18), AOR 1.39 (95% CI 1.05 - 1.85) Joint pain: OR 2.03 (95% CI 1.54 - 2.66), AOR 1.61 (95% CI 1.20 - 2.16)	III
Raman et al., 2012[101]	CS	Office workers	88/382	Previous wrist injury: OR 1.9 (95% CI 1.1 - 3.3)	III
Geoghegan et al., 2004[125]	CC	General practice patients	3391/13564	Wrist fracture: Univariate OR 2.58 (95% CI 1.91 - 3.48), Multivariate OR 2.29 (95% CI 1.67 - 3.12) Operated CTS: AOR 4.09 (95% CI 2.37 - 7.06)	III
Mattioli et al., 2009[115]	CC	General population	191/286	Trigger finger: Univariate OR 3.8 (95% CI 2.0 - 7.3), Multivariate OR 2.7 (95% CI 1.3 - 5.8)	III
No effect on risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	Wrist fracture: Univariate HR 2.4 (95% CI 0.92 - 6.12), p=0.07	I

Table 2.29 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Frost et al., 1998[79]	RC	Active workers (slaughterhouse & chemical)	81/1060	Wrist trauma (non-dominant hand): AOR 3.10 (95% CI 1.00 - 9.66)	II
Werner et al., 2005[114]	PC	Assembly workers	20/169	Wrist/hand/finger tendonitis at baseline: OR 4.74 (95% CI 1.09 - 20.43),p=0.04	II
Nordstrom et al., 1997[129]	CC	General population	206/211	Other MS condition: AOR 1.92 (95% CI 1.04 - 3.54), p=0.03; Multivariate OR 2.41 (95% CI 1.24 - 4.67), p=0.009	III
Ferry et al., 2000[117]	CC	Female survey responders	1264/1264	Neck pain: OR 1.68 (95% CI 1.08 - 2.62), AOR 1.15 (95% CI 0.71 - 1.86) Brachial Neuralgia: OR 1.89 (95% CI 1.09 - 3.30), AOR 1.65 (95% CI 0.90 - 3.02)	III
De Krom et al., 1990[116]	CC	General population	156/473	Wrist fracture: Risk Ratio 0.7 (95% CI 0.3 - 1.5)	III
Armstrong et al., 2008[81]	CS	Newly hired manual workers	131/940	Shoulder tendinitis: Logistic regression OR 2.55 (95% CI 0.97 - 6.73)	III
Mattioli et al., 2009[115]	CC	General population	191/286	Wrist fractures: Univariate OR 0.7 (95% CI 0.3 - 1.4)	III
Morgenstern et al., 1991[82]	CS	Female grocery checkers	127/931	Broken wrist: Multivariate OR 1.13 (95% CI 0.54 - 2.37), p=0.75	III

PC, prospective cohort; IRR, incidence rate ratio; CI, confidence interval; HR, hazard ratio CC, case control; OR, odds ratio; AOR, adjusted odds ratio; HR, hazard ratio; CS, cross sectional; DUE MSD, distal upper extremity musculoskeletal disorder

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

2.3.4.8 Other medical conditions

Thirteen studies investigated one or more other medical condition (not listed above) as being potential risk factors for CTS (Table 2.30). Of these, eleven studies found an increase in risk with various medical conditions[76,78–80,92,94,101,116,117,123,139]. Only one level I prospective study found an increase in risk with more than one predisposing condition[94]. Four level II studies investigated lymphoedema, hot flushes, endocrine conditions and any other medical conditions that predispose to CTS and found that these conditions increased the risk of developing CTS[76,78,79,92]. It should be kept in mind that “hot flushes” is likely to be the effect

of a hormonal condition and should therefore be interpreted with caution in the aetiology of CTS.

In contrast, two level III studies found headaches, fibrosis and coronary artery disease to lead to a decrease in CTS risk[117,123]. Furthermore, four studies found various other medical conditions were not associated with CTS risk[90,92,99,123].

A low level of certainty was assigned to each one of the abovementioned medical conditions, considering the lack of adequate good quality studies verifying these associations.

Table 2.30 Summary of all the studies that have investigated other medical conditions as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Bonfiglioli et al., 2013[94]	PC	Industrial and service worker groups	84/2837	Predisposing diseases ≥1: Univariate IRR 3.16 (95% CI 1.49 - 6.17), Multivariate IRR 1.91 (95% CI 1.26 - 2.91)	I
Mieog et al., 2012[92]	RC	Female breast cancer patients	79/4578	Lymphoedema: OR 4.36 (95% CI 1.27 - 14.92), p=0.002; AOR 2.69 (95% CI 95% CI 0.74 - 9.79), p=0.048 Hot flushes: OR 2.82 (95% CI 1.53 - 5.20), p<0.001; AOR 1.66 (95% CI 0.84 - 3.31), p=0.057	II
Violante et al., 2007[78]	PC	Working population	153/1939	Pathologies facilitating CTS onset: Univariate OR 3.4 (95% CI 2.2 - 5.1), Multivariate OR 2.3 (95% CI 1.5 - 3.6)	II
Nathan et al., 2002[76]	PC	Industrial workers	34/222	Endocrine condition: Univariate OR 1.35 (95% CI 0.48 - 3.83), p=0.56; Multivariate OR 0.23 (95% CI 0.04 - 1.24), p=0.080	II
Frost et al., 1998[79]	RC	Active workers (slaughterhouse & chemical)	81/1060	Any - Either hand: AOR 2.12 (95% CI 0.79 - 5.71) Dominant hand: AOR 2.30 (95% CI 0.65 - 8.17) Non-dominant hand: AOR 2.05 (95% CI 0.59 - 7.18)	II
Ferry et al., 2000[117]	CC	Female survey responders	1264/1264	Psychiatric illness - nonpsychotic: OR 1.31 (95% CI 1.11 - 1.54) Respiratory complaints: OR 1.68 (95% CI 1.38 - 2.04)	III

Table 2.30 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Tseng et al., 2012[80]	CC	General population	15802/31604	Uraemia: Univariate OR 2.31 (95% CI 1.99 - 2.68), Multivariate OR 1.52 (95% CI 1.28 - 1.76) Acromegaly: Univariate OR 2.26 (95% CI 2.01 - 2.54), Multivariate OR 1.47 (95% CI 1.29 - 1.66)	III
Raman et al., 2012[101]	CS	Office workers	88/382	Co-morbid conditions - 1: OR 3.5 (95% CI 1.8 - 6.8), AOR 4.9 (95% CI 2.0 - 12.3) 2: OR 4.0 (95% CI 1.8 - 9.2), AOR 3.3 (95% CI 1.1 - 9.7) ≥3: OR 14.6 (95% CI 5.8 - 36.8), AOR 14.9 (95% CI 4.8 - 46.5)	III
Shiri et al., 2011[123]	CS	General population	79/6175	High-sensitive C-reactive protein (high): AOR 1.7 (95% CI 1.1 - 2.8)	III
De Krom et al., 1990[116]	CC	General population	156/473	Varicosis (men): Risk Ratio 12.0 (95% CI 3.6 - 4.01)	III
Sekijima et al., 2011[139]	CC	CTS Patients and autopsy cases	100	TTR amyloid deposition: Logistic regression OR 15.79 (95% CI 3.29 - 75.68)	III
Decreased risk					
Ferry et al., 2000[117]	CC	Female survey responders	1264/1264	Headache: OR 0.71 (95% CI 0.58 - 0.87) Fibrosis: OR 1.44 (95% CI 1.16 - 1.79), AOR 1.27 (95% CI 1.01 - 1.59)	III
Shiri et al., 2011[123]	CS	General population	79/6175	Coronary artery disease: AOR 0.3 (95% CI 0.1 - 0.9)	III
No effect on risk					
Mieog et al., 2012[92]	RC	Female breast cancer patients	79/4578	Endocrine/metabolic medical history: OR 1.06 (95% CI 0.51 - 2.21), p=0.840 Osteoporosis: OR 1.28 (95% CI 0.28 - 5.96), p=0.670 Arthralgia: OR 0.94 (95% CI 0.15 - 6.01), p=0.93 Myalgia: OR 3.12 (95% CI 0.47 - 20.67), p=0.12	II
Mondelli et al., 2006[90]	CS	Female floor cleaners	70/75	Other diseases: Univariate OR 1.42 (95% CI 0.56 - 3.63); Multivariate OR 1.47 (95% CI 0.45 - 4.79)	III

Table 2.30 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Shiri et al., 2011[123]	CS	General population	79/6175	LDL Cholesterol - 130-189: AOR 1.1 (95% CI 0.6 - 1.9) ≥190: AOR 1.5 (95% CI 0.8 - 2.9) HDL Cholesterol - 40-60: AOR 0.9 (95% CI 0.5 - 1.7) ≤40: AOR 1.5 (95% CI 0.7 - 2.8) Total cholesterol - AOR 200-239: AOR 1.5 (95% CI 0.7 - 3.0) ≤240: AOR 1.8 (95% CI 0.9 - 3.6) Triglycerides - 151-199: AOR 1.1 (95% CI 0.5 - 2.3) ≥200: AOR 1.7 (95% CI 0.9 - 3.1) Insulin resistance - 2nd tertile: AOR 1.2 (95% CI 0.7 - 2.2) 3rd tertile: AOR 1.7 (95% CI 0.9 - 3.3) Metabolic syndrome: AOR 1.5 (95% CI 0.9 - 2.5) Intermittent claudication: AOR 0.4 (95% CI 0.1 - 2.7) Cerebrovascular disease: AOR 0.3 (95% CI 0.1 - 2.3) Heart failure: AOR 0.6 (95% CI 0.1 - 2.6) Arrhythmia: AOR 0.6 (95% CI 0.2 - 1.9) Valvular heart disease: AOR 07 (95% CI 0.1 - 3.2)	III
Maghsoudipour et al., 2008[99]	CS	Industrial workers	47/348	Rheumatologic disease: Univariate OR 0.88 (95% CI 0.85 - 0.91), p=0.61	III

PC, prospective cohort; IRR, incidence rate ratio; CI, confidence interval; HR, hazard ratio CC, case control; OR, odds ratio; AOR, adjusted odds ratio; HR, hazard ratio; CS, cross sectional; DUE MSD, distal upper extremity musculoskeletal disorder

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

2.3.5 Other

A total of 16 studies considered various other single risk factors that were not previously investigated (Table 2.31). Only one retrospective study (level II) found that the type of primary surgery a participant had, influenced their future risk of developing CTS[92]. A single case control study found that sleeping position on the side could lead to a decreased risk of CTS[130] and six other investigations found various variables to have no effect on the risk of CTS[92,103,107,119,123,129].

Each of these risk factors was assigned a low level of certainty, based on the little evidence available.

Table 2.31 Summary of all the studies that have investigated other, single factors as risk factor for carpal tunnel syndrome (CTS).

Risk factor	Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk						
Type of primary surgery	Mieog et al., 2012[92]	RC	Female breast cancer patients	79/4578	Other: OR 2.76 (95% CI 1.13 - 6.76), p=0.004; Multivariate OR 1.33 (95% CI 0.46 - 3.89), p=0.49	II
Marital status	Maghsoudipour et al., 2008[99]	CS	Industrial workers	47/348	Univariate OR 2.37 (95% CI 1.19 - 4.71), p=0.01; Multivariate OR 0.85 (95% CI 0.34 - 2.13) p=0.74	III
Internet use (leisure time)	Ali et al., 2006[27]	CS	Computer workers	85/563	OR 1.8 (95% CI 1.2 - 2.7), p=0.03; AOR 1.7 (95% CI 1.2 - 2.7), p=0.02	III
Median–ulnar peak latency >0.8 msec	Werner et al., 1997[87]	CS	Active workers (industrial & clerical sites)	158/348	OR 7.75 (95% CI 1.30 - 45.84), p=0.02	III
Having children	Bonfiglioli et al., 2007[94]	CS	Female supermarket cashiers	26/324	Multivariate OR 2.16 (95% CI 0.67 - 6.95), p=0.195	III
Waist circumference	Shiri et al., 2011[123]	CS	General population	79/6175	Increased: AOR 3.7 (95% CI 1.5 - 9.0) Obese: AOR 4.8 (95% CI 1.9 - 12.1)	III
Body fat	Anton et al., 2002[104]	CS	Dental hygienists	8/81	OR 1.17 (95% CI 0.99 - 1.38), AOR 1.23 (95% CI 1.00 - 1.52)	III
High parity	Roquelaure et al., 1997[140]	CC	Manufacturing workers	65/65	≥3: Logistic model OR 3.2 (95% CI 1.6 - 6.4), p=0.001	III
Consultations	Geoghegan et al., 2004[125]	CC	General practice patients	3391/13564	Con rate 2: OR 2.31 (95% CI 2.01 - 2.64) Con rate 3: OR 3.77 (95% CI 3.29 - 4.31) Con rate 4: OR 5.65 (95% CI 4.94 - 6.46)	III
Sleeping position	McCabe et al., 2011[130]	CC	Hospital patients	68/138	<60 years old: OR 8.7 (95% CI 1.9 - 39.4), p=0.05; AOR 8.0 (95% CI 1.9 - 39.4), p=0.016	III
Have home typewriter	Nordstrom et al., 1997[129]	CC	General population	206/211	AOR 0.69 (95% CI 0.47 - 1.02), p=0.06; Multivariate OR 0.71 (95% CI 0.46 - 1.09), p=0.117	III
Slimming course	De Krom et al., 1990[116]	CC	General population	156/473	Risk Ratio 2.10 (95% CI 1.41 - 3.12)	III
Vit B6 deficiency	Tseng et al., 2012[80]	CC	General population	15802/31604	Univariate OR 2.42 (95% CI 1.71 - 3.43), Multivariate OR 1.51 (95% CI 1.04 - 2.19)	III

Table 2.31 Continued

Risk factor	Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Decreased risk						
Sleeping position	McCabe et al., 2011[130]	CC	Hospital patients	68/138	>60 years old: OR 0.12 (95% CI 0.02 - 0.79), p<0.05	III
No effect on risk						
Type of primary surgery	Mieog et al., 2012[92]	RC	Female breast cancer patients	79/4578	Wide local excision: OR 2.03 (95% CI 1.02 4.02), p=0.0077; Multivariate OR 1.26 (95% CI 0.51 - 3.09), p=0.51	II
Trade union member	Nordstrom et al., 1997[129]	CC	General population	206/211	OR 1.61 (95% CI 0.91 - 2.87), p=0.10	III
Driving (hr per week)	Conlon & Rempel, 2005[103]	CS	Engineers	25/177	Univariate OR 0.91 (95% CI 0.81 - 1.02), p=0.091	III
Moderately distressing somatic symptoms (in past week)	Coggon et al., 2013[119]	CC	General population	475/799	1: AOR 1.1 (95% CI 0.8 - 1.6) ≥2: AOR 1.1 (95% CI 0.8 - 1.4)	III
Waist-to-hip ratio	Shiri et al., 2011[123]	CS	General population	79/6175	Increased: AOR 0.9 (95% CI 0.4 - 2.2) Obese: AOR 1.4 (95% CI 0.5 - 3.2)	III
Contraception (non-oral)	Chiang et al., 1993[107]	CS	Fish-processing workers	30/177	Tubal ligation: OR 0.6 (95% CI 0.2 - 2.0) Other: OR 1.1 (95% CI 0.4 - 2.8)	III

RC, retrospective cohort; OR, odds ratio; AOR, adjusted odds ratio; CI, confidence interval; CC, case control; CS, cross sectional

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

2.4 CONCLUSION

In conclusion, of the 28 non-occupational and non-psychosocial risk factors (excluding "other" risk factors) that were reviewed, only sex was found to have moderate certainty to have an effect on the modification of risk for CTS (Table 2.32). Specifically, the current research suggests that females are at increased risk of CTS. Interestingly, there was little evidence to support the hypothesis that the other non-occupational factors, specifically

obesity, diabetes and wrist ratio, commonly believed to increase risk of CTS, were associated. Future research in the form of good quality, prospective studies, should investigate these factors further. This systematic review also highlighted the limited number of good quality prospective studies investigating other non-occupational and non-psychosocial risk factors for CTS within the literature.

In addition this review also highlighted that the level of certainty for a familial predisposition or genetic component, which is the focus of this thesis, is currently low. Studies that only included self-diagnosed or suspected CTS, without a medical professional or nerve conduction studies, were excluded from this systematic review. Furthermore, very few studies investigated participants who have had carpal tunnel release surgery, which would be considered the most extreme phenotype, and would therefore be an ideal sub-population to investigate in future studies.

There are limitations to this systematic review. Firstly, many of the variables were considered only by univariate analyses compared to other which were considered by both uni- and multivariate analyses. Future systematic reviews could potentially only focus on multivariate analyses, which are considered to be of higher quality than univariate analyses. Secondly, in various sections, different variables were grouped under one umbrella risk factor (such as hormonal influences and hobbies). Although this is not ideal, this was done for simplicity purposes to avoid having an excessive amount single risk factors investigated only by one study. Lastly, groupings in this systematic review was made on the risk factor's effect on risk i.e. increased, decreased or no effect on risk. Although there are several methods of grouping risk factors, this method was chosen to increase the understanding of the effect of a particular risk factor on risk and to prevent confusion.

In conclusion, future, prospective studies with large sample sizes should aim to investigate these and other risk factors in order to create a better understanding of the role these factors play in the aetiology of CTS. Furthermore, a meta-analysis to investigate the combination and/or interaction of different studies would provide more information on the effect of different risk factors in this multifactorial condition. The next chapter of this thesis will review the occupational and psychosocial risk factors for CTS.

Table 2.32 Summary of the level of certainty of carpal tunnel syndrome risk factors

	Level of Certainty		
	High	**Moderate**	**Low**
(1) Biological			
	-	Sex	Ethnicity Height Weight BMI Age Hand/wrist structure/dimension Genetic/Familiar
(2) Behavioural and Social			
	-	-	Education/Social Alcohol use Exercise Hobbies Smoking Geographic location
(3) Medication			
	-	-	Corticosteroids Chemotherapy Contraceptives Hormone Replacement Therapy Radiotherapy Other
(4) Medical Conditions and Injuries			
	-	-	Diabetes Thyroid disorders Rheumatoid arthritis Osteoarthritis Hypertension Gout Previous MSD/injury Medical condition

CHAPTER 3: OCCUPATIONAL AND PSYCHOSOCIAL RISK FACTORS FOR CARPAL TUNNEL SYNDROME

3.1 INTRODUCTION

In the previous chapter the level of evidence and certainty, namely low, moderate and high, of 19 non-occupational and non-psychosocial risk factors in the aetiology of carpal tunnel syndrome (CTS) was systematically reviewed. The current research suggests that there is a moderate level of certainty that females are at increased risk of CTS. The current level of certainty of the remaining 18 factors, which include obesity, diabetes and wrist ratio, was however low. Occupational and psychosocial[20,26] factors are also often mentioned and investigated as potential risk factor for CTS[8]. Specifically those exposed to repetitive work, vibration exposure to the upper limb and forceful work, as well as individuals with poor psychological well-being with little job control are believed to be at increased risk for developing CTS[20,26]. Since there is, to our knowledge, also no recent systematic review of these risk factors in the scientific literature, the objective of this chapter of the thesis is therefore to critically assess the published evidence on occupational and psychosocial risk factors of CTS.

3.2 METHODS

The search strategy and method of data extraction, as well as the method for determining level of evidence and level of certainty was described in the previous chapter (section 2.2.1 and 2.2.2) with focus in the last appraisal step on occupational and/or psychosocial factors.

3.3 RESULTS AND DISCUSSION

The article selection process has been previously described in section 2.3 and is outlined for this systematic review in figure 3.1. Eighteen occupational risk factors and 11 psychosocial risk factors were identified from the 58 full text articles included in this review. The risk factors were divided into the following categories (1) current and previous occupations, (2) specific occupational exposure, (3) biomechanical factors and (4) psychosocial factors.

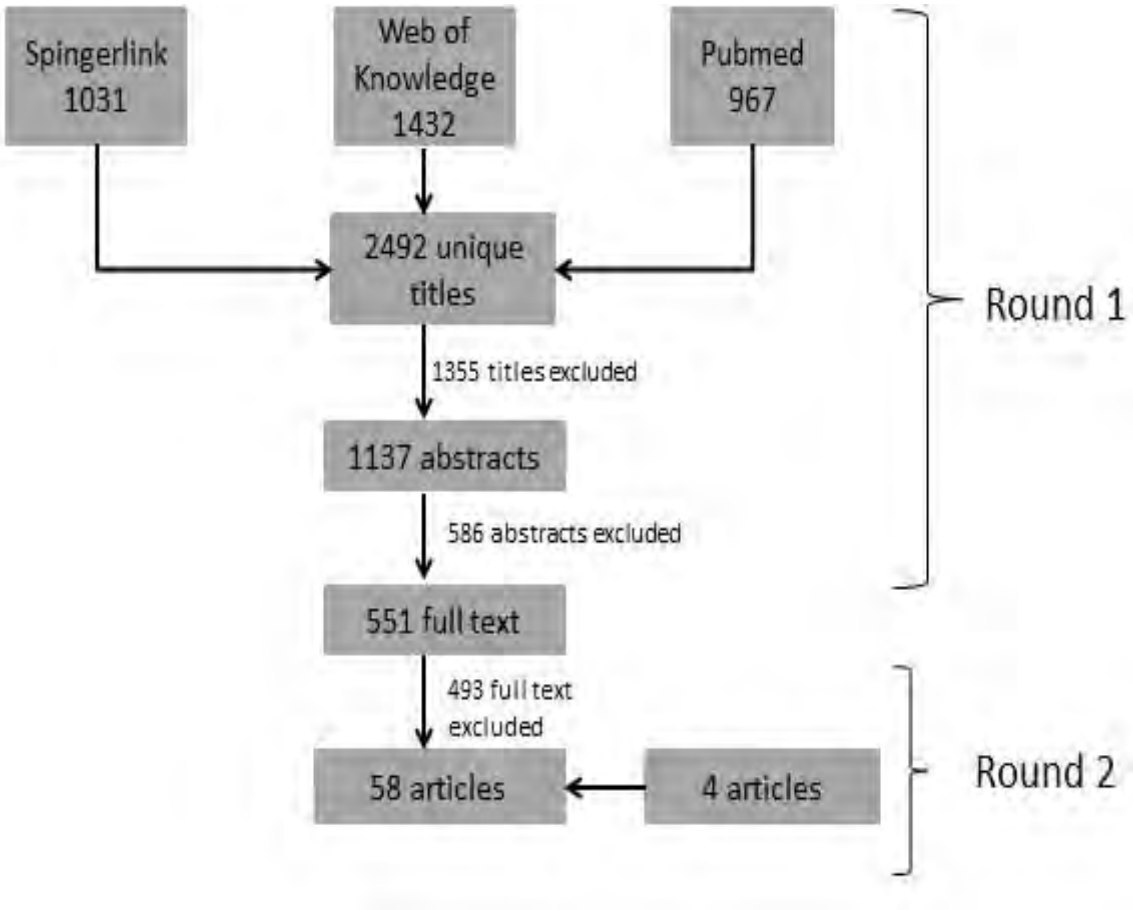

Figure 3.1 Outline of the literature search procedure. In round 1, a total of 2492 unique titles (duplicates excluded) were identified using the electronic database searched. After the three-step method, the number of articles were reduces to 1137 abstracts and then 551 full text articles. A total of 58 articles were included. When all the references of the articles that fit the inclusion criteria were analysed, using the same exclusion and exclusion criteria as previously described and three-step method (as in round one), an additional 4 articles on occupational and/or psychosocial risk factors were identified and included in the systematic review. A final selection of 58 articles was therefore included in this review.

3.3.1 Current and Previous Occupations

Most of the scientific literature has investigated an individual's current occupation as a risk factor for CTS. A few studies have specifically examined computer work as a risk factors. Studies have also investigated whether current or previous exposure to the same occupation is a risk factor.

3.3.1.1 Occupation

Twenty-three studies considered an individual's occupation as a potential risk factor for CTS (Table 3.1). Of these, only one prospective cohort, level I study found an increased risk of CTS with specific occupations, including housekeeping/cleaning, material handlers, food and beverage services, data processing operators and lorry and bus drivers[141]. Five level II[79,142–145] and twelve level III studies[27,80,81,86,87,102,109,115,121,146–148] also found that specific occupations lead to an increase in risk of CTS. It is interesting to note that blue-collar workers[80,115,144,145,147] as well as slaughterhouse workers (including poultry processing)[79,102,142] were at increased risk of CTS in various studies. These manual labour occupations are associated with repetitive hand/wrist movements which are believed to increase the risk of CTS.

The same prospective studies (one level I and two level II) found unemployed people, pensioners, non-manual workers and those in crafts, sales, managing or service professions to be at a decreased risk of developing CTS[141,144,145]. Interestingly, these are all occupations that have, by definition, minimal repetitive hand/wrist movements.

Two prospective, level I studies found no effect of various occupations including manual work, drivers, manufacturing, construction, fishermen etc. on CTS risk[141,149]. Similarly five level II studies[79,95,143–145] and nine level III studies found no effect of various occupations, which included those commonly believed to be associated with CTS because of the

repetitive nature of the work, such as poultry processing and industrial/manufacturing occupations, on CTS[24,27,80,84,88,100,109].

As a consequence of the multiple and often different occupations investigated in these studies and the conflicting results regarding some occupations' association with CTS, this risk factor was awarded a low level of certainty. However, there seems to be a trend towards an increased risk of CTS in blue-collar workers. Various different occupations were investigated together in this review and revealed contradictory information, a more in-depth systematic review on the individual occupations could potentially report different results and is thus warranted. Future studies should specifically investigate risk of CTS in individual occupational groups to determine whether these manual labour occupations, that often include high volumes of highly repetitive work, are associated with CTS.

Table 3.1 Summary of all the studies that have investigated specific occupations as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Rossignol et al., 1997[141]	PC	Hospital patients	238/~1.1 million	*Female* Housekeepers/cleaners: SIR 7.2 (95% CI 2.8 - 13.4) Material handlers: SIR 6.0 (95% CI 2.2 - 11.8) Food and beverage, processing: SIR 7.0 (95% CI 1.8 - 15.3) Food and beverage, service: SIR 3.6 (95% CI 1.4 - 6.7) *Male* Housekeepers/cleaners: SIR 67.5 (95% CI 17.3 - 148.0) Data processing operators: SIR 11.2 (95% CI 1.1 - 31.8) Material handlers: SIR 5.5 (95% CI 1.4 - 12.3) Lorry & Bus drivers: SIR 3.1 (95% CI 1.1 - 6.1)	I
Roquelaure et al., 2009[144]	PC	General population	1168/386910	*Female* Lower-grade white collar workers: RR 2.0 (95% CI 1.8 – 2.3) Blue collar workers: RR 2.9 (95% CI 2.5 - 3.4) Agriculture: RR 2.4 (95% CI 1.9 – 3.0) Manufacturing: RR 2.0 (95% CI 1.7 - 2.4) Service industries: RR 1.4 (95% CI 1.2 – 1.6) *Male* Blue collar workers: RR 3.8 (95% CI 3.0 – 4.7) Construction: RR 2.9 (95% CI 2.2 – 3.8) Manufacturing: RR 1.9 (95% CI 1.5 - 2.4)	II

Table 3.1 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Roquelaure et al., 2008[145]	PC	General population	1168/386910	*Female* Lower-grade white-collar workers: RR 2.5 (95% CI 2.2 – 3.0) Blue collar workers: RR 3.0 (95% CI 2.5 - 3.6) Agriculture: RR 2.5 (95% CI 2.0 - 3.2) Manufacturing: RR 2.1 (95% CI 1.7 - 2.5) Service industries: RR 1.7 (95% CI 1.5 - 2.1) *Male* Blue collar workers: RR 4.2 (95% CI 3.3 - 3.5) Construction: RR 3.0 (95% CI 2.2 - 4.0) Manufacturing: RR 2.0 (95% CI 1.5 - 2.5)	II
Wyatt et al., 2013[142]	PC	Lamb-freezing workers	285/300	All freezing workers: SIR 16.8 (95% CI 14.8 - 19.1) Production line workers: SIR 25.4 (95% CI 22.3 - 28.8) Slaughter men: SIR 5.4 (95% CI 3.1 - 8.6) Packers: SIR 22.8 (95% CI 19.0 - 27.2) Boners: SIR 51.6 (95% CI 43.0 - 61.6)	II
Bongers et al., 2007[143]	PC	General population	1987:113/355088 2001: 672/364326	*Female* Unskilled or semi-skilled workers: AOR 1.5 (95% CI 1.2 - 2.0), p=0.001	II
Frost et al., 1998[79]	RC	Active workers (slaughterhouse & chemical)	81/1060	*Either hand* Slaughterhouse workers (SHW): AOR 4.24 (95% CI 1.77 - 10.13), Non-deboning SHW: AOR 3.25 (95% CI 1.27 - 8.33) Deboning SHW: AOR 5.53 (95% CI 2.20 - 13.90) *Dominant hand* Deboning SHW: AOR 3.74 (95% CI 1.14 - 12.22) *Non-dominant hand* Slaughterhouse workers: AOR 5.31 (95% CI 1.60 - 17.61) Deboning SHW: AOR 8.43 (95% CI 2.44 - 29.07)	II
Armstrong et al., 2008[81]	CS	Newly hired manual workers	131/940	Working on assembly line Logistic regression OR 2.86 (95% CI 1.64 - 5.01)	III
Ali et al., 2006[27]	CS	Computer workers	85/563	*System administrator:* OR 2.5 (95% CI 1.2 - 5.2), p=0.01; AOR 2.4 (95% CI 1.2 - 4.8), p=0.010	III
Leclerc et al., 1998[109]	CS	Workers in Manual labour occupations (repetitive work)	151/1059	*Model 1* Assembly: OR 4.54 (95% CI 2.27 - 9.09) Clothing: OR 4.12 (95% CI 1.95 - 8.71) Food: OR 3.14 (95% CI 1.38 - 7.15) Packaging: OR 6.55 (95% CI 3.02 - 14.2)	III
Wolf et al., 2009[86]	CS	Military population	48957 [1]	*Army soldiers* Junior enlisted: Adjusted IRR 1.53 (95% CI 1.47 - 1.59) Senior enlisted: Ajusted IRR 3.18 (95% CI 3.06 -3.30) Senior officer: Adjusted IRR 4.98 (95% CI 2.60 - 2.85)	III
Jenkins et al., 2012[146]	CS	General population	1564/~358436	*Bilateral CTS* Technical/craft: OR 2.46 (95% CI 1.55 - 3.91), p<0.001 Semi-routine manual/service: OR 1.8 (95% CI 1.28 - 2.56), no p-value Unilateral CTS Retired: OR 1.91 (95% CI 1.52 - 2.39), p<0.001	III
Forst et al., 2007[121]	CS	Spine surgeons	107/264	*Surgeon using Kerrison Rongeur instrument:* Multivariate OR 2.72 (95% CI 1.54 - 4.81) Neurosurgeons: OR (95% CI 1.10 - 3.76)	III
Jenkins et al., 2012[148]	CS	Hand clinic patients	218/1346	*Female vs population* Managers/directors/senior officials: OR 3.5 (95% CI 1.9 - 6.7) Professional occupations: OR 3.6 (95% CI 2.4 - 5.4) Skilled trade occupations: OR 3.6 (95% CI 2.4 - 5.1) Elementary occupations: OR 2.9 (95% CI 2.0 - 4.0)	III

Table 3.1 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Werner et al., 1997[87]	CS	Active workers (industrial & clerical sites)	158/348	*MM5* Industrial: OR 2.0 (95% CI 1.3 - 3.2)	III
Cartwright et al., 2012[102]	CS	Latinos in Manual labour occupations	215/298 Possible 34/479 Definite	Poultry: AOR 2.51 (95% CI 1.80 - 3.50), p<0.001 Poultry job task Cutting, eviscerating, Wash-up, trimming, deboning: AOR 1.57, p=0.066; Receiving, hanging, killing, plucking: AOR 2.09, p=0.116; Multiple job tasks: AOR 2.66, p=0.004	III
Mattioli et al., 2009[115]	CC	General population	191/286	*Blue-collar/housewife* OR 8.0 (95% CI 4.5 - 14.2), Multivariate OR 7.1 (95% CI 4.0 - 12.7)	III
Tseng et al., 2012[80]	CC	General population	15802/31604	*Blue-collar* Univariate OR 1.36 (95% CI 1.30 - 1.42), Multivariate OR 1.25 (95% CI 1.18 - 1.31)	III
Mattioli et al., 2009[147]	CS	Hospital patients & census data	8801/3491199	*Female* Blue-collar workers 25-29 years old: RR 5.2 (95% CI 3.7 - 7.3) 30-34 years old: RR 4.9 (95%CI 3.8 - 9.3) 35-39 years old: RR 4.4 (95% CI 3.6 - 5.4) 40-44 years old: RR 3.5 (95% CI 3.0 - 4.2) 45-49 years old: RR 5.0 (4.3 - 5.9) 50-54 years old: RR 4.0 (95% CI 3.4 - 4.7) 55-59 years old: RR 3.6 (95% CI 2.9 - 4.6) Housewives 25-29 years old: RR 5.1 (95% CI 3.3 - 7.7) 30-34 years old: RR 4.6 (95% CI 3.4 - 6.0) 35-39 years old: RR 4.5 (95% CI 3.6 - 5.5) 40-44 years old: RR 3.6 (95% CI 3.0 - 4.3) 45-49 years old: RR 4.6 (95% CI 3.9 - 5.4) 50-54 years old: RR 3.4 (95% CI 3.0 - 4.0) 55-59 years old: RR 2.9 (95% CI 2.3 - 3.6) *Male* Blue-collar workers 25-29 years old : RR 5.9 (95% CI 2.5 - 13.5) 30-34 years old: RR 3.5 (95% CI 2.2 - 5.8) 35-39 years old: RR 7.1 (95% CI 4.4 - 11.6) 40-44 years old: RR 6.0 (95% CI 4.0 - 9.2) 45-49 years old: RR 4.7 (95% CI 3.4 - 6.5) 50-54 years old: RR 3.6 (95% CI 2.6 - 5.0) 55-59 years old: RR 5.1 (95% CI 3.4 - 7.6)	III
Decreased risk					
Rossignol et al., 1997[141]	PC	Hospital patients	238/~1.1 million	*Female* Not working (Unemployed): SIR 0.7 (95% CI 0.5 - 0.9) *Male* Non-manual workers: SIR 0.5 (95% CI 0.2 - 0.9) Not working (Unemployed): SIR 0.4 (95% CI 0.2 - 0.8)	I
Roquelaure et al., 2009[144]	PC	General population	1168/386910	*Female* Cafts, sales, managers: RR 0.5 (95% CI 0.3 – 0.9) Technicians/ass. professionals: RR 0.6 (95% CI 0.5 - 0.8) Pensioners/non-working people: RR0.2 (95% CI 0.2 – 0.3) *Male* Technicians/ass. Professionals: RR 0.6 (95% CI 0.4 - 0.8) Pensioners/non-working people: RR 0.2 (95% CI 0.1 – 0.4) Service industries: RR 0.5 (95% CI 0.4 - 0.7)	II

Table 3.1 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Roquelaure et al., 2008[145]	PC	General population	1168/386910	*Female* Technicians/ass. professionals: RR 0.6 (95% CI 0.5 - 0.8) *Male* Technicians/ass. Professionals: RR 0.6 (95% CI 0.4 - 0.8) Service industries: RR 0.6 (95% CI 0.6 - 0.8)	II
No effect on risk					
Kaerlev et al., 2008[149]	PC	Danish seamen and fishermen	194/8540	Fishermen: SIR 0.92 (95% CI 0.62 - 1.39) Officer: SIR1.34 (95% CI 0.65 - 2.79) Non-officer: SIR 1.03 (95% CI 0.63 - 1.67)	I
Rossignol et al., 1997[141]	PC	Hospital patients	238/~1.1 million	*Female* Data processing operators: SIR 3.1 (95% CI 1.0 - 6.3) Manual workers: SIR 1.8 (95% CI 1.4 - 2.2) Non-manual workers: SIR 0.8 (95% CI 0.6 - 1.1) Child care: SIR 3.9 (95% CI 1.0 - 8.6) Hairdressers: SIR 3.0 (95% CI 0.8 - 6.7) Specialised educators: SIR 2.2 (95% CI 0.6 - 4.8) Manufacturing: SIR 1.2 (95% CI 0.6 - 2.1) Clerical occupations: SIR 1.0 (95% CI 0.7 - 1.4) Health care occupations: SIR 0.9 (95% CI 0.4 - 1.4) *Male* Food and beverage, processing: SIR 2.7 (95% CI 0.3 - 7.8) Food and beverage, service: SIR 3.0 (95% CI 0.0 - 11.9) Manual workers: SIR 1.9 (95% CI 1.4 - 2.5) Lorry & Bus drivers: SIR 3.1 (95% CI 1.1 - 6.1) Hairdressers: SIR 4.2 (95% CI 0.0 - 16.5) Manufacturing: SIR 1.6 (95% CI 0.6 - 3.2) Clerical occupations: SIR 1.3 (95% CI 0.4 - 2.7) Health care occupations: SIR 0.7 (95% CI 0.0 - 2.8) Construction workers: SIR 1.1 (95% CI 0.1 - 2.8)	I
Roquelaure et al., 2009[144]	PC	General population	1168/386910	*Female* Farmers: RR 1.0 (95% CI 0.6 – 1.5) Professionals: RR 0.7 (95% CI 0.5 – 1.0) Construction: RR 2.3 (95% CI 1.0 – 5.2) *Male* Farmers: RR 1.2 (95% CI 0.7 - 2.1) Craftsmen/sales/managers: RR 0.6 (95% CI 0.3 - 1.0) Professionals: RR 0.7 (95% CI 0.4 - 1.2) Lower grade white collar workers: RR 1.3 (95% CI 0.8 - 2.0) Agriculture: RR 1.3 (95% CI 0.9 – 1.9)	II
Roquelaure et al., 2008[145]	PC	General population	1168/386910	*Female* Farmers: RR 1.2 (95% CI 0.8 - 2.0) Crafts/sales/managers: RR 0.5 (95% CI 0.3 - 1.2) Professionals: RR 0.9 (95% CI 0.6 - 1.4) Construction: RR 4.7 (95% CI 1.0 - 13.0) *Male* Farmers: RR 1.3 (95% CI 0.8 - 2.3) Craftsmen/sales/managers: RR 0.8 (95% CI 0.4 - 1.6) Professionals: RR 0.6 (95% CI 0.4 - 1.0) Lower grade white collar workers: RR 1.3 (95% CI 0.8 - 2.1) Agriculture: RR 1.4 (95% CI 0.9 - 2.0)	II
Cartwright et al., 2013[95]	PC	Latinos in Manual labour occupations	36/173	*Poultry:* AOR 1.81 (95% CI 0.83 - 3.98), p=0.139	II
Bongers et al., 2007[143]	PC	General population	1987:113/355088 2001: 672/364326	*Male* Unskilled or semi-skilled workers: AOR 1.1 (95% CI 0.7 - 1.6), p=0.820	II

Table 3.1 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Frost et al., 1998[79]	RC	Active workers (slaughterhouse & chemical)	81/1060	*Dominant hand* Slaughterhouse workers (SHW): AOR 3.26 (95% CI 1.09 - 9.71), Non-deboning SHW: AOR 2.90 (95% CI 0.90 - 9.39) Non-deboning SHW: AOR 3.01 (95% CI 0.82 - 11.30)	II
Ali et al., 2006[27]	CS	Computer workers	85/563	Software developer: OR 1.0 (95% CI 0.6 - 1.9), p=0.90 Others: OR 0.9 (95% CI 0.5 - 1.6), p=0.80	III
Bonfigliol i et al., 2007[100]	CS	Female supermarket cashiers	26/324	Part-time cashiers: Multivariate OR 1.06 (95% CI 0.35 - 3.21), p=0.916 Full-time cashiers: Multivariate OR 1.81 (95% CI 0.52 - 6.34), p=0.352	III
Tseng et al., 2012[80]	CC	General population	15802/3160 4	Other: Univarate OR 1.10 (95% CI 1.04 - 1.16), Multivariate OR 1.06 (95% CI 0.99 - 1.13)	III
Hakim et al., 2002[24]	CC	Female-female twin pairs	520/3154	Clerical: OR 1.11 (95% CI 0.72 - 1.73), AOR 1.13 (95% CI 0.90 - 1.43)	III
Leclerc et al., 1998[109]	CS	Workers in Manual labour occupations (repetitive work)	151/1059	*Model 2* Clothing: OR 1.64 (95% CI 0.93 - 2.91); Food: OR 0.62 (95% CI 0.28 - 1.36) Packaging: OR 1.43 (95% CI 0.78 - 2.61) *Model 3* Clothing: OR 1.25 (95% CI 0.74 - 2.12) Food: OR 0.64 (95% CI 0.29 - 1.40) Packaging: OR 1.78 (95% CI 0.98 - 3.25)	III
Jianmon gkol et al., 2005[88]	CS	Fishnet factory employees	96/566	Netmaker: OR 1.84 (95% CI 1.03 - 3.29), p=0.049	III
Rosecra nce et al., 2002[84]	CS	Construction workers	91/1024	Sheet metal workers: AOR 2.04 (95% CI 0.82 - 5.03) Operating engineers: AOR 1.00 (95% CI 0.46 - 2.18) Plumbers/pipefitters: AOR 1.18 (95% CI 0.53 - 2.04)	III
Jenkins et al., 2012[148]	CS	Hand clinic patients	218/1346	*Female vs population* Technical occupations: OR 1.8 (95% CI 1.0 - 3.4) Admin: OR 1.4 (95% CI 0.8 - 3.9) Service: OR 1.4 (95% CI 0.8 - 2.4) Sales/customer service: OR 1.7 (95% CI 0.9 - 3.5) Process/plant/machine: OR 1.3 (95% CI 0.8 - 2.2)	III
Werner et al., 1997[87]	CS	Active workers (industrial & clerical sites)	158/348	*MM8* Industrial: OR 1.7 (95% CI 0.9 - 3.4)	III

PC, prospective cohort; SIR, standardized incidence ratio; CI, confidence interval; RR, risk ratio; OR, odds ratio; AOR, adjusted odds ratio; RC, retrospective cohort; CS, cross sectional; CI, confidence interval

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

[1] The total number of participants was not reported

3.3.1.2 Computer work

Eight studies have specifically examined computer work as a risk factor for CTS (Table 3.2). Two level III studies found an increase in risk with an increase in number of years, hours per day and cumulative keyboard strokes[27,106]. Surprisingly, one level III study found a decreased risk of CTS with computer work of more than one hour per day, compared to those working on a computer less than one hour per day[85]. Six studies found several different forms of computer use to have no effect on the risk of CTS. Of these, only one was a prospective study, which specifically investigated keyboard use and found no effect in uni- and multivariate analyses[76].

As a result of the small number of studies investigating computer use as risk for CTS, this factor was awarded a low level of certainty.

Table 3.2 Summary of all the studies that have investigated computer use as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Ali et al., 2006[27]	CS	Computer workers	85/563	*Years of computer work* 4-8 years: OR 2.3 (95% CI 1.4 - 3.8), p=0.001; AOR 2.1 (95% CI 1.3 - 3.6), p=0.004 >8 years: OR 3.3 (95% CI 1.7 - 6.6), p<0.001; AOR 2.7 (95% CI 1.3 - 5.8), p=0.01 *Time per day* 8-12 hours per day: OR 3.4 (95% CI 1.2 - 9.5), p=0.020; AOR 3.6 (95% CI 1.3 - 10.3), p=0.02 > 12 hours per day: 4.9 (95% CI 1.5 - 16.2), p=0.009; AOR 4.4 (95% CI 1.3 - 14.9), p=0.02	III
Eleftheriou et al., 2012[106]	CS	Computer workers	A: 51/410[1] B: 167/294[2]	*Cumulative keyboard strokes* Case def A - ≥240500000: RR 2.38 (95% CI 1.38 - 4.12); Logistic regression: OR 2.23 (95% CI 1.09 - 4.52), p=0.026 Case def B - ≥149500000: RR 2.60 (95% CI 1.80 - 3.73); Logistic regression OR 2.41 (95% CI 1.36 - 4.25), p=0.002	III
Decreased risk					
Atroshi et al., 2007[85]	CS	General population	97/1906	*Time per day* ≥1 hour compared to <1 hour: Regression model - Adjusted PR 0.55 (95% CI 0.32 - 0.96), p=0.035	III
No effect on risk					
Nathan et al., 2002[76]	PC	Industrial workers	34/222	*Keyboard use* Univariate OR 0.95 (95% CI 0.74 - 1.23), p=0.71; Multivariate OR 0.88 (95% CI 0.52 - 1.47), p=0.62	II

Table 3.2 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Coggon et al., 2013[119]	CC	General population	475/799	*Time per day* >4 hours: AOR 0.7 (95% CI 0.6 - 1.0)	III
Raman et al., 2012[101]	CS	Office workers	88/382	*Time per day* >3 hours: OR 0.7 (95% CI 0.9 - 3.1)	III
Atroshi et al., 2007[85]	CS	General population	97/1906	*Time per day* <1 hour: Adjusted PR 0.93 (95% CI 0.52 - 1.70) 1-4 hour: Adjusted PR 0.55 (95% CI 0.26 - 1.20) ≥4 hour: AdjustedPR 0.52 (95% CI 0.23 - 1.20)	III
De Krom et al., 1990[116]	CC	General population	156/473	*Time per week* 1-7 hours: RR 0.9 (95% CI 0.6 - 1.4) 8-19 hours: RR 0.8 (95% CI 0.3 - 2.5) 20-40 hours: RR 0.7 (95% CI 0.1 - 6.0)	III
Conlon & Rempel, 2005[103]	CS	Engineers	25/177	*Time per week* 22-28 hours: Univariate OR 0.92 (95% CI 0.14 - 5.86), p=0.929; Multivariate OR 1.285 (95% CI 0.18 - 9.23), p=0.80 29-35 hours: Univariate OR 2.67 (95% CI 0.68 - 10.5), p=0.162; Multivariate OR 5.78 (95% CI 1.24 - 26.9), p=0.015 >35 hours: Univariate OR 3.58 (95% CI 0.93 - 13.8), p=0.064; Multivariate OR 6.53 (95% CI 1.44 - 29.7), p=0.004 *Years of computer work* Previous yr at >20 hrs/week on computer: Univariate OR 0.95 (95% CI 0.87 - 1.04), p=0.266 Total yr at > 20 hrs/wk on computer: Univariate OR 1.00 (95% CI 0.99 - 1.00), p=0.789 *Computer use at work* OR 1.73 (95% CI 0.94 - 3.18), p=0.76 *Additional hour on a computer per week* OR 1.01 (95% CI 0.95 - 1.08), p=0.739 *Typing speed* Univariate OR 0.98 (95% CI 0.95 - 1.00), p=0.104; Multivariate OR 0.96 (95% CI 0.93 - 0.98), p=0.004	III

CS, cross sectional; PC, prospective cohort; AOR, adjusted odds ratio; OR, odds ratio; CI, confidence interval; PR, prevalence ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

[1] Case definition A: Participants with personal medical history of CTS or surgery due to CTS

[2] Case definition B: Case definition A and participants identified through clinical examination (score ≥12)

3.3.1.3 Current or previous exposure

Sixteen studies investigated whether current or previous exposure to the same occupation, which includes overuse of the hand/wrist, modifies the risk of developing CTS (Table 3.3). Two prospective studies found that active workers who have worked less than one year as

well as those with previous exposure to biomechanical overload are at higher risk of developing CTS[74,78]. A retrospective study also found that the peak risk of CTS is during 6-10 years of exposure to the same occupation[79]. Furthermore, four cross-sectional studies (level III) also found increased exposure to lead to an increase in risk of CTS[82,90,121,124].

In contrast, one level III study found a decreased risk of CTS in members of the general population with a cumulative working hour count of 5464 to 15510 hours[129]. Furthermore, three prospective studies also found that exposure of more than 3.5 and 6 years, respectively, as well as any previous exposure, has no effect on risk of CTS in Danish seamen and industrial workers, respectively[74,76,149]. Eight level III studies also found no effect of previous and current exposure, cumulative working hours between 3048-4857 and 4480-5383 and job seniority, on the risk of developing CTS[82,88,90,99,100,104,124,129,133].

Considering the conflicting results when examining previous exposure as risk factor for CTS, it was assigned a low level of evidence and future research should aim to investigate this aspect further.

Table 3.3 Summary of all the studies that have investigated current or previous exposure as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	Years worked for recent hires (<1 year): Multivariate HR 3.08 (95% CI 1.55 - 6.12), p=0.001	I
Violante et al., 2007[78]	PC	Working population	153/1939	Previous exposure vs none: OR 1.6 (95% CI 1.1 - 2.4), Multivariate OR 1.4 (95% CI 0.9 - 2.1)	II
Frost et al., 1998[79]	RC	Active workers (slaughterhouse & chemical)	81/1060	Years of exposure 1-5 years: AOR 3.57 (95% CI 1.13 - 11.34) 6-10 years: AOR 5.354 (95% CI 2.01 - 14.20) 11-15 years: AOR 3.29 (95% CI 1.10 - 9.83) 16-20 years: AOR 4.18 (95% CI 1.11 - 15.80) >20 years: OR 4.44 (95% CI 1.49 - 13.22)	II
Mondelli et al., 2006[90]	CS	Female floor cleaners	70/75	Similar job at previous employer: Univariate OR 4.85 (95% CI 2.38 - 9.88); Multivariate OR 12.15 (95% CI 2.96 - 49.93)	III

Table 3.3 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Forst et al., 2007[121]	CS	Spine surgeons	107/264	>5 years: Multivariate OR 4.24 (95% CI 1.54 - 11.69)	III
Hou et al., 2007[124]	CS	Male visual terminal display workers	13/327	Job seniority 3-5 vs <3 years: Multivariate OR 4.6 (95% CI 1.2 - 16.9), p=0.023	III
Morgenstern et al., 1991[82]	CS	Female grocery checkers	127/931	>9 years: OR 3.13 (95% CI 1.94 - 5.08), AOR 1.74 (95% CI 0.96 - 3.16)	III
Decreased risk					
Nordstrom et al., 1997[129]	CC	General population	206/211	Cumulative work hours 5464-6507: AOR 0.41 (95% CI 0.20 - 0.85); Multivariate OR 0.43 (95% CI 0.18 - 1.05) 6647-15510: AOR 0.34 (95% CI 0.16 - 0.71); Multivariate OR 0.29 (95% CI 0.10 - 0.78)	III
No effect risk					
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	Length of employment (at current company) >3.5 and ≤7 years: Multivariate HR 0.63 (95% CI 0.39 - 1.03), p=0.06 >7 and ≤15 years: Multivariate HR 1.04 (95% CI 0.62 - 1.73), p=0.89 >15 years: Multivariate HR 0.86 (95% CI 0.49 - 1.50), p=0.59	I
Kaerlev et al., 2008[149]	PC	Danish seamen and fishermen	194/8540	6-12 years: RR 0.78 (95% CI 0.46 - 1.32) >12 years: RR 0.73 (95% CI 0.37 - 1.44) p=0.550	I
Nathan et al., 2002[76]	PC	Industrial workers	34/222	Univariate OR 1.11 (95% CI 0.84 - 1.47), p=0.44; Multivariate OR 0.96 (95% CI 0.66 - 1.40), p=0.84	II
Bonfigliol i et al., 2007[100]	CS	Female supermarket cashiers	26/324	Multivariate OR 0.95 (95% CI 0.84 - 1.07), p=0.395	III
Jianmongkol et al,. 2005[88]	CS	Fishnet factory employees	96/566	<4.5 years: OR 1.13 (95% CI 0.77 - 1.66), p=0.591	III
Mondelli et al., 2006[90]	CS	Female floor cleaners	70/75	Length of employment 2nd quartile: Univariate OR 1.58 995% CI 0.62 - 4.03), Multivariate OR 0.83 (95% CI 0.26 - 2.69) 3rd quartile: Univariate OR 1.33 (95% CI 0.51 - 3.46), Multivariate OR 0.77 (95% CI 0.24 - 2.43) 4th quartile: Univariate OR 2.25 995% CI 0.86 - 5.85), Multivariate OR 1.875 (95% CI 0.54 - 5.65)	III
Babski-Reeves & Crumpton-Young, 2003[133]	CS	Workers at a fish processing facility	33/73 hands	Length of employment: Logistic regression OR 1.02 (95% CI 1.00 - 1.04), p=0.084 BMI*length of employment: Logistic regression OR 1.00 (95% CI 1.00 - 1.00), p=0.032	III
Hou et al., 2007[124]	CS	Male visual terminal display workers	13/327	Job seniority >5 vs <3 years: Multivariate OR 2.7 (95% CI 0.6 - 12.4), p=0.211	III
Morgenstern et al., 1991[82]	CS	Female grocery checkers	127/931	5-9 years: OR 1.1 (95% CI 0.64 - 1.87), AOR 0.91 (95% CI 0.53 - 1.58)	III

Table 3.3 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Maghsoudipour et al., 2008[99]	CS	Industrial workers	47/348	Multivariate OR 0.93 (95% CI 0.79 - 1.01), p=0.45	III
Nordstrom et al., 1997[129]	CC	General population	206/211	Cumulative work hours 3048-4857: AOR 1.51 (95% CI 0.84 - 2.70); Multivariate OR 1.54 (95% CI 0.74 - 3.20) 4480-5383: AOR 0.46 (95% CI 0.23 - 0.93); Multivariate OR 0.29 (95% CI 0.12 - 0.72)	III
Anton et al., 2002[104]	CS	Dental hygienists	8/81	Years as dental hygienist: OR 1.14 (95% CI 1.04 - 1.26), AOR 1.08 (95% CI 0.91 - 1.28) Days per week as dental hygienist OR 1.30 (95% CI 0.58 - 2.94) Hours per week as dental hygienist: OR 1.04 (95% CI 0.94 - 1.15) Patients per day as dental hygienist: OR 1.35 (95% CI 1.01 - 1.80), AOR 1.73 (95% CI 1.00 - 2.99) Patients per week as dental hygienist OR 1.06 (95% CI 1.01 - 1.11), AOR 1.09 (95% CI 0.99 - 1.20) Mins per patient as dental hygienist: OR 0.98 (95% CI 0.91 - 1.05)	III

PC, prospective cohort; HR, hazard ratio; OR, odds ratio; CI, confidence interval; RR, risk ratio; CS, cross sectional; AOR, adjusted odds ratio; CC, case control;

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

3.3.2 Specific occupational procedures

A total of 27 studies, which will be reviewed in this section, have investigated various occupational procedures for its potential effect on the modification of risk of CTS.

3.3.2.1 Force exertion

Fifteen studies investigated the effect of force exertion on the risk of CTS (Table 3.4). One prospective study found that increased time spent performing forceful exertion had an increased association with CTS. More specifically, spending between 20 and 60% of the day in forceful exertion lead to a 2.8-fold increase in risk whereas spending more than 60% in forceful exertion lead to a 19.5-fold increase in risk of CTS[118]. Another ten studies investigated various forms of force exertion and found it to be associated with increased risk of CTS[81,97,99,107–109,127,128,140,150].

Five studies, of which only two were prospective[76,96], found that force exertion, including lifting force, frequency of force exertion and sometimes using sustained forceful motion, has no effect on risk of CTS[97,109,128].

Considering the large number of studies available showing an increase in risk compared to this higher quality studies reporting no effect on risk, force exertion as risk factor for CTS was assigned a low level of evidence.

Table 3.4 Summary of all the studies that have investigated force exertion as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Burt et al., 2013[118]	PC	Working population with low, medium or high level of hand activity	29/318	Model 1 ≥20% - <60%: Multivariate HR 2.83 (95% CI 1.18 - 6.79 >60%: Multivariate HR 19.57 (95% CI 5.96 - 64.24)	II
Leclerc et al., 2001[108]	PC	Workers in Manual labour occupations (repetitive work)	131/467	Tighten with force, Men: OR 4.09 (95% CI 1.43 - 11.70)	III
Chiang et al., 1993[107]	CS	Fish-processing workers	30/177	OR 1.8 (95% CI 1.1 - 2.9) Female: OR 1.6 (95% CI 1.1 - 3.0)	III
Thomsen et al., 2002[150]	CS	Workers in monotonous occupations	8/388	Multivariate OR 1.41 (95% CI 0.86 - 2.30), p=0.174	III
Roquelaure et al., 1997[140]	CC	Manufacturing workers	65/65	>1kg: Logistic model OR 9.0 (95% CI 2.4 - 33.4), p=0.001	III
Maghsoudipour et al., 2008[99]	CS	Industrial workers	47/348	>1 kgf: Univariate OR 13.31 (95% CI 4.67 - 37.90), p=0.00; Multivariate OR 6.38 (95% CI 1.91 2.02), p=0.003	III
Armstrong et al., 2008[81]	CS	Newly hired manual workers	131/940	Logistic regression OR 1.68 (95% CI 1.12 - 2.53)	III
Silverstein et al., 2009[97]	CS	Manufacturing & Healthcare workers	63/657	Lifting force (male), ≥10%: OR 6.0 (95% CI 1.3 - 28.0) Frequency of force exertion ≥1 - <5 (Female): OR 2.84 (95% CI 1.25 - 6.47) Duty cycle of forceful exertions, ≥15 (men): OR 5.37 (95% CI 1.16 - 24.86) Radial/ulnar deviation ≥4% AND lifting force ≥3%: OR 3.20 (95% CI 1.47 - 6.96), AOR 4.85 (95% CI 2.12 - 11.11)	III

Table 3.4 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Fung et al., 2007[128]	CC	Orthopaedic patients	166/111	Forceful sustained motion - Seldom: OR 2.62 (95% CI 1.24 - 6.08), AOR 2.29 (95% CI 0.90 - 5.85) Frequent: OR 2.93 (95% CI 1.42 - 6.02), AOR 2.59 (95% CI 1.14 - 5.85)	III
Wieslander et al., 1989[127]	CC	Hospital patients	38/76	High load on wrist Group 2 - >20 years: OR 6.6 (95% CI 1.4 - 14.7), p=0.01	III
Leclerc et al., 1998[109]	CS	Workers in Manual labour occupations (repetitive work)	151/1059	Just in time (Model 3): OR 2.24 (95% CI 1.40 - 3.57)	III
No effect risk					
Nathan et al., 2002[76]	PC	Industrial workers	34/222	Univariate OR 1.00 (95% CI 0.75 - 1.34), p=0.97; Multivariate OR 0.75 (95% CI 0.21 - 2.72), p=0.66	II
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	OR 1.1 (95% CI 0.3 - 3.3)	II
Leclerc et al., 1998[109]	CS	Workers in Manual labour occupations (repetitive work)	151/1059	Press with hand: Model 2: OR 1.41 (95% CI 0.92 - 2.15); Model 3: OR 1.37 (95% CI 1.04 - 2.43)	III
Silverstein et al., 2009[97]	CS	Manufacturing & Healthcare workers	63/657	*Lifting force* Female <10%: OR 2.29 (95% CI 1.07 - 4.88), >10%: OR 1.42 (95% CI 0.51 - 3.97) Male <10%: OR 3.07 (95% CI 0.63 - 14.90) *Frequency of force exertion* Female ≥5: OR 2.16 (95% CI 0.83 - 5.67) Duty cycle, ≥3-14: OR 2.01 (95% CI 0.84 - 4.81) ≥15: OR 2.22 (95% CI 0.93 - 5.32) Male ≥1 - <5: OR 1.68 (95% CI 0.52 - 5.44) ≥5: OR 2.99 (95% CI 0.97 - 9.23) Duty cycle, ≥3-14: OR 4.25 (95% CI 0.86 - 20.99) Radial/ulnar deviation ≥4% OR lifting force ≥3%: OR 1.98 (95% CI 0.98 - 4.13), AOR 2.35 (95% CI 1.09 - 5.04)	III
Fung et al., 2007[128]	CC	Orthopaedic patients	166/111	Forceful sustained motion - sometimes: OR 1.72 (95% CI 0.79 - 3.76), AOR 1.93 (95% CI 0.80 - 4.65)	III

PC, prospective cohort; HR, hazard ratio; OR, odds ratio; CI, confidence interval; RR, risk ratio; CS, cross sectional; AOR, adjusted odds ratio; CC, case control; kgf, kilogram force.

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

3.3.2.2 Breaks

Five studies investigating manual workers considered breaks during a working day as potentially modifying the risk of CTS (Table 3.5). Two level III studies found break time of more than 75 minutes and less than 15% of daily work time to be associated with increased risk of CTS[99,140] whereas one level II and two level III found breaks to have no effect on the risk of CTS[84,96,103].

Break time during a working day, as risk factor for CTS, was therefore assigned a low level of certainty.

Table 3.5 Summary of all the studies that have investigated breaks as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Maghsoudipour et al., 2008[99]	CS	Industrial workers	47/348	>75 min: Univariate OR 3.50 (95% CI 1.52 - 8.06), p=0.001; Multivariate OR 1.22 (95% CI 0.54 - 6.79), p=0.31	III
Roquelaure et al., 1997[140]	CC	Manufacturing workers	65/65	<15% of daily work time: Logistic model OR 6.0 (95% CI 1.8 - 20.2), p=0.004	III
No effect risk					
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	OR 2.5 (95% CI 0.8 - 8.1)	II
Conlon & Rempel, 2005[103]	CS	Engineers	25/177	Break time per day: Univariate OR 0.84 (95% CI 0.54 - 1.31), p=0.454; Multivariate OR 0.57 (95% CI 0.32 - 1.02), p=0.044 Total number of breaks per day: Univariate OR 1.19 (95% CI 0.74 - 1.91), p=0.472	III
Rosecrance et al., 2002[84]	CS	Construction workers	91/1024	Insufficient break time: OR 1.30, AOR 1.36 (95% CI 0.68 - 2.73)	III

CS, cross sectional; CC, case control; PC, prospective cohort; OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

3.3.2.3 Vibration and the use of power tools and/or machinery

Vibration and the use of power tools and/or machinery as risk factor for CTS were reviewed by twelve studies (Table 3.6). These studies included those considering exposure to vibration as well as those considering the use of power tools and/or machinery. Ten level III studies found that vibration exposure increases the risk of CTS[71,72,81,98,99,119,127,129,146,151]. Nordstrom et al. (1997) found that using power tools for more than 6 hours per day increases the risk of CTS, even after multivariate analyses, whereas using the same tools for less than 6 hours has no effect on risk[129]. Rigouin et al. (2013) found that only females were susceptible for CTS as a result of vibration[98], whereas Jenkins et al. (2012) found that it only had an effect in a participants who were socially deprived[146]. Another study considered vibration together with bending and twisting of the hand, but found that even without the extra hand movements, participants were still at higher risk to develop CTS[72]. The number of years of exposure to vibration also made no difference, with both exposure groups (1-20 years and >20 years) being at higher risk[127].

In contrast, the study by Rigouin et al. (2013) found that, contrary to females, vibration exposure in males made no difference to risk of CTS[98]. Similarly, the other studies, including two level II and two level III study, also found vibration or the use of power tools to have no effect on risk of CTS[76,96,129,151].

As a result of the low quality of the studies finding an increased risk and the limited number of studies contradicting that finding, vibration and the use of power tools and/or machinery as risk factor for CTS was awarded a low level of certainty.

Table 3.6 Summary of all the studies that have investigated vibration or using power tools/machinery as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Nordstrom et al., 1997[129]	CC	General population	206/211	Power tools 6-11 hr/day: AOR 2.52 (95% CI 1.13-5.62); Multivariate OR 3.30 (95% CI 1.11 - 9.80)	III
Maghsoudipour et al., 2008[99]	CS	Industrial workers	47/348	Power tools Univariate OR 6.74 (95% CI 3.41 - 13.31), p=0.00; Multivariate OR 3.23 (95% CI 1.46 - 7.15), p=0.004	III
Coggon et al., 2013[119]	CC	General population	475/799	Vibration: AOR 2.6 (95% CI 1.8 - 3.9); Multivariate OR 2.4 (95% CI 1.6 - 3.8)	III
Rigouin et al., 2013[98]	CS	Working population	156/3554	Vibration Female: Multivariate OR 2.44 (95% CI 1.11 - 5.38), p=0.026	III
Tanaka et al., 1997[71]	CS	General population	145/29929	Vibration: AOR 1.86 (95% CI 1.23 - 2.80)	III
Armstrong et al., 2008[81]	CS	Newly hired manual workers	131/940	Vibration: Logistic regression OR 1.88 (95% CI 1.23 - 2.85)	III
Shiri et al., 2009[151]	CS	Adult (>30yo) survey responders in Finland	~825/5429	Vibration Latest job: AOR 2.3 (95% CI 1.1 - 5.0)	III
Tanaka et al., 1995[72]	CS	Working population	675/176525	Vibration (Bend & twist included): Multivariate OR 1.81 (95% CI 1.27 - 2.57) Vibration (bend & twist excluded): Multivariate OR 3.00	III
Wieslander et al., 1989[127]	CC	Hospital patients	38/76	*Vibration* Group 1 1-20 years: OR 2.7 (95% CI 1.1 - 6.7), p=0.04 >20 years: OR 4.8 (95% CI 1.5 - 15.6), p=0.01 Group 2 1-20 years: OR 4.3 (95% CI 1.4 - 12.9), p=0.01 >20: OR 16.0 (95% CI 2.8 - 90.2), p=0.002	III
Jenkins et al., 2012[146]	CS	General population	1564/~358436	Vibration in socially deprived group: OR 2.33 (95% CI 1.84 - 2.95), p<0.001	III
No effect on risk					
Nathan et al., 2002[76]	PC	Industrial workers	34/222	Vibration: Univariate OR 2.28 (95% CI 0.84 - 6.29), p=0.100; Multivariate OR 3.73 (95% CI 1.04 - 13.33), p=0.04	II
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	Vibration: OR 1.8 (95% CI 0.2 - 10.8)	II
Rigouin et al., 2013[98]	CS	Working population	156/3554	Vibration Male: Multivariate OR 1.52 (95% CI 0.81 - 2.85), p=0.192	III
Shiri et al., 2009[151]	CS	Adult (>30yo) survey responders in Finland	~825/5429	Vibration Former job: AOR 1.7 (95% CI 0.6 - 4.6) Former and latest job: AOR 1.0 (95% CI 0.1 - 7.9)	III

Table 3.6 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Nordstrom et al., 1997[129]	CC	General population	206/211	Power tool use - Hr/day 0.08-0.75 hour: AOR 0.60 (95% CI 0.27 - 0.36); Multivariate OR 0.53 (95% CI 0.17 - 1.64) 1-2 hours: AOR 1.43 (95% CI 0.66 - 3.13); Multivariate OR 1.43 (95% CI 0.52 - 3.90) 2.5-5.5 hours: AOR 1.20 (95% CI 0.59 -2.45); Multivariate OR 1.58 (95% CI 0.63 - 4.00)	III

CC, case control; CS, cross sectional; OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

3.3.2.4 Physical limit/demand

Physical limit or demand, which includes number of working hours per week, was considered as risk factor for CTS by four studies (Table 3.7). Only one level III study that looked at female grocery checkers found working for more than 34 hours per week increased the risk of CTS 2.84-fold[82]. In contrast, one level II and three level III studies found working hours and high physical limit/demand to have no effect on the risk of developing CTS[82,84,96,103].

High physical limit/demand, including working hours per week, was assigned a low level of certainty as a result of the limited number of studies considering this factor in the risk of CTS.

Table 3.7 Summary of all the studies that have investigated high physical workload/limit (including hours per week at work) as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Morgenstern et al., 1991[82]	CS	Female grocery checkers	127/931	Hours per week at work >34 hr/wk: OR 2.84 (95% CI 1.77 - 5.54), AOR 1.86 (95% CI 1.12 - 3.08)	III
No effect on risk					
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	High physical workload: OR 0.6 (95% CI 0.2 - 2.0)	II
Rosecrance et al., 2002[84]	CS	Construction workers	91/1024	High physical limit: OR 1.44, AOR 1.37 (95% CI 0.82 - 2.27)	III
Conlon & Rempel, 2005[103]	CS	Engineers	25/177	Hours per week at work: Univariate OR 1.03 (95% CI 0.92 - 1.15), p=0.621	III
Morgenstern et al., 1991[82]	CS	Female grocery checkers	127/931	Hours per week at work: 26-34 hours: OR 1.62 (95% CI 1.02 - 2.57), AOR 1.53 (95% CI 0.95 - 2.44)	III

CS, cross sectional; OR, odds ratio; AOR, adjusted odds ratio; CI, confidence interval; PC, prospective cohort

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

3.3.2.5 Working conditions/Environment

Working conditions and work environment was investigated as potential risk factor for CTS by three studies (Table 3.8). None of the studies found any effect of these factors on risk of CTS, including various movements and hot/cold/humid conditions[84,96,120].

Working conditions and work environment was assigned a low level of certainty, due to the limited number of studies as well as the different variables that was investigating working conditions/environment as risk factors.

3.3.2.6 Specific occupational actions

Three studies looked at specific occupational actions associated with specific occupations, as a potential risk factor of CTS (Table 3.9). All three level III studies found these actions to have no effect on CTS risk. This includes specific dental procedures performed by dental hygienists[104], working with solvents[129] and using laser scanners and packing/lifting/unloading grocery bags in grocery checkers[82].

The limited number of studies considering specific occupational actions, as well as the wide variety of actions, lead to this risk factor to be assigned a low level of certainty.

Table 3.8 Summary of all the studies that have investigated working conditions and environment as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
No effect on risk					
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	High movement precision: OR 1.4 (95% CI 0.5 - 4.1) High visual load: OR 1.1 (95% CI 0.4 - 3.6) Lack of freedom to change movements: OR 2.4 (95% CI 0.6 - 11.2)	II
Rosecrance et al., 2002[84]	CS	Construction workers	91/1024	Hot/cold/humid environment: OR 1.62, AOR 1.37 (95% CI 0.83 - 2.26) Work fast, short period: OR 1.59, AOR 1.38 (95% CI 0.80 - 2.39) Akward/cramped work: OR 1.01, AOR 0.99 (95% CI 0.61 - 1.59) Work in same position: OR 1.13, AOR 1.11 (95% CI 0.70 - 1.75) Working whilst hurt/injured: OR 1.25, AOR 1.11 (95% CI 0.69 - 1.79) Work scheduling: OR 1.38, AOR 1.12 (95% CI 0.59 - 2.13) Tool characteristics: OR 1.50, AOR 1.39 (95% CI 0.81 - 2.41) Job training: OR 1.24, AOR 0.94 (95% CI 0.34 - 2.50)	III
Yagev et al., 2007[120]	CC	Hospital patients	127/102	Cold environment: Multivariate OR 3.52 (95% CI 1.08 - 11.47), p=0.037	III

PC, prospective control; OR, odds ratio; CI, confidence interval; CS, cross sectional; AOR, adjusted odds ratio; CC, case control

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

Table 3.9 Summary of all the studies that have investigated specific occupational actions as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
No effect on risk					
Anton et al., 2002[104]	CS	Dental hygienists	8/81	Hard calculus patients per week (hr): OR 0.99 (95% CI 0.88 - 1.12) Hours probing per week (hr): OR 0.93 (95% CI 0.60 - 1.44) Hours scaling per week (hr): OR 1.05 (95% CI 0.94 - 1.17) Hours polishing per week (hr): OR 1.04 (95% CI 0.82 - 1.32) Hours flossing per week (hr): OR 1.63 (95% CI 0.85 - 3.14) Hours teaching per week (hr): OR 1.03 (95% CI 0.70 - 1.52)	III
Nordstrom et al., 1997[129]	CC	General population	206/211	Contact with solvents (hr/day) 0.08-0.75 hours: OR 0.63 (95% CI 0.37 - 1.10); Multivariate OR 0.44 (95% CI 0.21 - 0.90) 1-11 hours: 1.43 (95% CI 0.79 - 2.59); Multivariate OR 0.80 (95% CI 0.36 - 1.79)	III
Morgenstern et al., 1991[82]	CS	Female grocery checkers	127/931	Using laser scanner: Multivariate OR 0.99 (95% CI 0.65 - 1.49), p=0.95 Unloading baskets: Multivariate OR 0.97 (95% CI 0.66 - 1.44), p=0.89 Load and lift grocery bags: Multivariate OR 0.94 (95% CI 0.35 - 2.57), p=0.91	III

CS, cross sectional; OR, odds ratio; CI, confidence interval; CC, case control

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

3.3.2.7 Job Rotation

Job rotation, defined as moving between a number of different job tasks through a certain period of time, as risk factor for CTS, was investigated by five studies (Table 3.10). Two of these studies, both level III, found that job rotation in male participants in the working population, as well as no job rotation, increase the risk of developing CTS[98,140]. In contrast, one level II and two level III studies found job and task rotation or lack thereof, has no effect on the risk of CTS[96,97,99].

Consequently, job rotation was assigned a low level of certainty as potential modifier of CTS risk.

Table 3.10 Summary of all the studies that have investigated job rotation as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Rigouin et al., 2013[98]	CS	Working population	156/3554	Male: Multivariate OR 2.45 (95% CI 1.41 - 4.24)	III
Roquelaure et al., 1997[140]	CC	Manufacturing workers	65/65	None: Logistic model OR 6.3 (95% CI 2.1 - 19.3), p=0.002	III
No effect risk					
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	No rotation: OR 0.5 (95% CI 0.1 - 1.8)	II
Maghsoudipour et al., 2008[99]	CS	Industrial workers	47/348	Univariate OR 0.90 (95% CI 0.49 - 1.67), p=0.75; Multivariate OR 0.72 (95% CI 0.33 - 1.58), p=0.42	III
Silverstein et al., 2009[97]	CS	Manufacturing & Healthcare workers	63/657	Work rotation: Female - AOR 1.24 (95% CI 0.60 - 2.55), Male - AOR 1.18 (95% CI 0.46 - 3.03) Task rotation: Female - AOR 1.30 (95% CI 0.61 - 2.77), Male - AOR 1.96 (95% CI 0.72 - 5.33)	III

CS, cross sectional; OR, odds ratio; CI, confidence interval; CC, case control; PC, prospective cohort; AOR, adjusted odds ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

3.3.2.8 Number of occupational risk factors

Two studies considered whether being exposed to multiple occupational risk factors influences the risk of CTS (Table 3.11). Both studies found that the risk of CTS increases with the addition of additional occupational risk factors[127,140] whereas being exposed to only one[127] or two or less[140], has no effect on risk.

Considering the limited number of studies looking specifically at the number of occupational risk factors, this factor was assigned a low level of certainty.

Table 3.11 Summary of all the studies that have investigated number of occupational risk factor as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Roquelaure et al., 1997[140]	CC	Manufacturing workers	65/65	3: OR 5.6 (95% CI 1.6 - 24.5) 4: OR 93.7 (95% CI 13.4 - 93.8) ≥5: OR 90.0 (95% CI 8.0 - 366.5)	III
Wieslander et al., 1989[127]	CC	Hospital patients	38/76	Group 1 2 risk factors: OR 3.3 (95% CI 1.2 - 9.1), p<0.001 >2 risk factors: OR 7.1 (95% CI 2.2 - 22.7), p<0.001	III
No effect risk					
Roquelaure et al., 1997[140]	CC	Manufacturing workers	65/65	≤2: OR 1.0 (95% CI 0.1 - 5.9)	III
Wieslander et al., 1989[127]	CC	Hospital patients	38/76	Group 1 1 risk factor: OR 1.7 (95% CI 0.7 - 4.4)	III

CC, case control; OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio PC, prospective cohort;

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

3.3.2.9 Other

A total of seven studies considered other specific occupational procedures as potential risk factors for CTS (Table 3.12). Two level III studies found an increase in CTS risk with elementary operations of 10 seconds and less and manual workstation supply[140] as well as working with temporary workers[98]. The five studies that found no effect on risk, including one level II and four level III studies, investigated cycle time[96,109], high loads on the wrist[127], neck position during work[119] and working overtime[133], respectively.

A low level of certainty was assigned to each one of the abovementioned factors, considering the lack of a large number of good quality studies verifying these associations.

Table 3.12 Summary of all the studies that have investigated other specific occupational procedures as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Roquelaure et al., 1997[140]	CC	Manufacturing workers	65/65	Elementary operation ≤10s: Logistic model OR 8.8 (95% CI 1.8 - 44.4), p=0.008 Manual workstation supply: Logistic model OR 5.0 (95% CI 2.2 - 21.2), p=0.001	III
Rigouin et al., 2013[98]	CS	Working population	156/3554	Working with temporary workers Female: Multivariate OR 1.99 (95% CI 1.23 - 3.25), p=0.005	III
No effect on risk					
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	Cycle time: <30s: OR 0.7 (95% CI 0.2 - 2.3)	II
Leclerc et al., 1998[109]	CS	Workers in Manual labour occupations (repetitive work)	151/1059	Cycle time: Model 2 30-59s: OR 1.03 (95% CI 0.56 - 1.89) 10-29s: OR 1.33 (95% CI 0.75 - 2.37) <10s: OR 1.90 (95% CI 1.04 - 3.48)	III
Wieslander et al., 1989[127]	CC	Hospital patients	38/76	High load on wrist Group 1 1-20 years: OR 1.7 (95% CI 0.7 - 3.9) >20 years: OR 2.1 (95% CI 0.8 - 5.5) Group 2 1-20 years: OR 2.1 (95% CI 0.8 - 5.2)	III
Coggon et al., 2013[119]	CC	General population	475/799	Neck position Neck bent forward, >2 hours: AOR 1.2 (95% CI 0.9 - 1.5) Work with neck twisted, >0.5 hours: AOR 1.3 (95% CI 1.0 - 1.6)	III
Babski-Reeves & Crumpton-Young, 2003[133]	CS	Workers at a fish processing facility	33/73 hands	Overtime Model 1: Logistic regression OR 0.64 (95% CI 0.43 - 0.96), p=0.030 Model 2: Logistic regression OR 0.82 (95% CI 0.68 - 1.00), p=0.047	III

CC, case control; OR, odds ratio; CI, confidence interval; CS, cross sectional; PC, prospective cohort; AOR, adjusted odds ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

3.3.3 Biomechanical Factors

Factors that influence specifically the upper limbs, such as overuse, awkward positioning, carrying weight, increased strain or force as well as specific biomechanical stressors were investigated by various studies.

3.3.3.1 Upper body movement

Repetitive upper body movement, with specific emphasis on the movement of the upper limbs, is believed to increase the risk of developing CTS and eighteen studies investigated this risk factor (Table 3.13). Only one level II study found that an elbow posture that deviates from the neutral position lead to an increased risk of CTS[114] whereas 10 level III studies[71,72,81,99,116,119,120,128,129,151] also found increased risk with various upper body/upper limb movements.

Similarly, one level II study[96] found no effect of wrist flexion, extension and deviation as well as rapid trigger movements on the risk of developing CTS. Furthermore, another eight level III studies[27,81,84,98,116,128,129,133] found no effect of these and other type of upper body and upper limb movements on the risk of CTS.

As a consequence of the lack of consensus about the effect of upper body and upper limb movements on risk of CTS, this risk factor was assigned a low level of certainty.

Table 3.13 Summary of all the studies that have investigated upper body movement as a risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Werner et al., 2005[114]	PC	Assembly workers	20/169	Elbow posture: OR 8.08 (95% CI 1.48 - 44.22), p=0.02	II
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	Wrist flexion >45°: OR 0.6 (95% CI 0.1 - 3.2) Wrist extension >45°: OR 1.2 (95% CI 0.4 - 3.4) Wrist deviation: OR 1.0 (95% CI 0.1 - 3.1) Rapid trigger movements: OR 2.8 (95% CI 0.6 - 11.5), Logistic model: OR 3.8 (95% CI 1.0 - 17.2), p=0.058	II
Coggon et al., 2013[119]	CC	General population	475/799	Bending/straightening of elbow > 1 hr/day: AOR 1.5 (95% CI 1.2 - 1.9) Hand/finger movement, > 4 hr/day: AOR 1.7 (95% CI 1.3 - 2.1); Multivariate OR 1.5 (95% CI 1.1 - 1.9)	III
Nordstrom et al., 1997[129]	CC	General population	206/211	Bending/twisting (hands) (hr/day) 3.5-6 hr/day: OR 2.33 (95% CI 1.24 - 4.36); Multivariate OR 2.65 (95% CI 1.83 - 5.92) 7-16 hr/day: OR 2.47 (95% CI 1.38 - 4.43); Multivariate OR 2.11 (95% CI 0.98 - 4.52)	III
Yagev et al., 2007[120]	CC	Hospital patients	127/102	Repetitive motion of wrist: Multivariate OR 2.15 (95% CI 1.14 - 4.07), p=0.018	III
Fung et al., 2007[128]	CC	Orthopaedic patients	166/111	Extension - frequent: OR 2.41 (95% CI 1.14 - 5.10), AOR 2.69 (95% CI 1.10 - 6.55) Flexion - seldom: OR 2.80 (95% CI 1.26 - 6.23), AOR 3.16 (95% CI 1.28 - 7.80) Flexion - sometimes: OR 2.92 (95% CI 1.35 - 6.29), AOR 2.12 (95% CI 0.90 - 4.96) Flexion - frequent: OR 5.17 (95% CI 2.34 - 11.45), AOR 4.44 (95% CI 1.83 - 10.73)	III
De Krom et al., 1990[116]	CC	General population	156/473	Working with flexed wrist (hrs/wk) 0-5 years ago 1-7 hours: RR 1.5 (95% CI 1.3 - 1.9) 8-19 hours: RR 3.0 (95% CI 1.8 - 4.9) 20-40 hours: RR 8.7 (95% CI 3.1 - 24.1) Working with extended wrist (hrs/wk) 0-5 years ago 20-40 hours: RR 5.4 (95% CI 1.1 - 27.4)	III
Maghsoudipour et al., 2008[99]	CS	Industrial workers	47/348	Bending/twisting (wrist) Univariate OR 18.62 (95% CI 2.53 - 136.88), p=0.00; Multivariate OR 5.62 (95% CI 0.56 - 55.6), p=0.14 Rapid hand movement Univariate OR: 3.17 (95% CI 1.30 - 7.69), p=0.004; Multivariate OR 4.44 (95% CI 1.41 - 14.02), p=0.01	III
Tanaka et al., 1997[71]	CS	General population	145/29929	Bending/twisting: AOR 5.50 (95% CI 3.21 - 9.42)	III
Armstrong et al., 2008[81]	CS	Newly hired manual workers	131/940	Twisting forearm: Logistic regression 1.78 (95% CI 1.18 - 2.69)	III
Tanaka et al., 1995[72]	CS	Working population	675/176525	Bend & twist (vibration included): Multivariate OR 5.23 (95% CI 3.44 - 7.96) Bend and twist (vibration excluded): Multivariate OR 5.99	III
Shiri et al., 2009[151]	CS	Adult (>30yo) survey responders in Finland	~825/5429	Repetitive movements of hand/wrist ≥2h/day Former job: AOR 2.5 (95% CI 1.1 - 5.7) Latest job: AOR 2.4 (95% CI 1.3 - 4.5) Both (former & latest job): AOR 2.8 (95% CI 1.4 - 5.6)	III

Table 3.13 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
No effect on risk					
Ali et al., 2006[27]	CS	Computer workers	85/563	Hand flexed or extended: OR 1.3 (95% CI 0.8 - 2.1), p=0.300	III
Armstrong et al., 2008[81]	CS	Newly hired manual workers	131/940	Bend/twist hands: Logistic regression OR 1.72 (95% CI 1.07 - 2.76)	III
Nordstrom et al., 1997[129]	CC	General population	206/211	Bend or twist hands (hr/day) 0.25-1.75 hours per day: OR 1.34 (95% CI 0.64 - 2.80); Multivariate OR 2.42 (95% CI 0.88 - 6.62) 2-3 hours per day: OR 1.23 (95% CI 0.60 - 2.53); Multivariate OR 1.27 (95% CI 0.50 - 3.26)	III
Rigouin et al., 2013[98]	CS	Working population	156/3554	Wrist bending and/or high physical demand *Female* One factor: Multivariate OR 1.64 (95% CI 0.97 - 2.78), Both factors: Multivariate OR 1.57 (95% CI 0.79 - 3.12), p=0.162 *Male* One factor: Multivariate OR 1.64 (95% CI 0.87 - 3.10), Both factors: Multivariate OR 2.21 (95% CI 1.04 - 4.68), p=0.103	III
Fung et al., 2007[128]	CC	Orthopaedic patients	166/111	Extension Seldom: OR 1.19 (95% CI 0.55 - 2.61), AOR 0.74 (95% CI 0.30 - 1.84) Sometimes: OR 1.04 (95% CI 0.50 - 2.18), AOR 0.75 (95% CI 0.32 - 1.77) Straight Seldom: OR 0.66 (95% CI 0.24 - 1.85), AOR 2.62 (95% CI 0.82 - 8.43) Sometimes: OR 0.47 (95% CI 0.17 - 1.31), AOR 1.44 (95% CI 0.72 - 2.87) Frequent: OR 0.43 (95% CI 0.15 - 1.21), AOR 1.02 (95% CI 0.50 - 2.08)	III
Babski-Reeves & Crumpton-Young, 2003[133]	CS	Workers at a fish processing facility	33/73 hands	Flexion & extension: Logistic regression OR 1.00 (95% CI 1.00 - 1.00), p=0.025 Flexion & extension repetitions: Logistic regression OR 1.00 (95% CI 1.00 - 1.00), p=0.003 Radial/Ulnar deviation repetitions: Logistic regression OR 1.00 (95% CI 1.00 - 1.00), p=0.003 Pronation (Model 1): Logistic regression OR 1.03 (95% CI 1.00 - 1.06), p=0.051 Pronation (Model 2): Logistic regression OR 1.06 (95% CI 1.02 - 1.11), p=0.006	III
De Krom et al., 1990[116]	CC	General population	156/473	Extended wrist (hr/wk) 1-7 hours: RR 1.4 (95% CI 1.0 - 1.9) 8-9 hours: RR 2.3 (95% CI 1.0 - 5.2) Extended & flexed wrist in combination 1-7 hours: RR 1.1 (95% CI 0.9 - 1.2) 8-19 hours: RR 1.2 (95% CI 0.8 - 1.7) 20-40 hours: RR 1.4 (95% CI 0.7 - 2.9)	III
Rosecrance et al., 2002[84]	CS	Construction workers	91/1024	Bend/twist back: OR 0.89, AOR 0.87 (95% CI 0.54 - 1.41)	III

PC, prospective cohort; OR, odds ratio; CI, confidence interval; CS, cross sectional; CI, confidence interval AOR, adjusted odds ratio;

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

3.3.3.2 Biomechanical stressors

Seven studies considered various biomechanical stressors as potentially modifying the risk of CTS (Table 3.14). Of the five investigations that found an increase in risk with certain biomechanical stressors, three were prospective studies (two level I and one level II). These studies considered specifically the working population and found that working between the action limit (AL) and threshold limit value (TLV), also known as the "borderline load"[94] as well as working above the TLV, also known as "unacceptable loads" are associated with an increased risk of CTS[78]. The threshold limit ratio was also associated with increased risk[118]. Two level III studies also considered different models and job categories that they found to be significant in increasing the risk of CTS[113,152].

Six investigations, including two level I and two level II studies, found various biomechanical stressors, including values about the TLV, to have no effect on CTS risk[78,93,94,96,113,152]. Considering the conflicting results of various biomechanical stressors as risk factor for CTS, this factor was assigned a low level of certainty.

Table 3.14 Summary of all the studies that have investigated biomechanical stressors as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Bonfigliol i et al., 2013[94]	PC	Industrial and service worker groups	84/2837	Between AL and TLV: IRR 2.03 (95% CI 1.57 - 2.61), Multivariate IRR 1.95 (95% CI 1.21 - 3.16)	I
Burt et al., 2013[118]	PC	Working population with low, medium or high level of hand activity	29/318	Threshold limit ratio, Per unit increase (Model 2) : Multivariate HR 1.40 (95% CI 1.11 - 1.78)	I

Table 3.14 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Violante et al., 2007[78]	PC	Working population	153/1939	Above TLV: OR 2.8 (95% CI 1.9 - 4.0), Multivariate OR 3.0 (95% CI 2.0 - 4.5)	II
Burt et al., 2011[113]	CS	Working population with low, medium or high level of hand activity	57/420	HAL TLV Model - TLV (Cat 3): Multivariate OR 2.96 (95% CI 1.51 - 5.80) Ratings based model - HAL TWA: Multivariate OR 2.21 (95% CI 1.17 - 4.15) Peak force match: Quantitative Model (Cat 3): Multivariate OR 2.74 (95% CI 1.32 - 5.68) Exerts/min: Quantitative Model (Cat 3) if BMI ≥ 30: Multivariate OR 3.35 (95% CI 1.14 - 9.87)	III
Yagev et al., 2001[152]	CS	Patients undergoing electrophysiological tests	123/246	*Job category* HF-LR vs LF-LR (Men): Multivariate OR 2.8 (95% CI 1.1 - 6.9), p=0.05 LF-HR vs LF-LR (Women): Multivariate OR 7.4 (95% CI 1.9 - 2.8), p=0.05 LF-HR vs LF, LR (All): Multivariate OR 4.7 (95% CI 1.8 - 12.5), p=0.05 HF-LR vs LF-LR (All): Multivariate OR 3.21 (95% CI 1.5 - 6.9), p=0.05	III
No effect on risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	*TLV for HAL Score* Per unit increase for score ≤ 12.5: HR 3.2 (95% CI 0.78 - 13.2), p=0.11 Per unit increase for score > 12.5: HR 0.21 (95% CI 0.02 - 2.69), p=0.10 *Strain Index* SI > 6.1: HR 2.2 (95% CI 0.92 - 5.37), p=0.07 Per unit increase for SI ≤ 13.5: HR 1.11 (95% CI 1.00 - 1.23), p=0.005 Per unit increase for SI > 13.5: HR 0.99 (95% CI 0.95 - 1.03), p=0.07	I
Bonfigliol i et al., 2013[94]	PC	Industrial and service worker groups	84/2837	Above TLV: IRR 2.53 (95% CI 0.97 - 6.60), Multivariate IRR 2.70 (95% CI 1.48 - 4.91)	I
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	Ergonomic Score Left hand, score >4: OR 0.6 (95% CI 0.2 - 1.6) Right hand, score >4: OR 0.8 (95% CI 0.3 - 2.6) Both hands, score >8: OR 0.8 (95% CI 0.3 - 2.4)	II
Violante et al., 2007[78]	PC	Working population	153/1939	Between AL and TLV: OR 1.2 (95% CI 0.8 - 2.0), Multivariate OR 1.5 (95% CI 0.9 - 2.5)	II
Yagev et al., 2001[152]	CS	Patients undergoing electrophysiological tests	123/246	Job category *Female* High frequency, low repetition (HF-LR) vs LF-LR: Multivariate OR 7.0 (95% CI 0.8 - 6.2) *Male* Low frequency, high repetition (LF-HR) vs Low frequency, low repetition (LF-LR): Multivariate OR 2.2 (95% CI 0.5 - 9.9)	III

Table 3.14 Continued

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Burt et al., 2011[113]	CS	Working population with low, medium or high level of hand activity	57/420	*HAL TLV Model* TLV Cat 2 vs 1: Multivariate OR 2.28 (95% CI 0.58 - 8.88) *Ratings based Model* HAL TWA (0-8), if female: Multivariate OR 1.03 (95% CI 0.83 - 1.28) HAL TWA (0-8), if male: Multivariate OR 1.38 (95% CI 1.05 - 1.81) Peak worker RPE: Multivariate OR 1.14 (95% CI 1.01 - 1.29) *Quantitative Model* Peak force match Cat 2 vs 1: Multivariate OR 1.33 (95% CI 0.58 - 3.04) Exerts/min Cat 2 vs 1 if BMI < 30: Multivariate OR 1.40 (95% CI 0.45 - 4.34) Exerts/min Cat 3 vs 1 if BMI < 30: Multivariate OR 1.13 (95% CI 0.44 - 2.93) Exerts/min Cat 2 vs 1 if BMI ≥ 30: Multivariate OR 2.92 (95% CI 0.90 - 9.46)	III

PC, prospective cohort; IRR, incidence rate ratio; OR, odds ratio; CI, confidence interval; CS, cross sectional; AOR, adjusted odds ratio; CC, case control; AL, action limit; TLV, threshold limit value; HAL, hand activity level; HF-LR, high frequency, low repetition; LF-HR, low frequency, high repetition; LF-LR, low frequency-low repetition; SI, strain index; HR, hazard ratio; TWA, time weighted average; RPE, rate of perceived exertion

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

3.3.3.3 Working overhead

Only three level III studies investigated working overhead as a potential risk factor of CTS (Table 3.15). Working overhead for more than 1 hour per day was found to increase the risk of CTS by Coggon et al. (2013)[119]. Adults whose former or current occupation involved working overhead was also at increased risk of CTS[151]. In contrast, working overhead was found to have no effect on risk by Rosecrance et al. (2002).

Considering the little information available, working overhead was assigned a low level of evidence.

Table 3.15 Summary of all the studies that have investigated working overhead as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Coggon et al., 2013[119]	CC	General population	475/799	>1 hr/day: AOR 1.8 (95% CI 1.3 - 2.5)	III
Shiri et al., 2009[151]	CS	Adult (>30yo) survey responders in Finland	~825/5429	Former jobs: AOR 2.4 (95% CI 1.3 - 4.6) Latest job: AOR 2.1 (95% CI 1.2 - 3.7) Both (former & latest job): AOR 2.7 (95% CI 1.4 - 5.2)	III
No effect on risk					
Rosecrance et al., 2002[84]	CS	Construction workers	91/1024	OR 1.66, AOR 1.70 (95% CI 1.06 - 2.72)	III

CS, cross sectional; CC, case control; OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

3.3.3.4 Lift/carry weight

Five studies investigated the effect of lifting and/or carrying weight as a potential modifier of CTS risk (Table 3.16). Three studies, including one prospective study looking at industrial workers, found this risk factor to be associated with an increase in CTS risk whereas three level III studies found it to have no effect on risk. Lifting and/or carrying weight was therefore assigned a low level of certainty.

Table 3.16 Summary of all the studies that have investigated lifting and/or carrying weight as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Nathan et al., 2002[76]	PC	Industrial workers	34/222	Univariate OR 0.99 (95% CI 0.76 - 1.29), p=0.94; Multivariate OR 1.17 (95% CI 0.37 - 3.67)	II
Armstrong et al., 2008[81]	CS	Newly hired manual workers	131/940	≥2 lbs: Logistic regression OR 3.31 (95% CI 1.54 - 7.12)	III
Shiri et al., 2009[151]	CS	Adult (>30yo) survey responders in Finland	~825/5429	>5kg, ≥2x/minute, 2h/day Latest job: AOR 2.6 (95% CI 1.5 - 4.6) Both (Former & current job): AOR 4.8 (95% CI 2.7 - 8.6) >20kg ≥10x/day - Latest job: AOR 1.9 (95% CI 1.1 - 3.3) Both (Former & Latest job): AOR 2.8 (95% CI 1.4 - 5.6)	III
No effect on risk					
Coggon et al., 2013[119]	CC	General population	475/799	≥5kg: AOR 1.3 (95% CI 1.0 - 1.6)	III
Shiri et al., 2009[151]	CS	Adult (>30yo) survey responders in Finland	~825/5429	>5kg, ≥2x/minute, 2h/day - Former job: AOR 2.0 (95% CI 0.9 - 4.0) >20kg, ≥10x/day - Former job: AOR 1.1 (95% CI 0.5 - 2.5)	III
Rosecrance et al., 2002[84]	CS	Construction workers	91/1024	OR 1.14, AOR 1.02 (95% CI 0.60 - 1.74)	III

PC, prospective cohort; CS, cross sectional; CC, case control; OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

3.3.3.5 Job strain

Two prospective cohort studies investigated job strain as potential risk factor for CTS (Table 3.17). Harris-Adamson et al. (2013) found high job strain, defined as high demand and low control, to increase the risk of CTS 1.86-fold whereas active (high demand and high control) and passive (low demand and low control) job strain had no effect on risk of CTS[74]. Similarly, Burt et al. (2013) found that high, compared to low/passive job strain, also has no effect on CTS risk[118].

As a result of the low number of studies considering job strain as risk factor, it was assigned a low level of certainty as potential modifier of CTS risk.

Table 3.17 Summary of all the studies that have investigated job strain as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	High: Multivariate HR 1.86 (95% CI 1.11 - 3.14), p=0.02	I
No effect on risk					
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	Active: Multivariate HR 1.48 (95% CI 0.83 - 2.66), p=0.18 Passive: Multivariate HR 1.23 (95% CI 0.67 - 2.27), p=0.50	I
Burt et al., 2013[118]	PC	Working population with low, medium or high level of hand activity	29/318	High (Model 2): Multivariate HR 2.3 (95% CI 1.00 - 4.54)	I

PC, prospective cohort; HR, hazard ratio; CI, confidence interval

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

3.3.3.6 Repetition

Eight studies considered repetition as potential modifier of CTS risk (Table 3.18). Only one level III study found an increase in risk in those participants exposed to repetitive movements of the wrist for more than 20 years[127]. The same study found no effect on risk in participants exposed for less than 20 years. Similarly, another 6 studies, of which two are of prospective design, found various forms of repetition, including performing a similar movement for more than 50% of work time, repetition combined with force and frequent repetitive exposure, to have no effect on the risk of CTS[76,84,96,105,107,121,128].

Considering the lack of evidence that repetition modifies the risk of CTS, this risk factor was assigned a low level of certainty. It is, however, interesting to note that although there was

not sufficient evidence to determine whether repetition is a true risk factor of CTS, there was a definite trend towards blue-collar workers (working class individuals performing manual labour) being more susceptible to developing CTS (section 3.3.1.1). Since manual labour occupations often involve highly repetitive hand/wrist action, future research should focus on repetitive motion specifically in manual labour occupations to determine whether there is an association.

Table 3.18 Summary of all the studies that have investigated repetition as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Wieslander et al., 1989[127]	CC	Hospital patients	38/76	Group 1 - >20 years: OR 4.6 (95% CI 1.8 - 11.9), p=0.002 Group 2 - >20 years: OR 9.6 (95% CI 2.8 - 33.0), p<0.001	III
No effect on risk					
Nathan et al., 2002[76]	PC	Industrial workers	34/222	Univariate OR 1.05 (95% CI 1.39 - 0.79), p=0.73; Multivariate OR 1.14 (95% CI 0.59 - 2.20), p=0.69	II
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	Similar movement for >50% of work time: OR 0.9 (95% CI 0.2 - 4.8)	II
Chiang et al., 1993[107]	CS	Fish-processing workers	30/177	Repetition x force OR 1.1 (95% CI 0.7 - 1.8) Female: OR 1.5 (95% CI 0.8 - 2.8)	III
Rosecrance et al., 2002[84]	CS	Construction workers	91/1024	OR 1.65, AOR 1.54 (95% CI 0.92 - 2.56)	III
Forst et al., 2007[121]	CS	Spine surgeons	107/264	Multivariate OR 1.84 (95% CI 1.06 - 3.19), p=0.030	III
Wieslander et al., 1989[127]	CC	Hospital patients	38/76	1-20 years (Group 1): OR 1.5 (95% CI 0.5 - 4.4) 1-20 years (Group 2): OR 2.3 (95% CI 0.7 - 7.9)	III
Fung et al., 2007[128]	CC	Orthopaedic patients	166/111	Seldom: OR 1.11 (95% CI 0.44 - 2.78), AOR 1.07 (95% CI 0.37 - 3.15) Sometimes: OR 1.11 (95% CI 0.47 - 2.64), AOR 0.68 (95% CI 0.24 - 1.91) Frequent: OR 1.45 (95% CI 0.68 - 3.13), AOR 0.93 (95% CI 0.37 - 2.36)	III
Latko et al., 1999[105]	CS	Workers at manufacturing facility	19/333	OR 1.22 (95% CI 0.98 - 1.53)	III

Legend appears on following page.

From previous page:

CC, case control; CS, cross sectional; OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

3.3.3.7 Grip force

Five studies investigated grip force, including finger and power grip force, on the risk of developing CTS (Table 3.19).

Table 3.19 Summary of all the studies that have investigated grip force as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Silverstein et al., 2009[97]	CS	Manufacturing & Healthcare workers	63/657	Power grip force (female): OR 2.90 (95% CI 1.44 - 5.87)	III
Shiri et al., 2009[151]	CS	Adult (>30yo) survey responders in Finland	~825/5429	Grip forces ≥1h/day - Latest job: AOR 2.8 (95% CI 1.7 - 4.7) Both (former & latest job): AOR 3.4 (95% CI 1.7 - 6.6)	III
No effect on risk					
Armstrong et al., 2008[81]	CS	Newly hired manual workers	131/940	Finger in pinch grip: Logistic regression OR 1.24 (95% CI 1.24 (95% CI 0.82 - 1.86) Use finger/thumb for pressing :Logistic regression OR 1.19 (95% CI 0.80 - 1.76)	III
Silverstein et al., 2009[97]	CS	Manufacturing & Healthcare workers	63/657	Pinchgrip force (female): OR 2.03 (95% CI 1.01 - 4.08), Men: OR 1.00 (95% CI 0.40 - 2.45) Powergrip force (male): OR 2.43 (95% CI 1.02 - 5.80)	III
Shiri et al., 2009[151]	CS	Adult (>30yo) survey responders in Finland	~825/5429	≥1h/day - Former job: AOR 2.2 (95% CI 1.0 - 4.6)	III
Rosecrance et al., 2002[84]	CS	Construction workers	91/1024	Grasp small objects: OR 1.91, AOR 1.44 (95% CI 0.82 - 2.83)	III
De Krom et al., 1990[116]	CC	General population	156/473	1-7 hours: RR 0.9 (95% CI 0.8 - 1.1) 8-19 hours: RR 0.8 (95% CI 0.5 - 1.3) 20-40 hours: RR 0.7 (95% CI 0.3 - 1.6)	III

CS, cross sectional; OR, odds ratio; CI, confidence interval

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

Only two cross-sectional studies found that females exposed to power grip force, as well as participants exposed to grip forces for an hour or more per day in their latest as well as former and latest job, to be at increased risk of CTS[97,151]. These same studies found pinch grip force in females and power grip force in males, as well as exposure of an hour or more of grip force in the participant's former job, to have no effect on CTS risk[97,151]. Similarly, another three level III studies also found various forms of grip force to have no effect on CTS risk[81,84,116]. As a result of the limited number of studies available showing grip force to alter the risk of CTS, it was awarded a low level of certainty to modify the risk of CTS.

3.3.4 Psychosocial factors

Although not traditionally seen as a major risk factor for CTS, psychosocial factors have been implicated as potentially playing a role in CTS risk with certain psychosocial factors such as low job control being associated with increased risk[20,26].

3.3.4.1 General and Mental health

Four studies considered the effect of general health on the risk of CTS (Table 3.20), while two level III studies investigated the association of mental or psychological problems with risk of CTS (Table 3.20). Two prospective cohort studies found that general health being "worse than others" [93] and good[74], respectively, was associated with an increase in risk of CTS. These same studies found that having somewhat better, or similar health as others[93], or having fair or poor health[74] did not have any effect on risk of CTS. Similarly, two lower quality (level II and level III, respectively) studies found general health to not have any effect[74,101]. Leclerc et al. (1998) found that having psychological problems lead to an increase in CTS risk, whereas only having 'some' problems had no effect on risk[109]. A study by Coggon et al. (2013) also found that intermediate or poor mental health had no effect on the risk of developing CTS[119]. Only one prospective cohort study specifically considered depression as a potential risk factor for CTS and found that three different levels of depression have different effects on the risk of developing CTS[93]. More specifically, always

feeling depressed had a 5.2-fold increase in risk whereas never being depressed reduced the risk of developing CTS 10-fold. Often having feelings of depression had no effect on risk[93].

Physical and mental exhaustion as potential risk factors for CTS was investigated by two level I studies (Table 3.20). Both studies found that various levels of exhaustion had no effect on CTS risk[74,93] and as a result, exhaustion as risk factor for CTS was awarded a low level of certainty.

Considering the limited number of studies, and the contradictory findings, general and mental health as modifier of CTS risk was awarded a low level of certainty. Considering that only one study has investigated depression as risk factor for CTS, it was also assigned a low level of certainty.

Table 3.20 Summary of all the studies that have investigated general health as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	General health - Worse than others: HR 3.04 (95% CI 1.27 - 7.25), p=0.03 Depressed - Always: HR 5.2 (95% CI 1.22 - 21.97), p=0.03	I
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	General health - Good: IRR 1.7 (95% CI 1.20 - 2.44); Multivariate HR 1.71 (95% CI 1.21 - 2.42), p=0.00	I
Leclerc et al., 1998[109]	CS	Workers in Manual labour occupations (repetitive work)	151/1059	Mental health - problems Model 1: OR 2.32 (95% CI 1.48 - 3.63) Model 2: OR 2.32 (95% CI 1.40 3.82) Model 3: OR 2.34 (95% CI 1.42 - 3.85)	III
Decreased risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	Depressed - Never: HR 0.1 (95% CI 0.01 - 0.68), p=0.02	I

Table 3.20 Continued

Article	Study design	Population	Number of CTS/CONa	Results	Level of Evidence
No effect on risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	General health - Somewhat better than others: HR 1.6 (95% CI 0.45 - 5.85), p=0.45 General health - the same as others: HR 1.7 (95% CI 0.47 - 5.83), p=0.43 Depressed - Often: HR 1.1 (95% CI 0.50 - 2.49), p=0.78	I
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	General health - Fair/poor: IRR 1.76 (95% CI 1.03 - 2.91), Multivariate HR 1.52 (95% CI 0.91 - 2.54), p=0.11	I
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	Physical exhaustion - Moderate to severe: Multivariate HR 1.45 (95% CI 1.05 - 2.00), p=0.03 Mental exhaustion - Moderate to severe: Multivariate HR 1.34 (95% CI 0.96 - 1.87), p=0.08	I
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	Physical exhaustion Seldom: HR 1.2 (95% CI 0.36 - 4.28), p=0.73 Often: HR 1.8 (95% CI 0.50 - 6.23), p=0.38 Always: HR 3.4 (95% CI 0.81 - 14.18), p=0.10 Mental exhaustion Seldom: HR 1.1 (95% CI 0.47 - 2.67), p=0.80 Often: HR 2.7 (95% CI 1.07 - 6.59), p=0.04 Always: HR 3.7 (95% CI 0.78 - 17.34), p=0.10	I
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	GHQ score ≥14/36: OR 1.2 (95% CI 0.4 - 3.7) GHQ score ≥90%: OR 3.1 (95% CI 0.7 - 9.3); Logistic model: OR 4.3 (95% CI 1.0 - 18.6), p=0.49	II
Raman et al., 2012[101]	CS	Office workers	88/382	General health - Excellent/very good: OR 0.4 (95% CI 0.2 - 3.3) General health - Fair/poor: OR 1.8 (95% CI 1.0 - 3.3)	III
Leclerc et al., 1998[109]	CS	Workers in Manual labour occupations (repetitive work)	151/1059	Mental health - some problems Model 1: OR 1.36 (95% CI 0.91 - 2.04) Model 2: OR 1.15 (95% CI 0.73 - 1.82) Model 3: OR 1.16 (95% CI 0.74 - 1.82)	III
Coggon et al., 2013[119]	CC	General population	475/799	Mental health - intermediate: AOR 1.2 (95% CI 0.9 - 1.7); Multivariate OR 1.3 (95% CI 0.9 - 1.7) Mental health - poor: AOR 1.3 (95% CI 1.0 - 1.8); Multivariate OR 1.4 (95% CI 1.0 - 1.9)	III

PC, prospective cohort; CI, confidence interval; HR, hazard ratio; IRR, incidence rate ratio; GHQ, general health questionnaire; CS, cross sectional; OR, odds ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

3.3.4.2 Job satisfaction and psychological demand

Only three studies investigated job satisfaction in the working population, to determine whether it has any effect on CTS risk (Table 3.21). One level I study found that being dissatisfied or very dissatisfied in a particular occupation increases the risk of developing

CTS, but that being satisfied or apathetic has no effect on risk[93]. On the contrary, another level I and one level III study found that job satisfaction had no effect on risk[74,119]. Three studies investigated psychological demand in the working population as a potential modifier of CTS risk (Table 3.21). Only one level III study found that women working under high demand are at increased risk of developing CTS[98]. In contrast, one level II and one level III study found that high work demand and high decision latitude, both aspects of psychological demand, have no effect on the risk of CTS[96,97].

Job satisfaction was awarded a low level of certainty, owing to the limited number of studies showing its effect on CTS risk. Similarly, owing to the limited number of good quality studies, psychological demand was allocated a low level of certainty.

3.3.4.3 Support

Support, as risk factor for CTS, was considered by seven studies (Table 3.22). Two studies considered whether supervisor support, in a working population, plays a role in CTS risk. Both studies (level I and level III, respectively) showed that little or no support from the supervisor lead to an increase in CTS risk[93,119] whilst a supervisor seldom or often showing appreciation had no effect on the risk of developing this condition[93]. Furthermore, the employer's attitude, specifically whether participants feel that their employer cares (about them), was investigated by two studies. One level III, case control study, found that when participants slightly or strongly disagreed when asked whether they felt that their employer care, they were at increased risk whereas there was no risk when participants agreed with the statement, or when they had no specific employer[129]. In contrast, a prospective cohort study found that regardless of the answer given by participants, the employer attitude had no effect on CTS risk[93]. When social and/or co-worker support was considered, five studies (including two level I and two level II studies) found that various levels support had no effect on developing CTS.

Support, from the employer, supervisor and co-workers, as risk factor for CTS, was awarded a low level of certainty.

Table 3.21 Summary of all the studies that have investigated job satisfaction as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	Job satisfaction Dissatisfied/Very dissatisfied: HR 7.2 (95% CI 1.45 - 35.78), p=0.02	I
Rigouin et al., 2013[98]	CS	Working population	156/3554	Psychological demand: Women: Multivariate OR 1.90 (95% CI 1.17 - 3.09), p=0.009	III
No effect on risk					
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	Job satisfaction Satisfied: Multivariate HR 1.43 (95% CI 1.03 - 1.99), p=0.03 Dissatisfied/very dissatisfied: Multivariate HR 1.28 (95% CI 0.79 - 2.08), p= 0.31	I
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	Job satisfaction Satisfied: HR 2.9 (95% CI 0.86 - 9.61), p=0.31 Neither/Nor: HR 2.0 (95% CI 0.52 - 7.76), p=0.09	I
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	Psychological demand: OR 1.0 (95% CI 0.3 - 3.1) High work demand: OR 1.7 (95% CI 0.5 - 5.4)	II
Silverstein et al., 2009[97]	CS	Manufacturing & Healthcare workers	63/657	Psychological demand - High decision latitude: Women: AOR 1.24 (95% CI 0.61 - 2.51) Men: AOR 0.56 (95% CI 0.23 - 1.37)	III
Coggon et al., 2013[119]	CC	General population	475/799	Job satisfaction Dissatisfied: AOR 1.1 (95% CI 0.8 - 1.5)	III

PC, prospective cohort; CI, confidence interval; HR, hazard ratio; CC, case control; AOR, adjusted odds ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

Table 3.22 Summary of all the studies that have investigated support as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Increased risk					
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	Supervisor shows appreciation - never: HR 4.4 (95% CI 1.13 - 16.9), p=0.51	I
Nordstrom et al., 1997[129]	CC	General population	206/211	Employer cares Slightly disagree: AOR 2.63 (95% CI 1.18 - 5.88) Strongly disagree: AOR 4.17 (95% CI 1.31 - 13.29)	III
No effect on risk					
Harris-Adamson et al., 2013[74]	PC	Industrial workers	204/3311	Social support - High: Multivariate HR 0.54 (95% CI 0.31 - 0.95), p=0.03	I
Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	*Employer cares* Strongly agree: HR 0.3 (95% CI 0.09 - 1.03), p=0.06 Neither/Nor: HR 0.6 (95% CI 0.23 - 1.64), p=0.33 Disagree: HR 1.5 (95% CI 0.50 - 4.33), p=0.48 Strongly disagree: HR 1.4 (95% CI 0.42 - 4.74), p=0.58 *Supervisor shows appreciation* Seldom: HR 1.5 (95% CI 0.42 - 5.60), p=0.03 Often: HR 2.3 (95% CI 0.67 - 8.04), p=0.18 *Social support* Never: HR 1.0 (95% CI 0.13 - 7.58, p=0.99 Often: HR 1.7 (95% CI 0.88 - 3.40), p=0.11	I
Werner et al., 2005[114]	PC	Assembly workers	20/169	Social support - OR 0.69 (95% CI 0.48 - 0.99), p=0.04	II
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	Social support - Low score: OR 1.6 (95% CI 0.5 - 4.9)	II
Coggon et al., 2013[119]	CC	General population	475/799	Supervisor - Little support: AOR 1.7 (95% CI 1.2 - 2.4); Multivariate OR 1.6 (95% CI 1.1 - 2.3)	III
Silverstein et al., 2009[97]	CS	Manufacturing & Healthcare workers	63/657	Social support Women: AOR 0.66 (95% CI 0.32 - 1.34) Men: AOR 1.81 (95% CI 0.75 - 4.34)	III
Nordstrom et al., 1997[129]	CC	General population	206/211	Employer cares Slightly agree: AOR 1.52 (95% CI 0.89 - 2.60) No such person: AOR 0.78 (95% CI 0.37 - 1.67)	III

PC, prospective cohort; HR, hazard ratio; CI, confidence interval; CC, case control; AOR, adjusted odds ratio; OR, odds ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

3.3.4.4 Job control & targets/deadlines/bonuses

Three studies investigated whether job control has an effect on CTS risk (Table 3.23). Job control is defined as the amount of control the worker has in determining how and when work is done including the timetable and breaks[119]. Having little control over task control and having ones work strongly controlled by supervisors both had no effect on CTS risk, in a study by Roquelaure et al. (2001)[96]. In a different study, a score was to the level of control each participant has where a lower score indicated less control compared to a higher score which indicated more control. This study concluded found that a lower score was associated with a decreased risk of developing CTS, whilst a higher score had no effect[129]. Similarly, two studies investigated whether having specific targets, deadlines and bonuses had any effect on the risk of developing CTS (Table 3.23). Both studies found that it had no effect on CTS risk[98,119] and it was therefore assigned a low level of certainty.

Considering the little evidence showing that job control and targets/deadlines/bonuses influence the risk of CTS, it was awarded a low level of certainty as modifier of CTS risk.

3.3.4.5 Other psychosocial factors

A total of four studies considered various other single risk factors. This includes family problems, general tension and occupation-related psychosocial factors such as having strong structural constraints, lack of job clarity, low skill discretion, little recovery time and whether the participant would recommend their job to others or whether they would take the same job (Table 3.24). These studies, one level I, one level II and two level III, considered these single psychosocial factors but found them to have no effect on CTS risk[93,96–98].

Each of these risk factors was assigned a low level of certainty, based on the little evidence available.

Table 3.23 Summary of all the studies that have investigated job control as risk factor for carpal tunnel syndrome (CTS).

Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
Decreased risk					
Nordstrom et al., 1997[129]	CC	General population	206/211	Job control 2.8-3.4: AOR 0.80 (95% CI 0.44 - 1.47); Multivariate OR 1.05 (95% CI 0.48 - 2.27)	III
No effect on risk					
Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	Task control - Low score: OR 1.3 (95% CI 0.4 - 3.8) Work strongly controlled by supervisors: OR 0.5 (95% CI 0.2 - 1.3), Logistic model: OR 0.5 (95% CI 0.2 - 1.3), p=0.139	II
Coggon et al., 2013[119]	CC	General population	475/799	Job control Little: AOR 1.6 (95% CI 1.2 - 2.1); Multivariate OR 1.4 (95% CI 1.1 - 2.0) Targets, deadlines and bonuses AOR 1.0 (95% CI 0.8 - 1.2)	III
Nordstrom et al., 1997[129]	CC	General population	206/211	Job control 3.6-3.8: AOR 0.36 (95% CI 0.18 - 0.71); Multivariate OR 0.34 (95% CI 0.14 - 0.82) 4-4.4: AOR 0.46 (95% CI 0.24 - 0.86); Multivariate OR 0.64 (95% CI 0.29 - 1.42) 4.6-4.8: AOR 0.42 (95% CI 0.21 - 0.83); Multivariate OR 0.35 (95% CI 0.14 - 0.91)	III
Rigouin et al., 2013[98]	CS	Working population	156/3554	Targets, deadlines and bonuses Men: Multivariate OR 1.93 (95% CI 1.08 - 3.46), p=0.026	III

CC, case control; AOR, adjusted odds ratio; CI, confidence interval; OR, odds ratio

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

Table 3.24 Summary of all the studies that have investigated other psychosocial risk factors for carpal tunnel syndrome (CTS).

Risk factor	Article	Study design	Population	Number of CTS/CON[a]	Results	Level of Evidence
No effect on risk						
Family problems	Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	Seldom: HR 1.6 (95% CI 0.62 - 4.32), p=0.32 Often: HR 1.4 (95% CI 0.44 - 4.73), p=0.41 Always: HR 2.0 (95% CI 0.39 - 10.30	I
Recommend their job to others	Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	Strongly recommend: HR 0.2 (95% CI 0.03 - 1.54), p=0.12 Neither/Nor: HR 1.1 (95% CI 0.47 - 2.43), p=0.87 Discourage: HR 2.0 (95% CI 0.73 - 5.44), p=0.18 Strongly discourage: HR 2.5 (95% CI 0.83 - 7.45), p=0.10	I
Would take the same job again	Garg et al., 2012[93]	PC	Working population (manufacturing)	35/501	Likely: HR 3.2 (95% CI 0.93 - 10.81), p=0.07 Neither/Nor: HR 2.3 (95% CI 0.51 - 10.17), p=0.28 Unlikely: HR 3.1 (95% CI 0.78 - 12.46), p=0.11 Very unlikely: HR 3.8 (95% CI 0.90 - 15.78), p=0.07	I
Tension	Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	Permanent tension during work: OR 0.3 (95% CI 0.4 - 4.1) Permanent time pressure: OR 1.3 (95% CI 0.6 - 11.2)	II
Lack of job clarity	Roquelaure et al., 2001[96]	PC	Blue-collar workers	34/100	OR 0.8 (95% CI 0.3 - 2.3)	II
Low skill discretion	Rigouin et al., 2013[98]	CS	Working population	156/3554	Men: Multivariate OR 1.77 (95% CI 1.01 - 3.11), p=0.047	III
Little recovery time	Rigouin et al., 2013[98]	CS	Working population	156/3554	Men: Multivariate OR 2.02 (95% CI 0.81 - 5.07) Women: Multivariate OR 1.73 (95% CI 0.88 - 3.42)	III
Strong structural constraints	Silverstein et al., 2009[97]	CS	Manufacturing & Healthcare workers	63/657	Women: AOR 1.04 (95% CI 0.51 - 2.12) Men: AOR 1.28 (95% CI 0.52 - 3.16)	III

PC, prospective cohort; HR, hazard ratio; CI, confidence interval; CS, cross sectional; OR, odds ratio AOR, adjusted odds ratio;

[a] CON in case-control studies represent the controls, while in the other study designs it represents the total study population without the CTS cases.

3.4 CONCLUSION

In conclusion, 29 occupational and psychosocial risk factors (excluding “other” risk factors) were reviewed. All the risk factors were found to only have a low certainty of having an effect on the modification of risk for CTS (Table 3.25). Interestingly, there was little evidence to support the hypothesis that the occupational factors, specifically repetitive action of the hands/wrists and working with vibratory tools, commonly believed to increase risk of CTS were associated. However, it is very likely that this finding is not as a result of the lack of a true association but rather the lack of good quality, prospective studies investigating these factors in the literature and this should be the focus of future research.

Furthermore, this review also highlighted that the overall level of certainty for occupational involvement, which is partly the focus of his thesis, is currently low. It is however interesting to note that blue-collar workers, specifically, seemed to be at increased risk of CTS. Several blue-collar worker occupations are often associated with repetitive work, and future studies should focus on this group of workers.

As stated in the previous chapter, only studies that included diagnosed CTS (by a medical professional, electro-diagnostic examination or surgical cases) were included in this systematic review and very few studies investigated specifically participants who have had carpal tunnel release surgery, which would be considered the most extreme phenotype.

As mentioned in the previous chapter, there are limitations to this systematic review. 1) Many of the variables were considered only by univariate analyses compared to other which were considered by both uni- and multivariate analyses. Future systematic reviews could potentially only focus on multivariate analyses, which are considered to be of higher quality than univariate analyses. 2) In various sections, different variables were grouped under one umbrella risk factor (such as occupation or exposure). Although this is not ideal, this was

done for simplicity purposes to avoid having an excessive amount single risk factors investigated only by one study. Lastly, groupings in this systematic review was made on the risk factor's effect on risk i.e. increased, decreased or no effect on risk. Although there are several methods of grouping risk factors, this method was chosen to increase the understanding of the effect of a particular risk factor on risk and to prevent confusion.

In conclusion, large, prospective studies focusing on occupational and psychosocial risk factors are needed to investigate whether these factors do, in fact, play a role in the aetiology of CTS. Furthermore, a meta-analysis to investigate the combination and/or interaction of different studies would provide more information on the effect of different risk factors in this multifactorial condition.

Table 3.25 Summary of the level of certainty of carpal tunnel syndrome risk factors

Level of Certainty		
High	**Moderate**	**Low**
(1) Previous and current Occupations		
-	-	Occupation
-	-	Computer work
-	-	Current/Previous exposure
(2) Specific Occupational Exposure		
-	-	Force Exertion
-	-	Breaks
-	-	Vibration
-	-	Physical limit/demand
-	-	Working conditions/Environment
-	-	Specific Occupational actions
-	-	Job rotation
-	-	Number of Occupational factors
(3) Biomechanical factors		
-	-	Upper body movement
-	-	Biomechanical stressors
-	-	Working overhead
-	-	Lift/carry weight
-	-	Job strain
-	-	Repetition
-	-	Grip force
(4) Psychosocial		
-	-	General and Mental health
-	-	Job satisfaction and psychological demand
-	-	Support
-	-	Job control and Targets/deadlines/bonuses

CHAPTER 4: MATRIX METALLOPROTEINASE (*MMP*) GENES ON CHROMOSOME 11q22 ARE NOT ASSOCIATED WITH RISK OF CARPAL TUNNEL SYNDROME

4.1 INTRODUCTION

As reviewed in chapter one, previous studies have proposed a possible involvement of tendon or tendon-structures in the aetiology of CTS (Section 1.6). In support of this both tendinopathy and tenosynovitis have been proposed as being comorbid conditions or a precursor of CTS[12]. Furthermore, flexor tenosynovitis can lead to an increase in carpal tunnel pressure[3] which could lead to compression of the median nerve and it has also been suggested that fibrosis of the SSCT could be a cause of CTS[13]. Several investigators have also suggested that there could be a familial or genetic predisposition to CTS[24,153–156]. Since investigators have previously shown that several genetic DNA sequences variants are associated with chronic Achilles tendinopathy[157], it has therefore been hypothesised that some of these variants could also be associated with CTS (Section 1.6).

Specifically, variants rs679620 (A/G, E45K), rs591058 (T/C) and rs650108 (G/A) within the matrix metalloproteinases 3 (*MMP3*), as well as variant rs4789932 (C/T) tissue inhibitors of metalloproteinases 2 (*TIMP2*) genes have been reported to be associated chronic Achilles tendinopathy[58,158]. The MMPs, a family of 25 endopeptidases, are involved in the remodelling of the collagen fibril, the basic building block of tendons and other connective tissues (refer to figure 1.2). This remodelling is dependent on a balance between tissue degradation and formation maintained by, among other proteins, the MMPs and their antagonists, TIMPs[61–63]. Several other genes encoding for the MMPs, which include amongst others *MMP10*, *MMP1* and *MMP12*, are clustered together with *MMP3* on human chromosome 11q22 (Figure 4.1). The stromelysins, MMP3 and MMP10 are key MMPs, able to degrade several structurally diverse extracellular matrix (ECM) components such as types

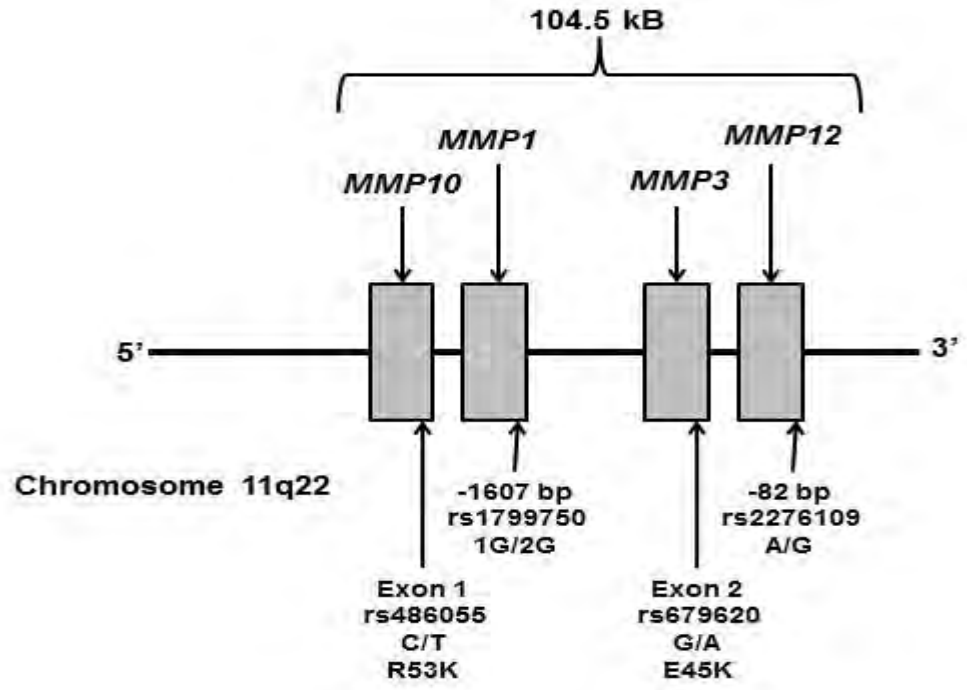

Figure 4.1 The cluster of four *MMP* genes spanning a 105.4 kb region on chromosome 11q22[59], which were investigated. The grey boxes represent the genes each containing one of the four polymorphisms investigated in this study. All four genes are orientated in the 3'–5' direction. The *MMP3* and *MMP10* single nucleotide polymorphisms (SNPs) are both non-synonymous, while the *MMP1* and *MMP12* polymorphisms are both functional promoter variants. Figure adapted from Posthumus et al. (2012)[59].

I, II, III, IV, V, VII, IX, X, XI collagens, laminin, fibronectin, elastin, gelatins and proteoglycans[61–63]. In addition MMP3 and MMP10 are also able to activate several MMPs by removing the propeptide from the pro-MMP[159–161]. MMP1 is a collagenase, responsible for degrading the triple-helical region of most subtypes of collagen whereas MMP12 is a metalloelastase, responsible for the degradation of elastin and other ECM proteins[62].

In addition to the independent association of *MMP3* variants with chronic Achilles tendinopathy a haplotype constructed from the *MMP10* C/T rs486055, *MMP1* G/GG rs1799750, *MMP3* G/A rs679620 and *MMP12* A/G rs2276109 variants are also associated with altered risk for anterior cruciate ligament (ACL) ruptures[59]. The *MMP1* (G/GG)

rs1799750 variant is also associated with knee osteoarthritis in the Turkish population with the G allele being over-represented in the patients and the GG allele being over-represented in the control group[162]. Although the results should be interpreted with caution because of the small sample size, the GG/GG genotype of *MMP1* rs1799750[163] and the T allele of the -799 *MMP-8* variant (rs11225395, C/T)[164] is associated with increased risk for primary posterior tibial (PPT) tendinopathy in Brazilian females.

The *MMP3* and *MMP10* variants both contain non-synonymous SNPs. *MMP3* rs679620 (G/A) results in a glutamate to lysine substitution at position 45[59] whilst MMP10 rs486055 (C/T) results in a arginine to lysine substitution at position 53[59]. *MMP1* and *MMP12* both contain functional promoter polymorphisms, rs1799750 (G/GG)[165] and rs2276109 (A/G)[166], that alters transcriptional activity with the GG and the A alleles resulting in increased transcriptional activity of the *MMP1* and *MMP12* genes respectively [165,166].

Considering (i) the important role of that the MMPs play in ECM homeostasis, (ii) the possibility of a tendon/tendon-like structure involvement in the aetiology of CTS, and (iii) independent and/or gene-gene associations of *MMP10*, *MMP1*, *MMP3* and *MMP12* have been reported for other musculoskeletal disorders, the aim of the study presented in this chapter was to determine whether the *MMP10* rs486055 (C/T), *MMP1* rs1799750 (G/GG), *MMP3* rs679620 (A/G) and *MMP12* rs2276109 (A/G) variants are associated with CTS.

4.2 METHODS

4.2.1 Participants

A total of 103 self-reported Coloured participants (94 female and 9 male), with a history of bi- or unilateral carpal tunnel release surgery (CTS), were recruited for this study and subsequent studies of this thesis from various Occupational Health clinics in the Western Cape region of South Africa from May 2012 to March 2013. Bilateral carpal tunnel release surgery was performed in 53.2% (47 female and 3 male) of the CTS participants, while 35.1% (30 female and 3 male) only had surgery on their dominant hand and 9.6% (7 female and 2 male) had surgery on their non-dominant hand. Two (2.1%) participants, one male and one female, who reported being ambidextrous had carpal tunnel surgery on their right hand. Nine of the participants did not report the operated hand. Seven (7.1%) of the 99 CTS participants who answered the question were pregnant at the onset of CTS symptoms.

South African populations who self-identify as Coloured have a complex history of ancestrally derived admixture. This ethnic group within the Western Cape region of South Africa is ancestrally derived from admixtures of one or more of the indigenous African populations (Khoe- and San-speaking or Bantu-speaking), immigrants from Western Europe, or slave labourers from West Africa, Indonesia, Madagascar, Java, India and Malaysia. The term “Coloured” in South Africa is therefore a name that encompasses a wide range of people who are unique to this country[7]. This admixed study sample investigated is predicted to potentially contain more genetic variation compared to the South African populations of Caucasian ancestry[246]. For this reason, this admixed population can be considered ideal to use in the characterisation and refining of the genetic interval containing functional genomic motifs that are relevant to both the common occupational and recreational, multifactorial soft tissue injuries[246].

The diagnosis of CTS was initially made by an Occupational Medical Practitioner (OMP) and then confirmed by an Orthopaedic Surgeon. Nerve conduction studies were performed in some cases but since it is not a requirement of the Commissioner for Workman's compensation in South Africa, this information was not recorded. In addition, 150 apparently healthy, self-reported Coloured participants (133 female and 17 male) without any reported history of CTS symptoms or surgery were recruited as control (CON) participants from appropriate industries within the Western Cape region during the same period. The CON and CTS participants were matched for the type of occupation and years of exposure for wrist activity. Although a case-control ratio of 1:1 is generally accepted as sufficient, increasing the number of controls per case will improve the quality of the study. In this study, every CTS case was matched to 1.5 control (CON) participants.

Prior to participation in this study, all participants were informed about the procedures and gave written informed consent (according to the Declaration of Helsinki) (Appendix A). In addition, a questionnaire containing personal details as well as self-reported personal and family medical history questionnaires was completed by each participant (Appendix A). This study was approved by the Human Research Ethics Committee of the Faculty of Health Sciences within the University of Cape Town (HREC 158/2011) (Appendix A). This case-control genetic association study is reported using the recommendations outlined in the genetic association study specific STREGA initiative[167].

The CTS and CON groups were matched for age of surgery (age of recruitment was used for the CON group), sex, height and country of birth (Table 4.1). Both groups were similarly matched for weight and BMI after adjusting for the significant difference ($p<0.001$) in age of recruitment between the CTS (45.6 ± 10.6, n=103) and CON (40.3 ± 9.7, n=149) groups. Twenty-three percent (n=23) and 34.2% (n=50) of the CTS and CON participants were

overweight (BMI: >25 to ≤30 kg.m^{-2}), respectively, while 58.4% (n=59) CTS and 44.5% (n=65) CON participants were obese (BMI: >30 kg.m^{-2}).

Participants within the CTS (27.2%, n=28) and CON (15.3%, n=23) groups self-reported similar histories (p=0.118) of medical condition(s), such as diabetes (9.7 % CTS, n=10 vs 8.0% CON, n=12), osteoarthritis (OA, 7.8 % CTS, n=8 vs 5.3% CON, n=8), rheumatoid arthritis (RA, 3.9 % CTS, n=4 vs 0.7% CON, n=1), thyroid disorders (1.9 % CTS, n=2 vs 0.7% CON, n=1), diabetes and OA (1.0 % CTS, n=1 vs 0.0% CON), RA and OA (0.0 % CTS vs 0.7% CON, n=1), diabetes and RA (1.0 % CTS, n=1 vs 0.0% CON), diabetes and thyroid disorder (1.0 % CTS, n=1 vs 0.0% CON), RA and systemic lupus erthematosus (1.0 % CTS, n=1 vs 0.0% CON), suggested to be associated with CTS.

Table 4.1 General characteristics of the carpal tunnel syndrome (CTS) and control (CON) groups.

	CTS (n=103)	**CON** (n=150)	p-value
Age of recruitment (yrs)	45.6 ± 10.6 (103)	40.3 ± 9.7 (148)	<0.001
Age of surgery (yrs)	42.1 ± 10.7 (91)	40.3 ± 9.7 (148)[a]	0.181
Sex (% Female)	91.3 (103)	88.7 (150)	0.535
Height (cm)	159.9 ± 7.6 (101)	160.4 ± 7.7 (148)	0.684
Weight (kg)	82.8 ± 18.0 (102)	78.5 ± 19.1 (147)	0.193[b]
BMI (kg/m^2)	32.4 ± 6.9 (101)	30.4 ± 6.8 (146)	0.905[b]
Country of birth (% SA)	99.0 (99)[c]	100.0 (140)	0.414

Values are expressed as a mean ± standard deviation or a frequency (%). The number of participants (n) with non-missing data is indicated in parentheses. The maximum number (n) of participants in each group is also indicated.

Significant p-values are indicated in bold.

[a] age at recruitment.

[b] co-varied for age at recruitment.

[c] One participant was born in Namibia.

yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, meter; SA, South Africa.

Sixty-two percent (n=64) of the CTS and 42.2 % (n=62) of the CON participants self-reported a history of one or more other medical conditions (p=0.102). These included hypertension (46.6% CTS, n=48 vs 26.0% CON, n=39), hypercholesterolemia (18.4% CTS, n=19 vs 3.3% CON, n=5), asthma (4.9% CTS, n=5 vs 3.3% CON, n=5), angina (6.8% CTS, n=7 vs 0.7% CON, n=1) and other conditions (4.9% CTS, n=5 vs 4.7% CON, n=7), which included hypotension, malignant disease, anaemia, aortic valve stenosis, epilepsy, kidney disease, spinal stenosis, Hirschsprung's disease and autonomic insufficiency.

The majority of the participants (27.2% CTS, n=28 and 40.3% CON, n=75) included in this study with non-missing data were general poultry processing workers or general workers within other industries where repetitive action is performed with the upper limbs (Table 4.2). The other major self-reported occupations included administration (22.3% CTS, n=23 and 11.6% CON, n=17) and nursing (10.7% CTS, n=11 and 8.2% CON, n=12). As summarised in Table 4.2 the remaining participants were recruited from several high risk occupations which included work where the hands/wrists are used for a high percentage of the normal working day.

Table 4.2 Occupations of the carpal tunnel syndrome (CTS) and control (CON) participants.

Occupation	CTS	CON
	n=103	n=146
General worker		
Poultry processing	22.3 (23)	22.6 (49)
Other industries [a]	4.9 (5)	17.7 (26)
Administrator	22.3 (23)	11.6 (17)
Nurse	10.7 (11)	8.2 (12)
Packer	8.7 (9)	6.8 (10)
Seamstress	7.8 (8)	7.5 (11)
Operator	7.8 (8)	10.2 (15)
Food Handler	4.9 (5)	2.7 (4)
Other [b]	10.7 (11)	1.4 (2)

Values are expressed as a frequency (%) with the number of participants (n) in parentheses. The maximum number (N) of participants with non-missing data in each group is also indicated.

[a] includes bottle packer/cleaners, general workers in dairy industry, barrel makers and rotators, cleaners, fitters, wrappers.

[b] includes sanders, engineering clerks, security, cashiers, kitchen attendant, ironing clothes, fire fighters.

There was no significant difference (p=0.077) in the number of participants who reported spending their whole working day (100%) performing manual labour requiring the use of their hands between the CTS (78.0%, n=79 of 101) and CON (87.9%, n=131 of 149) groups. There was however a significant difference (p=0.015) between CTS (Median = 50%, interquartile range 5% - 100%) and CON (Median = 100%, interquartile range 10% - 100%) groups for the percentage time spent standing during a normal working day. There was no significant difference (p=0.295) in number of participants who reported repetitive leisure

activities of the wrist (e.g. knitting/crocheting, gardening and kneading/rolling dough) within the CTS (51.5%, n=50 of 97) and CON (43.9%, n=65 of 148) groups.

There were two related participants in both the CTS and the CON groups (mother and daughter). Similar results were obtained when the analyses were repeated after excluding one of the related participants.

4.2.2 DNA Extraction

Approximately 5 ml of venous blood was collected into an EDTA vacutainer tube from each participant by venepuncture of the forearm. DNA was extracted at the MRC/UCT Research Unit for Exercise Science & Sports Medicine, University of Cape Town, South Africa, as previously described by Lahiri and Nurnberger[168] with some modifications as described by Mokone et al.[44]. Briefly, blood samples were transferred to 15ml sterile polypropylene tubes to which 10ml TKM1 buffer (10mM Tris-HCl pH 7.6, 10mM KCl, 10mM MgCl2 and 2mM EDTA) containing 2.5% Nonidet P-40, was added to lyse the red blood cells. Samples were then incubated at room temperature for 10 minutes after which the white blood cells (WBC) were pelleted by centrifuging at 1200 X g for 10 minutes at room temperature. Samples were then washed at least once with one volume TKM1 buffer. The washed WBC pellets were resuspended in 800µl TKM2 buffer (10mM Tris-HCl pH 7.6, 10mM KCl, 10mM MgCl2, 0.4M NaCl2 and 2mM EDTA) containing 50µl of 10% SDS. Samples were then incubated for at least 60 minutes at 55^0C for lyses of the WBC. After the addition of 150µl of 5M NaClO4 and 500µl chloroform, samples were mixed thoroughly by means of vortexing. All samples were transferred to new 1.5ml microfuge tubes and proteins were precipitated by centrifuging at 15000 X g (13 000 rpm) for 5 minutes at room temperature. Five hundred µl of the top aqueous layer from each sample was transferred to a new 1.5ml microfuge tube containing 1ml of absolute ethanol in order to precipitate the DNA. The DNA was then pelleted by centrifugation at 15000 X g for 2 minutes at room temperature after which it was air dried for approximately 30 minutes before being resuspended in 200µl TE buffer (10mM Tris-HCl,

1mM EDTA, pH 8.0). Finally, each tube was incubated at 65^0C for 15 minutes in a heating block. DNA was then stored at 4^0C until PCR analysis. Purity and concentration of a subset of DNA samples was calculated using nanodrop analysis on the Biotek Synergy HT (Life Technologies, Applied Biosystems, Foster City, California, USA).

4.2.3 Genotyping

All DNA samples (n=253) were all genotyped at the MRC/UCT Research Unit for Exercise Science & Sports Medicine, University of Cape Town, South Africa for the *MMP10* rs486055 (C/T), *MMP1* rs1799750 (1G/2G), *MMP3* rs679620 (A/G) and *MMP12* rs2276109 (A/G) variants as previously described[59] using custom designed fluorescence-based Taqman® polymerase chain reaction (PCR) assays (Applied Biosystems, Foster City, California, USA) (Figure 4.1). Allele-specific primer and probe sets (Table 4.3) were used along with a pre-made PCR mastermix containing ampliTaq DNA polymerase Gold (Applied Biosystems, Foster City, California, USA) in a final reaction of 8µl following the manufacturer's recommended cycling conditions which consisted of a 95°C hold step for 10 minutes, followed by 40 cycles of a denaturing step of 92°C for 15 seconds and a 60°C annealing/extension step of 1 minute. The PCR reactions were carried out in the Applied Biosystems StepOnePlus real-time PCR System (Life Technologies, Applied Biosystems, Foster City, California, USA) (Figure 4.2).

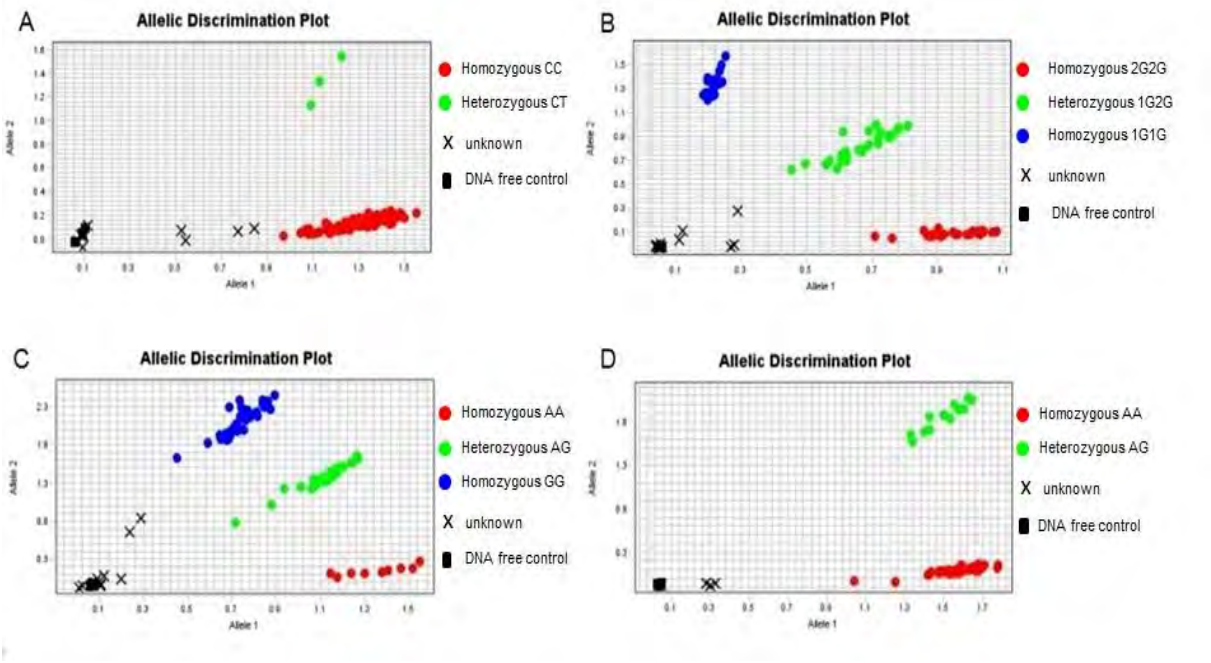

Figure 4.2 Typical allelic discrimination plots using the Taqman® Genotyping assay for (A) *MMP10* rs486055 (C/T), (B) *MMP1* rs1799750 (G/GG), (C) *MMP3* rs679620 (A/G) and (D) *MMP12* rs2276109 (A/G) on the StepOnePlus™ Real-time PCR System.

A number of positive controls of known genotype and DNA-free controls were randomly included on PCR plate for quality control purposes. In addition, a subset of samples (approximately 10%) was genotyped twice using the same methodology to ensure genotyping was consistent. In order to avoid genotyping errors, samples that failed twice to amplify during PCR for a particular variant were considered to be unsuccessfully genotyped and no further attempts were made to genotype them at that specific locus. A total of 85% (n=88 of 103) CTS and 86% (n=129 of 150) CON participants were successfully genotyped for *MMP1* rs1799750 whilst 92% (n=95 or 103) CTS and 85% (n=127 of 150) CON participants were successfully genotyped for *MMP3* rs679620. Ninety-four percent (94%, n=97 of 103) of CTS and 87% (n=131 of 150) CON participants were successfully genotyped for *MMP10* rs486055 and finally, 93% (n=96 of 103) CTS and 87% (n=131 of 150) CON participants were successfully genotyped for *MMP12* rs2276109.

Table 4.3 *MMP* Primer and probe sequences used for genotyping

Polymorphism	Primer/Probe	Sequence
MMP10 rs486055	Forward	GGAACTTCTGCATTCCTTGGATTTTT
	Reverse	ACAACCTCGAAAAGGATGTGAAACA
	Probe	VIC-ACTGTCCTTTCTTCTAAAC
	Probe	FAM-ACTGTCCTTTTTTCTAAAC
MMP1 rs1799750	Forward	ACATGTTATGCCACTTAGATGAGGAAA
	Reverse	CGTCAAGACTGATATCTTACTCATAAACAATACTTC
	Probe	VIC-TGAGATAAGTCATATCCTTTC
	Probe	FAM-TGAGATAAGTCATATCTTTC
MMP3 rs679620	Forward	GTAAGAGTGACCTAAAAACTATACTTATTCTGTTAGAAA
	Reverse	ACCACTGTCCTTTCTCCTAACAAAC
	Probe	VIC-TGTTTCACATCTTTTTTGAGGTC
	Probe	FAM-TTCACATCTTTTTCGAGGTC
MMP12 rs2276109	Forward	TGCTTTTGTTTGCATGTTTTTGAGATAGA
	Reverse	CCGGGTTCTGTGAATATGAATCCT
	Probe	VIC®-TGAGTGACTCATAGTTGAT
	Probe	FAM®-AGTGACTCACAGTTGAT

Primer and probe sets were incorporated into a PCR mastermix and used as described in section 4.2.3.

4.2.4 Statistical Analysis

No allele frequency data was available for the Coloured, South African population in the public databases (http://www.ncbi.nlm.nih.gov/snp/). For this reason, the sample size for this study was calculated based on the range of the reported minor allele frequencies (0.6 to 10.5% for *MMP10* rs486055, 37.5 to 47.9% for *MMP1* rs1799750, 23.0 to 48.3% for *MMP3* rs679620 and 0.0 to 15.9 for *MMP12* rs2276109 [Table 4.4]) previously described for populations in this public database. Quanto V.1.2.4 was used to determine the statistical power for a given sample size and minor allele frequency[26]. A sample size of approximately

100 cases and 150 controls was found to be adequate to detect a genetic effect size ranging from 2.05 to 2.80 at a power of 80% and a significance level of 5%, assuming a minor allele frequencies ranging from 0.5 to 48.3%.

Data was analysed using STATISTICA (version 11, StatSoft Inc., Tulsa, Oklahoma, USA) and Graphpad Prism (version 5, GraphPad Software, San Diego, CA, USA, http://www.graphpad.com). A Pearson's chi-squared test or a Fisher's exact test was used to determine any significant differences between the genotype distributions or any other categorical data of the groups. An analysis of variance (ANOVA) was used to detect any significant differences between CTS and CON groups for continuous data. Where appropriate, values were adjusted for the effect of age at recruitment. A Mann-Whitney U test was used to detect significant differences between the CTS and CON groups for non-parametric data. Statistical significance was accepted at $p<0.05$. Hardy-Weinberg equilibrium (HWE) was established using the program Genepop web version 4.0.10 (http://genepop.curtin.edu.au/)[169,170]. Linkage disequilibrium (LD) was calculated using CubeX: cubic exact solution (www.oege.org/software/cubex/)[171]. Inferred haplotypes were constructed from *MMP10* rs486055 (C/T), *MMP1* rs1799750 (G/GG), *MMP3* rs679620 (A/G) and *MMP12* rs2276109 (A/G) variants using Chaplin (version 1.2.2, Emory University School of Medicine, Atlanta, Georgia, USA)[172,173]. No adjustments were made for multiple testing considering no obvious appropriate method currently exists[174,175]. The Bonferroni adjustment was considered too conservative since the statistical tests in this and following studies are all performed on the same group of participants[175]. Adjustment for multiple testing was also considered inappropriate since there is an *a priori* hypothesis that the gene variants investigated in this study are associated with the CTS phenotype[174].

Table 4.4 Minor allele frequencies of the investigated *MMP* polymorphisms in different populations.

Polymorphism	Population	Population size	Minor Allele	Minor allele frequency (%)
rs486055 (C/T)	HAPMAP-YRI	116	T	0.9
	HAPMAP-GIH	172	T	5.2
	HAPMAP-LWK	180	T	0.6
	HAPMAP-MEX	100	T	5.0
	HAPMAP-MKK	286	T	1.7
	HAPMAP-TSI	172	T	10.5
rs1799750 (G/GG)	CAUC1	60	GG	43.3
	AFR1	48	GG	37.5
	HISP1	46	G	47.0
	PAC1	48	G	47.9
rs679620 (G/A)	HAPMAP-YRI	216	A	38.9
	HAPMAP-GIH	174	A	25.3
	HAPMAP-LWK	174	A	23.0
	HAPMAP-MKK	282	A	30.5
	HAPMAP-TSI	172	A	48.3
rs2276109 (A/G)	HAPMAP-YRI	120	G	0.0
	HAPMAP-GIH	176	G	7.4
	HAPMAP-MEX	100	G	9.0
	HAPMAP-MKK	286	G	3.1
	HAPMAP-TSI	176	G	15.9

HAPMAP-YR, Yoruba in Ibadan Nigeria; HAPMAP-GIH, Gujarati Indians in Houston TX USA; HAPMAP-LWK, Luhya in Webuye Kenya; HAPMAP-MEX, Mexican ancestry in Los Angeles CA USA; HAPMAP-MKK, Maasai in Kinyawa Kenya; HAPMAP-TSI, Toscani in Italy; CAUC1, individuals of self-described CAUCASIAN heritage; AFR1, of self-described AFRICAN/AFRICAN AMERICAN heritage; HISP1, individuals of self-described HISPANIC heritage; PAC12, individuals of self-described PACIFIC RIM heritage

4.3 RESULTS

4.3.1 General characteristics

Similar to all the participants recruited for this thesis (section 4.2.1), the CTS and CON groups of all participants successfully genotyped for at least one of the investigated variants were matched for age of surgery (age of recruitment was used for the CON group), sex, height and country of birth (Table 4.5). As with the entire cohort, both groups were similarly matched for weight and BMI after adjusting for the significant difference ($p<0.001$) in age of recruitment between the CTS (45.5 ± 10.7, n=101) and CON (40.5 ± 9.8, n=141) groups. Twenty-two percent (n=22) and 34.0% (n=47) of the CTS and CON participants were overweight (BMI: >25 to ≤30 $kg.m^{-2}$), respectively, while 58.5% (n=58) CTS and 43.5% (n=60) CON participants were obese (BMI: >30 $kg.m^{-2}$).

Similar to the all the participants recruited for this thesis (section 4.2.1), bilateral carpal tunnel release surgery was performed in 50 (53.2%) of the CTS participants successfully genotyped for at least one of the investigated variants, while 33 (35.1%) only had surgery on their dominant hand and 9 (9.6%) had surgery on their non-dominant hand. Two (2.1%) participants who reported being ambidextrous had carpal tunnel surgery on their right hand.

Participants within the CTS (25.7%, n=26) and CON (14.9%, n=21) groups self-reported similar histories (p=0.266) of medical condition(s), such as diabetes (8.9 % CTS, n=9 vs 7.8% CON, n=11), osteoarthritis (OA, 7.9 % CTS, n=8 vs 5.0% CON, n=7), rheumatoid arthritis (RA, 4.0 % CTS, n=4 vs 0.7% CON, n=1), thyroid disorders (1.0 % CTS, n=1 vs 0.7% CON, n=1), diabetes and OA (1.0 % CTS, n=1 vs 0.0% CON), RA and OA (0.0 % CTS vs 0.7% CON, n=1), diabetes and RA (1.0 % CTS, n=1 vs 0.0% CON), diabetes and thyroid disorder (1.0 % CTS, n=1 vs 0.0% CON), RA and systemic lupus erthematosus (1.0 % CTS, n=1 vs 0.0% CON), suggested to be associated with CTS.

Table 4.5 General characteristics of the carpal tunnel syndrome (CTS) and control (CON) groups of participants successfully genotyped for at least one of the investigated variants for the variants investigated in this study.

	CTS (n=101)	**CON** (n=141)	p-value
Age of recruitment (yrs)	45.5 ± 10.7 (101)	40.5 ± 9.8 (140)	**<0.001**
Age of surgery (yrs)	42.0 ± 10.7 (90)	40.5 ± 9.8 (140)[a]	0.284
Sex (% Female)	91.1 (101)	87.9 (141)	0.530
Height (cm)	160.0 ± 7.7 (99)	160.5 ± 7.9 (140)	0.622
Weight (kg)	82.8 ± 18.1 (100)	78.4 ± 19.0 (139)	0.185[b]
BMI (kg/m^2)	32.4 ± 7.0 (99)	30.3 ± 6.7 (138)	0.138[b]
Country of birth (% SA)	99.0 (97)[c]	100.0 (132)	0.426

Values are expressed as a mean ± standard deviation or a frequency (%). The number of participants (n) with non-missing data is indicated in parentheses. The maximum number (n) of participants in each group is also indicated.

Significant p-values are indicated in bold.

[a] age at recruitment.

[b] co-varied for age at recruitment.

[c] One participant was born in Namibia.

yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, meter; SA, South Africa.

Sixty-two percent (n=62) of the CTS and 42.0% (n=58) of the CON participants self-reported a history of one or more other medical conditions (p=0.004). These included hypertension (45.5% CTS, n=46 vs 26.6% CON, n=37), hypercholesterolemia (18.8% CTS, n=19 vs 3.6% CON, n=5), asthma (5.0% CTS, n=5 vs 3.4% CON, n=5), angina (6.9% CTS, n=7 vs 0.7% CON, n=1) and other conditions (5.0% CTS, n=5 vs 5.0% CON, n=7), which included hypotension, malignant disease, anaemia, aortic valve stenosis, epilepsy, kidney disease, spinal stenosis, Hirschsprung's disease and autonomic insufficiency.

Similar to all the participants recruited for this thesis (section 4.2.1), the majority of the participants (27.7% CTS, n=28 and 51.8% CON, n=75) who were successfully genotyped for at least one of the four *MMP* variants, with non-missing data were general poultry processing workers or general workers within other industries where repetitive action is performed with the upper limbs (Table 4.6). The other major self-reported occupations included administration (21.8% CTS, n=22 and 10.6% CON, n=15) and nursing (9.9% CTS, n=10 and 8.5% CON, n=12). As summarised in Table 4.6 the remaining participants were recruited from several high risk occupations which included work where the hands/wrists are used for a high percentage of the normal working day.

Similar to all participants recruited for this thesis (section 4.2.1), there was also no significant difference (p=0.115) between the number of participants, successfully genotyped for at least one of the four *MMP* variants, who reported spending their whole working day (100%) performing manual labour requiring the use of their hands between the CTS (78.8%, n=78 of 99) and CON (87.1%, n=122 of 140). Similarly, there was a significant different (p=0.041) between CTS (Median = 50%, interquartile range 10% - 100%) and CON (Median = 100%, interquartile range 10% - 100%) participants who were successfully genotyped, for the percentage time spent standing during a normal working day. There was no significant difference (p=0.351) in number of successfully genotyped participants who reported repetitive leisure activities of the wrist (e.g. knitting/crocheting, gardening and kneading/rolling dough) within the CTS (55.6%, n=49 of 95) and CON (32.9%, n=60 of 140) groups.

There were no genotype effects on any of the other physiological characteristics of the participants (Supplementary Tables 4.1-4.4, Appendix B).

Table 4.6 Occupations of the carpal tunnel syndrome (CTS) and control (CON) participants who were successfully genotyped for at least one of the variants investigated in this study.

Occupation	CTS	CON
	n=101	n=137
General worker		
Poultry processing	22.7 (23)	31.9 (45)
Other industries [a]	5.0 (5)	18.4 (26)
Administrator	21.8 (22)	10.6 (15)
Nurse	9.9 (10)	8.5 (12)
Packer	8.9 (9)	6.4 (9)
Seamstress	7.9 (8)	7.8 (11)
Operator	7.9 (8)	9.2 (13)
Food Handler	5.0 (5)	2.8 (4)
Other [b]	10.9 (11)	1.4 (2)

Values are expressed as a frequency (%) with the number of participants (n) in parentheses. The maximum number (N) of participants with non-missing data in each group is also indicated.

[a] includes bottle packer/cleaners, general workers in dairy industry, barrel makers and rotators, cleaners, fitters, wrappers.

[b] includes sanders, engineering clerks, security, cashiers, kitchen attendant, ironing clothes, fire fighters.

4.3.2 Genotypes

There were no significant differences between in any of the genotypes and allele distributions between the CTS and CON groups for any of the *MMP* variants. All four variants were in Hardy-Weinberg equilibrium (Table 4.7). Similar genotype distributions were observed when participants with a history of a medical condition believed to be associated with CTS were excluded from the analysis (Supplementary table 4.5, Appendix B).

Table 4.7 Genotype frequency distributions of the *MMP10* rs486055, *MMP1* rs1799750, *MMP3* rs679620 and *MMP12* rs2276109 polymorphism in carpal tunnel syndrome (CTS) and control (CON) groups for all participants (All) as well as the female participants (Female).

	All		Female	
	CTS	**CON**	**CTS**	**CON**
***MMP10* rs486055**	n=97	n=131	n=88	n=114
CC Genotype	91.8 (89)	90.1 (118)	92.1 (81)	88.6 (101)
CT Genotype	8.3 (8)	9.9 (13)	8.0 (7)	11.4 (13)
TT Genotype	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Genotype p-value	0.818		0.482	
T Minor Allele	4.1 (8)	5.0 (13)	4.0 (7)	5.7 (13)
Allele p-value	0.673		0.428	
HWE	1.000		1.000	
***MMP1* rs1799750**	n=88	n=129	n=79	n=113
GG Genotype	22.7 (20)	17.8 (23)	24.1 (19)	19.5 (22)
GGG Genotype	42.1 (37)	47.3 (61)	40.5 (32)	48.7 (55)
GGGG Genotype	35.2 (31)	34.9 (45)	35.4 (28)	31.9 (36)
Genotype p-value	0.621		0.517	
G Minor Allele	43.8 (77)	41.5 (107)	44.3 (70)	43.8 (99)
Allele p-value	0.637		0.923	
HWE	0.269		0.311	
***MMP3* rs679620**	n=95	n=127	n=86	n=110
GG Genotype	42.3 (43)	51.2 (65)	46.5 (40)	47.3 (52)
GA Genotype	46.3 (44)	37.8 (48)	44.2 (38)	40.9 (45)
AA Genotype	8.4 (8)	11.0 (14)	9.3 (8)	11.8 (13)
Genotype p-value	0.424		0.813	
A Minor Allele	31.6 (60)	29.9 (76)	31.4 (54)	32.3 (71)
Allele p-value	0.708		0.853	
HWE	0.754		0.742	
***MMP12* rs2276109**	n=96	n=131	n=87	n=114
AA Genotype	87.5 (84)	90.1 (118)	87.4 (76)	88.6 (101)
AG Genotype	12.5 (12)	9.9 (13)	12.6 (11)	11.4 (13)
GG Genotype	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Genotype p-value	0.668		0.829	
G Minor Allele	6.3 (12)	5.0 (13)	6.3 (11)	5.7 (13)
Allele p-value	0.552		0.795	
HWE	1.000		1.000	

Values are expressed as a frequency (%) with the number of participants (n) in parentheses. The maximum number (N) of participants in each group is also indicated. Significant p-values are indicated in bold. HWE, Hardy-Weinberg equilibrium.

The *MMP12* rs2276109 variant was in linkage disequilibrium (LD) with *MMP3* rs679620 (CTS D'=1.000 and CON D'=1.000), *MMP1* rs1799750 (CTS D'=1.000 and CON D'=0.674) and *MMP10* rs486055 (CTS D'=1.000 and CON D'=1.000). Similarly *MMP1* rs1799750 was in LD with *MMP10* rs486055 (CTS D'=0.661 and CON D'=0.535). However the *MMP3* rs679620 variant was not in LD with *MMP10* rs486055 (CTS D'=0.169 and CON D'=0.667) nor with *MMP1* rs1799750 (CTS D'=0.536 and CON D'=0.393).

4.3.3 Inferred Haplotypes

Five of the inferred haplotypes constructed from the four variants out of a possible sixteen had a frequency greater than 2% (Figure 4.3A). There were no significant differences between the CTS and CON groups for any of the haplotypes. Inferred haplotypes were also constructed from the two most informative variants, *MMP1* rs1799750 and *MMP3* rs679620. All four haplotypes had a frequency greater than 2% (Figure 4.3B). There were also no significant differences between the CTS and CON groups for any of the haplotypes.

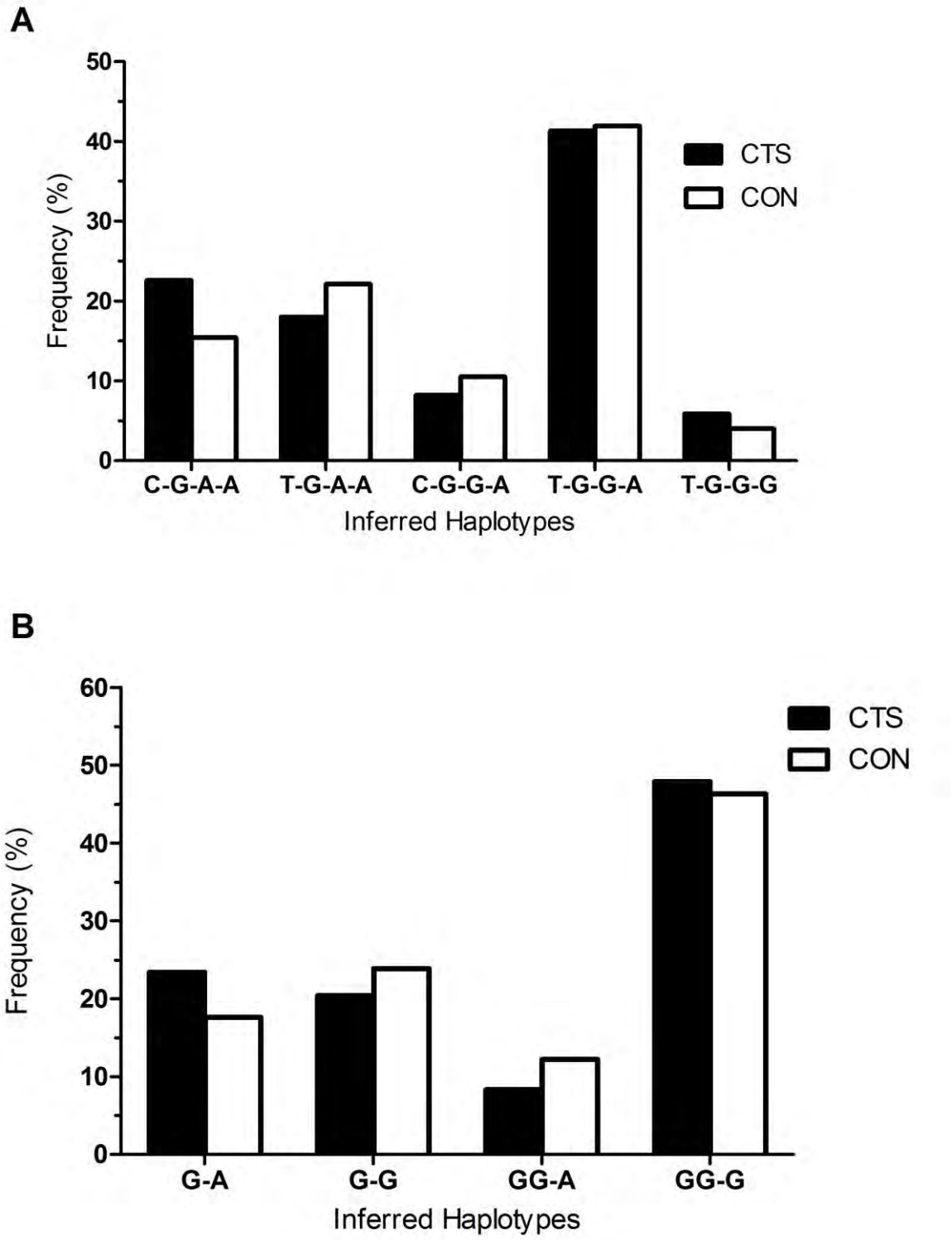

Figure 4.3 Inferred haplotypes constructed from the **(A)** *MMP10* rs486055, *MMP1* rs1799750, *MMP3* rs679620 and *MMP12* rs2276109 polymorphisms and **(B)** the *MMP1* rs1799750 and *MMP3* rs679620 polymorphisms in the carpal tunnel syndrome (CTS, black bars) and control (CON, white bars) groups.

4.4 DISCUSSION

The main finding of this study was there was no individual association between the *MMP10* rs486055, *MMP1* rs1799750, *MMP3* rs679620 or *MMP12* rs276109 variants and CTS. Similarly, there were also no associations between any of the inferred haplotypes constructed from all four variants or from *MMP1* rs1799750 and *MMP3* rs679620 with CTS.

A previous study has shown that the *MMP3* rs679620 variant and two other *MMP3* variants were independently associated with risk of Achilles tendinopathy (AT) and that rs679620 also interacts with a variant (rs12722, C/T) with a functional region of the *COL5A1* gene, which codes for the α1 chain of type V collagen, to alter the risk of AT[58]. Furthermore, inferred haplotypes constructed of the same four *MMP* variants investigated in this study have previously been associated with altered risk of anterior cruciate ligament (ACL) injuries[59]. The *MMP1* rs1799750 variant is also independently associated with primary posterior tibial (PPT) tendinopathy in Brazilian females[163]. Considering these findings, and the suggested role of flexor tendon involvement in the compression of the median nerve in CTS[3,176], the four *MMP* gene variants investigated in this study were considered plausible candidates for potential association with altered risk of CTS. The *MMP10* rs486055 and *MMP3* rs679620 variants are both non-synonymous polymorphisms that result in amino acid changes within pro-MMP10 and pro-MMP3, respectively[59], whereas the *MMP1* rs1799750 and *MMP12* rs2276109 variants are both promoter polymorphisms that have been shown to alter transcriptional activity[165,166]. The *MMP1* rs1799750 polymorphism is manifested as either the presence or absence of an extra guanine at -160 7bp that creates, in the presence of the extra guanine (GG), an Ets binding site (5'-GGA-3') that leads to increased transcription. The GG allele has been shown to have significantly higher transcription of *MMP1* than the G allele[165]. The *MMP12* rs2276109 polymorphism in turn influences the

binding of transcription factor activator protein-1 (AP-1) which is involved in regulating gene expression. More specifically, the A allele has been shown to increase the affinity of AP-1[166].

The *MMP10* rs486055 and *MMP3* rs679620 variants are both non-synonymous SNPs. *MMP3* rs679620 results in a G to A substitution that causes a change from glutamate to lysine whereas the *MMP10* rs486055 G to A substitution results in a change from an arginine to a lysine. The specific consequence of these amino acid changes remains unknown, but neither of these non-synonymous SNPs were predicted to be damaging when analyzed using the SIFT (Sorting Intolerant From Tolerant) program[59,177]. The four variants included in this study have all been independently associated with complex phenotypes, including rheumatoid arthritis[178], osteoarthritis[162], lumbar disk degeneration[179] and idiopathic scoliosis[180]

Although, in this study, there was no association between the investigated variants and CTS, it does not exclude the possibility that other variants within the *MMP1*, *MMP3*, *MMP10* and *MMP12* could potentially be associated with altered risk of CTS. One such example is the *MMP3* 5A/6A polymorphism. This variants has been associated with several multifactorial conditions[62] and plays in important role in the regulation of MMP gene expression[181].

Several other *MMP* genes, including *MMP7*, *MMP8*, *MMP13* and *MMP20*, also form part of the cluster of *MMP* genes on chromosome 11, together with the four investigated in this study. As previously mentioned, the *MMP8* rsrs11225395 variant is associated with primary posterior tibial (PPT) tendinopathy in Brazilian females[164]. Furthermore, variants within other *MMPs*, which include *MMP2* and *MMP9*, have been associated with vascular remodelling. MMP2 is produced and activated in the flexor tenosynovium in early CTS[182]. Variants within these other *MMP* genes should therefore also be considered in future work.

The balance between degradation, mediated by the MMPs, and inhibition of degradation, mediated by the TIMPs is very important in the maintenance of the ECM[62]. It has been proposed that an over- or under production of MMPs in comparison to TIMPs are related to pathology[62] but it is unknown whether a change in production of MMPs will alter the risk of CTS. Considering the important role of the TIMPs in the maintenance of the ECM, future work should also consider variants in the family of the four TIMP genes, especially considering the previous association of TIMP2 with Achilles tendinopathy[158]. The ADAMs (a disintegrin and metalloproteinase) and ADAMTSs (a disintegrin and metalloproteinase with thrombospondin motifs) are, like the MMPs and TIMPs also proteins involved in the regulation of the ECM and other connective tissues. Variants within the ADAM and ADAMTS genes could therefore also be considered as ideal candidates for the investigation of connective tissue disorders and should be investigated in the future.

In conclusion, the four *MMP* gene variants investigated in this study were not associated with risk of CTS in a South African Coloured population. However, this finding does not exclude other variants within the same and other *MMP* genes to potentially be associated with CTS.

CHAPTER 5: THE *COL5A1* GENE IS ASSOCIATED WITH INCREASED RISK OF CARPAL TUNNEL SYNDROME

The data presented in this chapter was published in the following peer-reviewed article: Marilize Burger, Hanli de Wet, Malcolm Collins. The *COL5A1* gene is associated with increased risk of carpal tunnel syndrome. **Clinical Rheumatology,** In Press.

5.1 INTRODUCTION

In the previous chapter variants within four *MMP* genes were shown not to be associated with altered risk for CTS in the population investigated in this thesis. These genes encode for distinct endopeptidases, which are involved in collagen fibril remodelling. The collagen fibril consists predominately of type I collagen together with other collagens, such as type V collagen, and non-collagen macromolecules (refer to figure 1.2). The major isoform of type V collagen, which regulates fibrillogenesis[41], is a heterotrimer consisting of two α1 and one α2 chains, which are encoded by the *COL5A1* and *COL5A2* genes respectively[42].

Two functional copies of the *COL5A1* gene are required for normal development during embryogenesis, since mutations that inactivate both copies of the gene result in death *in utero* in a murine model[183,184]. Mutations, which inactivate a single copy of the gene (known as haploinsufficiency), cause a severe autosomal dominant condition known as classical type Ehlers-Danlos syndrome (EDS)[42]. This condition is characterized by, amongst other features, skin hyper-extensibility and joint hypermobility[42]. In addition, common DNA sequence variants within *COL5A1* contribute to more complex, less severe multifactorial traits, which arise as a result of the interaction of environmental exposures on the genetic background[157,185]. Specifically, several variants within the *COL5A1* 3'-untranslated region

(UTR) have been associated with chronic Achilles tendinopathy[44,45], and other exercise-associated phenotypes[185–188].

Specifically the CC genotype of the *COL5A1* 3'-UTR rs12722 (C/T) variant (Figure 5.1) was associated with decreased risk of developing chronic Achilles tendinopathy[44,45], anterior cruciate ligament ruptures in females[46], an age-related increase in sit-and-reach range of motion[189] and exercise associated muscle cramping[186], while the TT genotype was associated with superior endurance running performance[187,188]. Furthermore, the -/- genotype of the downstream *COL5A1* 3'-UTR rs71746744 (-/AGGG) variant (Figure 5.1) was significantly over-represented in the participants with chronic Achilles tendinopathy[185], while the AGGG/AGGG genotype was shown to be associated with increased running performance and a decreased range of motion (ROM)[190]. The -/- and TT genotypes of two other tightly linked *COL5A1* 3'-UTR variants, rs16399 (ATCT/-) and rs1134170 (A/T) respectively, downstream of rs71746744 have also been shown to be associated with chronic Achilles tendinopathy[185]. Although seemingly unrelated, these phenotypes are all directly or indirectly associated with the mechanical properties of musculoskeletal soft tissue[184].

In addition, it has been proposed that these *COL5A1* 3'-UTR variants are believed to be functional, altering *COL5A1* messenger RNA (mRNA) stability within the cytoplasm of the tenocyte[191]. Specifically, the *COL5A1* rs12722 T, rs71746744 AGGG, rs16399 deletion (-) and rs1134170 T alleles are associated with increased *COL5A1* mRNA stability[185]. Altered *COL5A1* mRNA stability has been proposed to result in altered type V collagen production, changes in collagen fibril diameter and packing density, and potentially altering the mechanical properties of connective tissues[184].

Since it has been suggested that there could be a genetic predisposition to CTS[24,153–156,192],

and that the flexor tendons within the carpal tunnel structure could be directly involved in the aetiology[3,12,176], it is therefore tempting to speculate that DNA sequence variants within the functional *COL5A1* 3'-UTR are also associated with CTS. The aim of this study was to determine whether three *COL5A1* DNA sequence variants, rs13946 (T/C), rs12722 (C/T) and rs71746744 (-/AGGG), are associated with CTS (Figure 5.1). Since it has previously been reported that rs71746744, rs16399 and rs1134170 are tightly linked, only rs71746744 was included in this study. Although rs13946 (C/T) was not previously associated with chronic Achilles tendinopathy[44,45], it was nevertheless identified as one of the functional variants within the *COL5A1* 3'-UTR[191], and was therefore included in this study. Based on previous findings, it was hypothesised that the CC genotype of rs12722 will be under-represented in the CTS population, while the -/- genotype of rs71746744 will be over-represented.

The primary aim of this study was to determine whether three *COL5A1* DNA sequence variants, rs13946 (T/C), rs12722 (C/T) and rs71746744 (-/AGGG), are associated with CTS (Figure 5.2). Since it has previously been reported that rs71746744, rs16399 and rs1134170 are tightly linked, only rs71746744 was included in this study. Based on previous findings, we hypothesise that the CC genotype of rs12722 will be under-represented in the CTS population, while the -/- genotype of rs71746744 will be over-represented. Since it has previously been reported that *COL5A1* and *MMP3* variants interact to modulate risk for chronic Achilles tendinopathy[58], the secondary aim of this study was to investigate whether any of the four *COL5A1* rs12722 - *MMP* gene variant interactions modulate the risk of CTS.

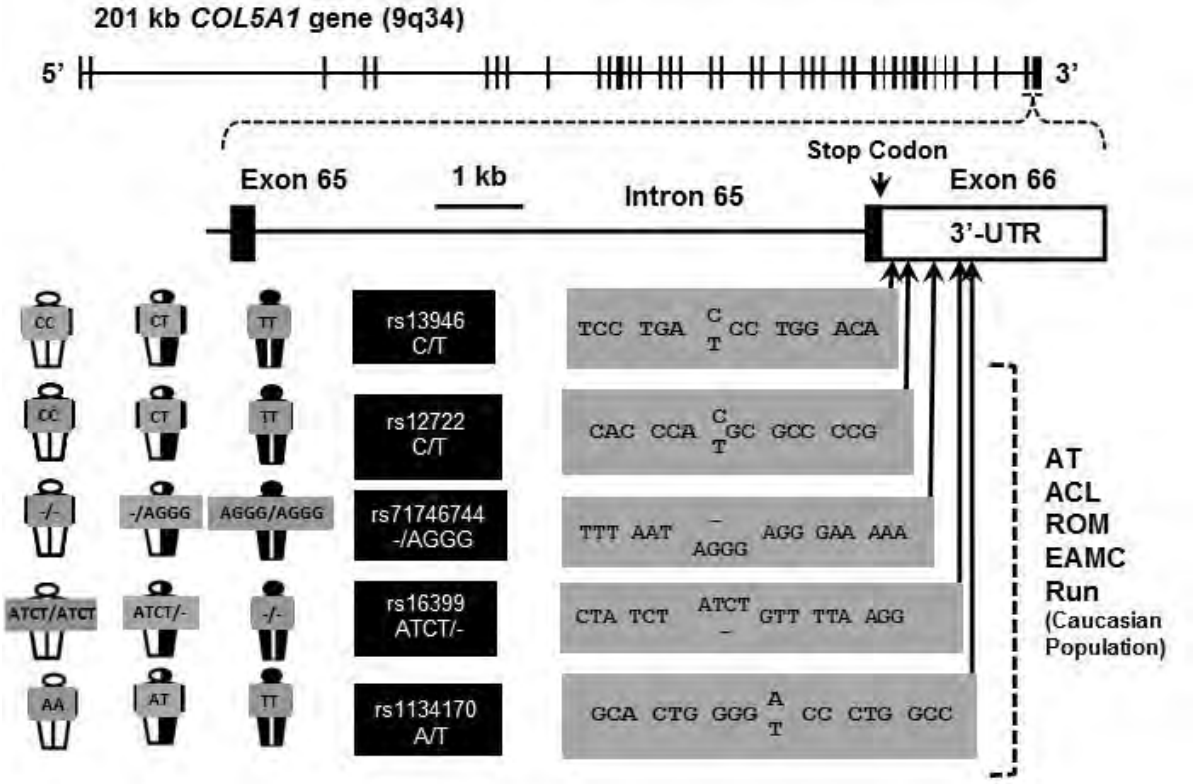

Figure 5.1 A schematic representation of the exon (vertical lines) and intron (horizontal lines) boundaries of the *COL5A1* gene located on chromosome 9q34. The 3'-end of the gene spanning exons 65 to 66 has been enlarged. Exon 66 encodes for the terminal amino acids of the α1(V) chain (black box), the stop codon and the 3'-untranslated region (UTR) (clear box). The five variants (indicated in black boxes), (i) rs13946 (T/C), (ii) rs12722 (C/T), (iii) rs71746744 (-/AGGG), (iv) rs16399 (ATCT/-) and (v) rs1134170 (A/T), discussed in this chapter are all located within the 3'-UTR. Only rs13946, rs12722 and rs71746744 were included in this study. The sequences flanking the variants and the polymorphic nucleotide changes, with the wild type nucleotide indicated on top, are indicated in the grey boxes. As illustrated in the figure, each variant produces three possible genotypes. The phenotypes, as well as the population group investigated, shown to be associated with specific variants within the *COL5A1* 3'-UTR are indicated by dashed brackets.

```
ACCAAGAAAG GCTACCAGAA GACGGTTCTG GAGATCGACA CCCCCAAAGT GGAGCAGGTG 60
                    Forward Primer  DpnII
CCCATCGTGG ACATCATGTT CAATGACTTC GGTGAAGCGT CACAGAAATT TGGATTTGAA 120
GTGGGGCCGG CTTGCTTCAT GGGCTAGGAG CCGCCGAGCC CGGGCTCCCG AGAGCAACCT 180
CGTGACCTCA GCATGCCATT CGTTCGTGAG TGTCCCGTGC ACGTCCTGAC CCTGGACAGT 240
                                  rs13946 (C/T)  DpnII
GAAGGCTTCT CCCTCCCCTC CCACCTGACT TCATCTACGC CTCGGCACCA CGGGGTGTGG 300
GACCCCAGCC CGGAGAGAAC AGAGGGAAGG AGCCGCGCCC CCACCTGGAG CTGAATCACA 360
                                  BstUI
                       rs146776422(C/T) rs55748801(G/A)
TGACCTAGCT GCACCCCAGC GCCTGGGCCC GCCCCACGCT CTGTCCACAC CCACGCGCCC 420
                                            rs12772 (C/T) BstUI
CGGGAGCGGG GCCATGCCTC CAGCCCCCCA GCTCGCCCGA CCCATCCTGT TCGTGAATAG 480
GTCTCAGGGG TTGGGGGAGG GACTGCCAGA TTTGGACACT ATATTTTTTT CTAAATTCAA 540
CTTGAAGATG TGTATTTCCC CTGACCTTCA AAAAATGTTC CAAGGTAAGC CTCGTAAAGG 600
TCATCCCACC ATCACCAAAG CCTCCGTTTT TAACAACCTC CAACACGATC CATTTAGAGG 660
                                                    DpnII
CCAAATGTCA TTCTGCAGGT GCCTTCCCGA TGGATTAAAG GTGCTTATGT TTTTGTGAGT 720
         Reverse Primer
TTTAAGTAAA TATTTGTATT GTATTGTTAT AAATGTTAAG TGTGCCTGGC TTTCAATCAT 780
GCACGGAAAC CCAGTCTCAG TCCCACGGAC AGAATGGGCG AGGCATGGAT TCTGGGTTGC 840
AGTACCGTTC TGATTAGAAA TAGGAAGTCT CCCCACCCCC GCCCTGGCCA AGAACGTGCA 900
ATAAATTGGA AGTTTGCCCC GGGGCAGCAA GAATTTATGC TGCCATTGAA AAGCAGGTAC 960
CAGTGCCCCT TTTCAGACAG TTTTTGATTC GCTCTAGACT TTTTTTTTTT TTAATAGGGA 1020
                                                  rs71746744 (-/AGGG)
```

Figure 5.2 Nucleotide sequence of the 5'-end (nucleotides 1 to 1020) of the *COL5A1* exon 66 (pubmed accession no. NM_000093) containing the 3'-untranslated region (UTR). The sequence highlighted in grey (nucleotides 1-147) represents the translated region of exon 66 and the stop codon, TAG (underlined). Nucleotide numbers are annotated on the right side of the sequence. The positions of key variants within the 3'-UTR are highlighted in black with the text in white. The sequence contains the wild-type nucleotides of the polymorphic sites. The accession numbers and nucleotide substitutions of the selected variants are indicated. The reverse and forward primers used to amplify a 667 bp PCR product are also indicated (bold underlined). The *Dpn*II and *Bst*UI restriction sites within the PCR product are annotated on the sequence (double underlined). Both the *Bst*UI and the middle *Dpn*II restriction sites are polymorphic.

5.2 METHODS

5.2.1 Participants & DNA Extraction

The same participants, described in section 4.2.1, were also analysed in this study. DNA extraction was performed as described in section 4.2.2.

5.2.2 Genotyping

All DNA samples (n=253) were all genotyped for the *COL5A1* rs13946 (T/C), rs12722 (C/T) and rs71746744 (I/II) variants at the MRC/UCT Research Unit for Exercise Science & Sports Medicine, University of Cape Town, South Africa. At least 6 positive controls of known genotype and 4 DNA-free controls were randomly included on each PCR plate for quality control purposes. In addition, a subset of samples (approximately 10%) was genotyped twice using the same methodology to ensure genotyping was consistent. In order to avoid genotyping errors, samples that failed twice to amplify during PCR for a particular variant were considered to be unsuccessfully genotyped and no further attempts were made to genotype them at that specific locus.

5.2.2.1 COL5A1 rs13946 and rs12722

A 667 bp DNA amplicon containing the rs13946 single nucleotide polymorphism (SNP, C/T) and rs12722I SNP (C/T) RFLPs within the *COL5A1* 3'-UTR was PCR (polymerase chain reaction) amplified as previously described by Greenspan and Pasquinelli (1994) [193] and modified by Mokone et al. (2006)[44]. The PCR reaction was performed in a final volume of 60µl containing at least 100ng DNA, 20pmol of the forward and reverse primers (Table 5.1), 2.0mM $MgCl_2$, 50mM KCl, 10mM Tris-HCl (pH 8.3), 200µmol of dNTPs (dATP, dTTP, dCTP and dGTP) and 1 unit of DNA Taq polymerase (New England Biolabs, Ipswich, Massachusetts, USA). The amplification was performed with an initial denaturing step for 3 minutes at 94°C, 35 x cycles of denaturing for 1 min at 94°C, annealing for 1 minute at 53°C,

extension for 1.5 minute at 72°C and the final extension step for 8 minutes at 72°C on a thermal cycler (Hybrid, PCR Express, Middle sex, UK).

Table 5.1 Primer and probe sequences used for genotyping of variants within the *COL5A1* 3'-untranslated region

SNP	Primers (forward/reverse)/ Probes	Restriction Enzyme	DNA fragments (bp)
rs13946	5'-GAAGACGGTTCTGGAGATCG-3'	*Dpn*II	C – 612, 40, 15
	5'-GAAGGCACCTGCAGAATGAC-3'		T– 418, 194, 40, 15
rs12722	5'-GAAGACGGTTCTGGAGATCG-3'	*BstUI*	T – 351, 316
	5'-GAAGGCACCTGCAGAATGAC-3'		C – 316, 271, 80
rs71746744	5'-GCCCCTTTTCAGACAGTTTTTGATT-3'	N/A	N/A
	5'-CAAACCTGTGTTTTAGTCTTAAGTGCAT-3'		
	Insertion: 5'-VIC-TTTCCCTCCCTATTAAAA-3'		
	Deletion: 5'-VIC-CAAATTTTTTCCCTATTAAAA-3'		

Primer and probe (where applicable) sets were incorporated into a PCR mastermix and used as described in materials and methods. The restriction endonuclease and DNA fragment sizes are indicated where applicable.
SNP, single nucleotide polymorphism
Bp, base pair

The 667 bp PCR products were digested with the restriction endonucleases, *Dpn*II or *Bst*UI, and the resultant amplicons were resolved together with a 100bp molecular weight marker and SYBER® *Gold* nucleic acid gel stain (Invitrogen Molecular Probes™, Oregon, USA) on 6% non-denaturing polyacrylamide gels (Figure 5.3A and B). The gels were photographed under UV light using an Uvitec photodocumentation system (Uvitec Limited, Cambridge, UK) and genotypes were determined based on the resultant DNA fragment sizes (Table 5.2). Eighty-eight % (n=91 of 103) of the cases and 85 % (n=127 of 150) of the controls were successfully genotyped for *COL5A1* rs13946, whereas 96% (n=99 of 103) of the cases and 94% (n=141 of 150) of the controls were successfully genotyped for rs12722.

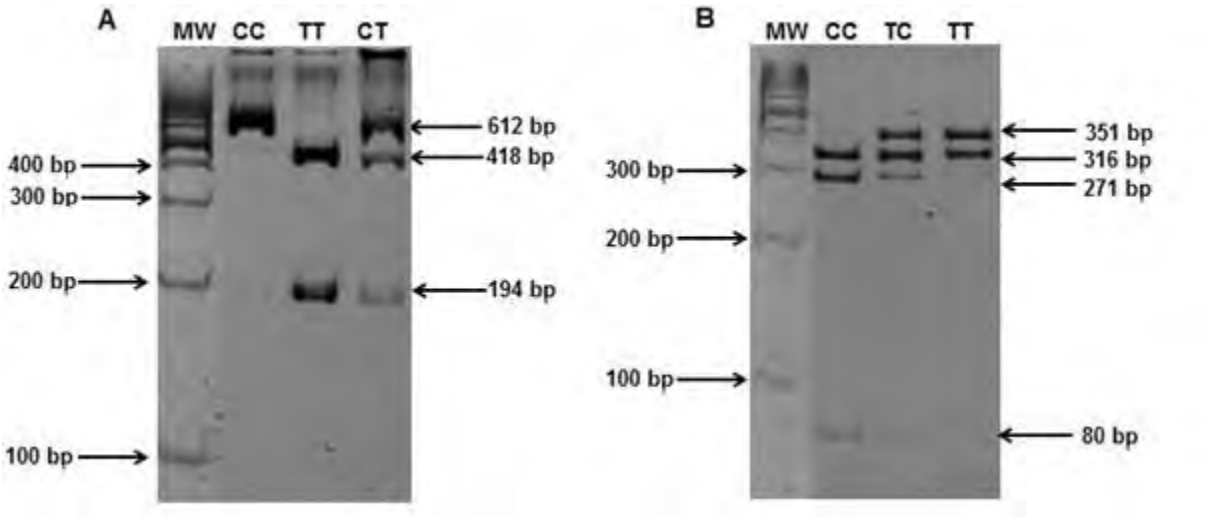

Figure 5.3 Typical 6% non-denaturing polyacrylamide gels showing the genotypes of the (A) *COL5A1 Dpn*II rs13946 and (B) *COL5A1 Bst*UI rs12722 restriction fragment length polymorphisms (RFLPs). Digestion of the 667 bp PCR product with **(A)** *Dpn*II produce 612 bp, 40 bp and 15 bp fragments for the T-allele and 418 bp, 144 bp, 40 bp and 15 bp for the C-allele and **(B)** with *BstU*I. Within this population group, the 667 bp PCR product contains two informative polymorphic *Bst*UI restriction sites. The first site contains the well characterised SNP rs12722 (C/T). The *Bst*UI site is present in the C allele and destroyed in the T allele. The second upstream polymorphic *Bst*UI site overlaps two rare adjacent SNPs, rs146776422 (C/T) and rs55748801 (G/A). The CG wild-type (designated as W in this study) alleles of these two adjacent SNPs contains a *Bst*UI site which is destroyed by either of the alternative nucleotides (CA, TG or TA, designated as M in this study). Four alleles of the *Bst*UI RFLP are therefore produced, which have been designated WC, WT, MC and MT (Table 3.2). The WC alleles produce 316, 271 and 80 bp fragments, while the WT alleles produce 351 and 316 bp fragments. The MC and MT alleles produce 398 and 271 bp and 667 bp fragments respectively (not shown).

The genotypes of three participants designated with a WM + CT genotype using the *Bst*UI RFLP method was further confirmed by Sanger sequencing of the PCR amplified 667bp DNA fragment by the Central Analytical Facility (University of Stellenbosch, Stellenbosch, South Africa) as previously described [191]. BioEdit 7.0.5.2 (www.mbio.ncsu.edu/bioedit/bioedit.html) was used to analyze the obtained sequence information (Figure 5.4).

Table 5.2 The DNA fragment sizes of the BstUI digested 667 bp PCR product of the *COL5A1* 3'-untranslated region, which contains two informative polymorphic *Bst*UI restriction sites. The first site contains variant rs12722 (C/T). The *Bst*UI site is present in the C allele and destroyed in the T allele. The second site contains two rare adjacent variants, rs146776422 (C/T) and rs55748801 (G/A). The CG wild-type (designated as W) allele of these two adjacent variants contains a *Bst*UI site, while the site is destroyed by either the alternative sequence variations (CA, TG or TA, designated as M). Four alleles are therefore produced after digestion with *Bst*UI, which have been designated WC, WT, MC and MT. The sizes of the digested fragments are indicated with a tick. The corresponding genotypes are also indicated.

Allele Phases	Fragment Sizes (bp)						Genotype
	667	396	351	316	271	80	
WC				✓	✓	✓	-
WT			✓	✓			-
MC		✓			✓		-
MT	✓						-
WC, WC				✓	✓	✓	WW + CC
WC, WT			✓	✓	✓	✓	WW + CT
WC, MC		✓		✓	✓	✓	WM + CC
WC, MT	✓			✓	✓	✓	WM + CT
WT, WT			✓	✓			WW + TT
WT, MC		✓	✓	✓	✓		WM + CT
WT, MT	✓		✓	✓			WM + TT
MC, MC		✓			✓		MM + CC
MT, MC	✓	✓			✓		MM + CT
MT, MT	✓						MM + TT

```
COL5A1              CGTGACCTCA GCATGCCATT CGTTCGTGAG TGTCCCGTGC ACGTCCTGAC CCTGGACAGT   240
Variant                                   T                           T (rs13946)
Restriction Site                                                    DpnII
Participant A       ---------- ---------- ---------- ---------- ---------T ----------
Participant B       ---------- ---------- ---------- ---------- ---------T ----------
Participant C       ---------- ---------- ---------- ---------- ---------T ----------

COL5A1              GAAGGCTTCT CCCTCCCCTC CCACCTGACT TCATCTACGC CTCGGCACCA CGGGGTGTGG   300
Variant                                                           T    A
Participant A       ---------- ---------- ---------- ---------- ---------- ----------
Participant B       ---------- ---------- ---------- ---------- ---------- ----------
Participant C       ---------- ---------- ---------- ---------- ---------- ----------

COL5A1              GACCCCACCC CGGAGAGAAC AGAGGGAAGG AGCCGCGCCC CCACCTGGAG CTGAATCACA   360
Variant                    C              (rs146776422) TA (rs55748801)
Restriction Site                                  BstUI
Participant A       ---------- ---------- ---------- -----Y---- ---------- ----------
Participant B       ---------- ---------- ---------- -----Y---- ---------- ----------
Participant C       ---------- ---------- ---------- -----Y---- ---------- ----------

COL5A1              TGACCTAGCT GCACCCCAGC GCCTGGGCCC GCCCCACGCT CTGTCCACAC CCACGCGCCC   420
Variant                                                                    T (rs12722)
Restriction Site                                                           BstUI
Participant A       ---------- ---------- ---------- ---------- ---------- ---Y------
Participant B       ---------- ---------- ---------- ---------- ---------- ---Y------
Participant C       ---------- ---------- ---------- ---------- ---------- ---Y------

COL5A1              CGGGAGCGGG GCCATGCCTC CAGCCCCCCA GCTCGCCCGA CCCATCCTGT TCGTGAATAG   480
Variant              GG                                      T
Participant A       ---------- ---------- ---------- ---------- ---------- ----------
Participant B       ---------- ---------- ---------- ---------- ---------- ----------
Participant C       ---------- ---------- ---------- ---------- ---------- ----------
```

Figure 5.4 DNA sequence, corresponding to nucleotides 181 to 480, of the *COL5A1* exon 66 (pubmed accession no. NM_000093). Nucleotide numbers are annotated on the right side of the sequence. The positions of variants within this region of the 3'-UTR are highlighted in black with the text in white. The sequence contains the wild-type nucleotides of the polymorphic sites. The minor nucleotides are annotated below the sequence. The accession numbers of selected variants are indicated. The polymorphic *Dpn*II and *Bst*UI restriction sites are also indicated (underlined). The corresponding sequence data from participants A, B and C are aligned, with the dashes (-) indicating identical sequence. Since total genomic DNA was sequences from each participant, the Y represents a C for one copy of the gene and a T on the other copy.

5.2.2.2 COL5A1 rs71746744 (-/AGGG) variant

DNA samples were genotyped for the *COL5A1* rs71746744 (-/AGGG) variant in the 3'-UTR of the *COL5A1* gene using a custom designed fluorescence-based Taqman® polymerase chain reaction (PCR) assay (Applied Biosystems, Foster City, California, USA). Allele-specific primer and probe sets (Table 5.1) were used along with a pre-made PCR mastermix containing ampliTaq DNA polymerase Gold (Applied Biosystems, Foster City, California, USA) in a final reaction of 8 µl following the manufacturer's recommended cycling conditions as described in section 4.2.3. The PCR reactions were carried out in the Applied Biosystems

StepOnePlus real-time PCR System (Life Technologies, Applied Biosystems, Foster City, California, USA). Eighty-one % (n=83 of 103) of the cases and 83% (n=124 of 150) of the controls were successfully genotyped for *COL5A1* rs71746744 (Figure 5.5).

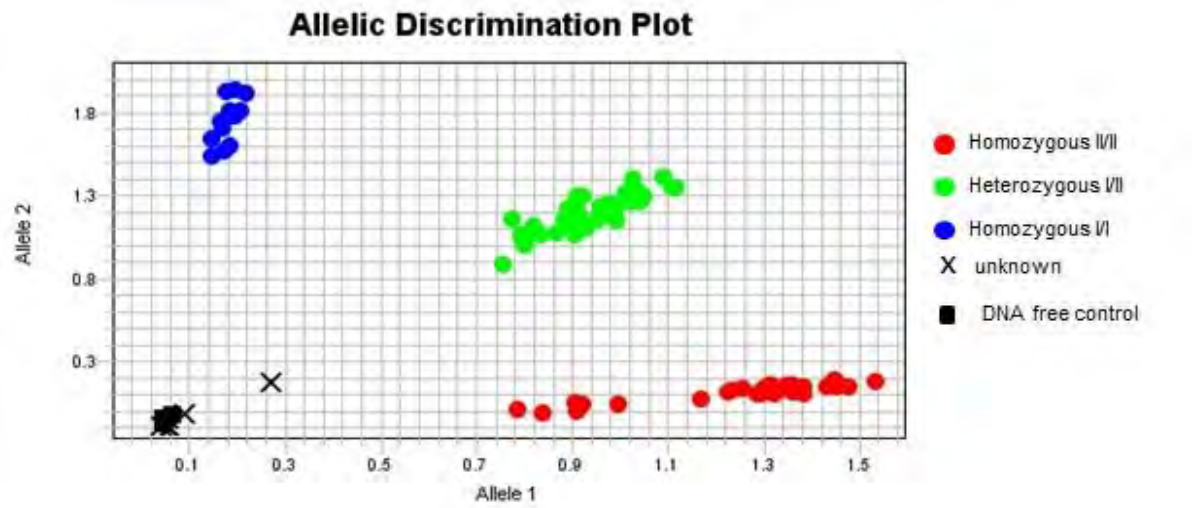

Figure 5.5 Typical allelic discrimination plots using the Taqman® Genotyping assay for *COL5A1* rs71746744 (-/AGGG)

5.2.3 Statistical Analysis

No allele frequency data was available for the Coloured, South African population in the public databases (http://www.ncbi.nlm.nih.gov/snp/). For this reason, the sample size for this study was calculated based on the range of the reported minor allele frequencies, 10.5 to 43.0% for *COL5A1* rs13946, 13.6 to 43.4% for *COL5A1* rs12722 and 14.2% for *COL5A1* rs71746744, previously described for populations in this public database (Table 5.3). Quanto V.1.2.4 was used to determine the statistical power for a given sample size and minor allele frequency[26]. A sample size of approximately 100 cases and 150 controls was found to be adequate to detect a genetic effect size ranging from 2.05 to 2.80 at a power of 80% and a significance level of 5%, assuming a minor allele frequency ranging from 0.5 to 50.0%.

Table 5.3 Minor allele frequencies of the investigated polymorphisms in different populations.

Polymorphism	Population	Population size	Minor Allele	Minor allele frequency (%)
rs13946 (T/C)	HAPMAP-YRI	226	C	38.4
	HAPMAP-GIH	176	C	21.6
	HAPMAP-LWK	180	C	12.8
	HAPMAP-ASW	98	C	11.5
	HAPMAP-MEX	100	C	43.0
	HAPMAP-MKK	286	C	10.5
	HAPMAP-TSI	176	C	23.3
	HAPMAP-CEU	226	C	23.5
rs12722 (C/T)	HAPMAP-YRI	118	T	13.6
	HAPMAP-JPT	82	T	15.9
	HAPMAP-HCB	86	T	22.1
	HAPMAP-CEU	106	C	43.4
rs71746744 (-/AGGG)	South African	106	I	14.2[1]

HAPMAP-YR, Yoruba in Ibadan Nigeria; HAPMAP-GIH, Gujarati Indians in Houston TX USA; HAPMAP-LWK, Luhya in Webuye Kenya; HAPMAP-ASW, African ancestry in Northwest USA; HAPMAP-MEX, Mexican ancestry in Los Angeles CA USA; HAPMAP-MKK, Maasai in Kinyawa Kenya; HAPMAP-TSI, Toscani in Italy; HAPMAP-CEU, Utah residents with Northern and Western European ancestry; HAPMAP-JPT, Japanese in Tokyo, Japan; HAPMAP-HCB, Han Chinese in Beijing, China.

[1] Participants in a South African road running event[190].

Data was analysed using STATISTICA (version 11, StatSoft Inc., Tulsa, Oklahoma, USA) and Graphpad Prism (version 5, GraphPad Software, San Diego, CA, USA, http://www.graphpad.com). A Pearson's chi-squared test or a Fisher's exact test was used to determine any significant differences between the genotype distributions or any other categorical data of the groups. An analysis of variance (ANOVA) was used to detect any significant differences between CTS and CON groups for continuous data. Where appropriate, values were adjusted for the effect of age at recruitment. A Mann-Whitney U

test was used to detect significant differences between the CTS and CON groups for non-parametric data. Statistical significance was accepted at $p<0.05$. Hardy-Weinberg equilibrium (HWE) was established using the program Genepop web version 4.0.10 (http://genepop.curtin.edu.au/)[169,170] Linkage disequilibrium (LD) was calculated using CubeX: cubic exact solution (www.oege.org/software/cubex/)[171]. Inferred haplotypes were constructed from rs13946 (C/T), rs146776422/rs55748801 (W/M), rs12722 (C/T) and rs71746744 (-/AGGG), as well as rs12722 and each of the four *MMP* variants (*MMP10* rs486055, *MMP1* rs1799750, *MMP3* rs679620 and *MMP12* rs2276109) genotyped in Chapter 4, using Chaplin (version 1.2.2, Emory University School of Medicine, Atlanta, Georgia, USA)[172,173] and Hapstat software (version 3.0, University of North Carolina at Chapel Hill, North Carolina, USA)[194]. No adjustments were made for multiple testing considering no obvious appropriate method currently exists[174,175]. The Bonferroni adjustment was considered too conservative since the statistical tests in this and following studies are all performed on the same group of participants[175]. Adjustment for multiple testing was also considered inappropriate since there is an *a priori* hypothesis that the gene variants investigated in this study are associated with the CTS phenotype[174].

5.3 RESULTS

5.3.1 General characteristics

The general characteristics of the participants recruited for this thesis were previously reported in section 4.2.1. The general characteristics for all participants successfully genotyped for at least one of the investigated variants in this chapter are shown in Table 5.4 and were similar to all the participants recruited for this thesis (Table 4.1). Briefly, both groups were matched for age of surgery (age of recruitment was used for the CON group), sex, height and country of birth (Table 5.4). Both groups were also matched for weight and BMI after adjusting for the significant difference ($p<0.001$) in age of recruitment between the CTS (45.6 ± 10.6, n=103) and CON (40.3 ± 9.7, n=148) groups. There were two related participants in both the CTS and the CON groups (mother and daughter). Similar results were obtained when the analyses were repeated after excluding one of the related participants.

Participants with a TT genotype for *COL5A1* rs12722 were significantly older ($p=0.025$) at recruitment (48.2 ± 12.3 n=21) than those with either a CT (41.8 ± 10.3, n=102) or CC genotype (41.7 ± 10.0, n=116). There were no additional genotype effects on age of recruitment, age of surgery, height, weight or BMI for *COL5A1* rs13946, rs146776422/rs55748801 , rs12722, rs71746744, or the combined rs146776422/rs55748801 and rs12722 genotypes (Supplementary tables 5.1 – 5.5, Appendix B). There were also similar *COL5A1* genotype distributions between the normal weight, over-weight and obese CON and/or CTS participants (data not shown).

Table 5.4 General characteristics of the carpal tunnel syndrome (CTS) and control (CON) groups successfully genotyped for at least one of the investigated variants.

	CTS (n=103)	**CON** (n=149)	**p-value**
Age of recruitment (yrs)	45.6 ± 10.6 (103)	40.3 ± 9.7 (148)	**<0.001**
Age of surgery (yrs)	42.1 ± 10.7 (91)	40.3 ± 9.7 (148)[a]	0.197
Sex (% Female)	91.3 (103)	88.6 (149)	0.535
Height (cm)	159.9 ± 7.6 (101)	160.4 ± 7.7 (148)	0.632
Weight (kg)	82.8 ± 18.0 (102)	78.5 ± 19.1 (147)	0.198[b]
BMI (kg/m^2)	32.4 ± 6.9 (101)	30.4 ± 6.8 (146)	0.155[b]
Country of birth (% SA)	99.0 (99)[c]	100.0 (140)	0.414

Values are expressed as a mean ± standard deviation or a frequency (%). The number of participants (n) with non-missing data is indicated in parentheses. The maximum number (N) of participants in each group is also indicated.

Significant p-values are indicated in bold.

[a] age at recruitment.

[b] co-varied for age at recruitment.

[c] One participant was born in Namibia.

yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, meter; SA, South Africa

Similar to the all the participants recruited for this thesis (section 4.2.1), bilateral carpal tunnel release surgery was performed in 50 (53.2%) of the CTS participants successfully genotyped for at least one of the investigated variants, while 33 (35.1%) only had surgery on their dominant hand and 9 (9.6%) had surgery on their non-dominant hand. Two (2.1%) participants who reported being ambidextrous had carpal tunnel surgery on their right hand.

Participants within the CTS (27.2%, n=28) and CON (15.4%, n=23) groups, who were successfully genotyped for at least one of the investigated variants, self-reported similar histories (p=0.199) of medical condition(s), such as diabetes (9.7 % CTS, n=10 vs 8.1% CON, n=12), osteoarthritis (OA, 7.8 % CTS, n=8 vs 5.4% CON, n=8), rheumatoid arthritis

(RA, 3.9 % CTS, n=4 vs 0.7% CON, n=1), thyroid disorders (1.9 % CTS, n=2 vs 0.7% CON, n=1), diabetes and OA (1.0 % CTS, n=1 vs 0.0% CON), RA and OA (0.0 % CTS vs 0.7% CON, n=1), diabetes and RA (1.0 % CTS, n=1 vs 0.0% CON), diabetes and thyroid disorder (1.0 % CTS, n=1 vs 0.0% CON), RA and systemic lupus erthematosus (1.0 % CTS, n=1 vs 0.0% CON), suggested to be associated with CTS.

Sixty-two percent (n=64) of the CTS and 42.5 % (n=62) of the CON participants who were successfully genotyped for at least one of the investigated variants self-reported a history of one or more other medical conditions (p=0.003). These included hypertension (46.6% CTS, n=48 vs 26.2% CON, n=39), hypercholesterolemia (18.4% CTS, n=19 vs 3.4% CON, n=5), asthma (4.9% CTS, n=5 vs 3.4% CON, n=5), angina (6.8% CTS, n=7 vs 0.7% CON, n=1) and other conditions (4.9% CTS, n=5 vs 4.7% CON, n=7), which included hypotension, malignant disease, anaemia, aortic valve stenosis, epilepsy, kidney disease, spinal stenosis, Hirschsprung's disease and autonomic insufficiency.

Similar to all the participants recruited for this thesis (section 4.2.1, table 4.2), the majority of the participants (27.2% CTS, n=28 and 50.3% CON, n=75) who were successfully genotyped for at least one of the five investigated variants, with non-missing data were general poultry processing workers or general workers within other industries where repetitive action is performed with the upper limbs. The other major self-reported occupations included administration (22.3% CTS, n=23 and 11.4% CON, n=17) and nursing (10.7% CTS, n=11 and 8.1% CON, n=12).

Similar to all participants recruited for this thesis (section 4.2.1), there was also no significant difference (p=0.053) between the number of participants, successfully genotyped for at least one of the three investigated variants, who reported spending their whole working day (100%) performing manual labour requiring the use of their hands between the CTS (78.0%,

n=79 of 101) and CON (87.8%, n=130 of 148). Similarly, there was a significant different (p=0.017) between CTS (Median = 50%, interquartile range 5% - 100%) and CON (Median = 100%, interquartile range 10% - 100%) participants who were successfully genotyped, for the percentage time spent standing during a normal working day. There was no significant difference (p=0.295) in number of successfully genotyped participants who reported repetitive leisure activities of the wrist (e.g. knitting/crocheting, gardening and kneading/rolling dough) within the CTS (51.5%, n=50 of 97) and CON (44.2%, n=65 of 147) groups.

5.3.2 Genotypes

The TT genotype of *COL5A1* rs13946 was significantly over-represented in the CON (69.3%) compared to the CTS (50.6%) group (p=0.007, OR=0.45, 95% CI=0.26-0.79, Sensitivity=69.3%, Specificity=49.5%, Table 5.5). Similarly the TT genotype was also significantly over-represented in the CON when only the female participants were analysed (p=0.004). There were however no significant differences in the genotype distributions between the CTS and CON groups for *COL5A1* rs146776422/rs55748801 (p=0.835), rs12722 (p=0.273) and rs71746744 (p=0.986) (Table 5.5). Similarly there were also no significant differences in any of these genotypes distributions between the CTS and CON groups when only the female participants were analysed. All four variants were in Hardy-Weinberg equilibrium. Similar genotype distributions were observed when participants with a history of a medical condition believed to be associated with CTS were excluded from the analysis (Supplementary table 5.6, Appendix B).

Table 5.5 Genotype frequency distributions of the *COL5A1* 3'-untranslated region (UTR) rs13946 (C/T), rs12722 (C/T) and rs71746744 (-/AGGG), as well as the adjacent rs146776422 (C/T) and rs55748801 (G/A), variants in carpal tunnel syndrome (CTS) and control (CON) groups for all participants (All) as well as the female participants (Female).

***COL5A1* Genotype**	**All**		**Female**	
	CTS	**CON**	**CTS**	**CON**
rs13946	n=91	n=127	n=83	n=110
TT	50.6 (46)	69.3 (88)	50.6 (42)	71.8 (79)
CT	40.7 (37)	27.6 (35)	42.2 (35)	25.5 (28)
CC	8.8 (8)	3.2 (4)	7.2 (6)	2.7 (3)
Genotype p-value [b]	**0.007**		**0.004**	
C Minor allele	29.1 (53)	16.9 (43)	28.3 (47)	15.5 (34)
Allele p-value	**0.003**		**0.002**	
HWE	0.554		0.828	
rs146776422/rs55748801	n=98	n=139	n=89	n=123
WW (CG/CG) [a]	89.9 (88)	88.5 (123)	89.9 (80)	87.8 (108)
WM	10.2 (10)	10.8 (15)	10.1 (9)	11.4 (14)
MM	0.0 (0)	0.7 (1)	0.0 (0)	0.8 (1)
Genotype p-value [c]	0.835		0.668	
M Minor allele	5.1 (10)	6.1 (17)	5.1 (9)	6.5 (16)
Allele p-value	0.692		0.677	
HWE	0.544		0.530	
rs12722	n=99	n=141	n=90	n=125
CC	43.4 (43)	51.8 (73)	43.3 (39)	53.6 (67)
CT	44.4 (44)	41.1 (58)	45.6 (41)	40.0 (50)
TT	12.1 (12)	7.1 (10)	11.1 (10)	6.4 (8)
Genotype p-value	0.273		0.236	
T Minor allele	34.3 (68)	27.7 (78)	33.9 (61)	26.4 (66)
Allele p-value	0.131		0.108	
HWE	1.000		0.873	
rs71746744	n=83	n=124	n=74	n=108
AGGG/AGGG	39.8 (33)	39.5 (49)	37.8 (28)	38.0 (41)
AGGG/-	43.4 (36)	42.7 (53)	44.6 (33)	44.4 (48)
-/-	16.9 (14)	17.7 (22)	17.6 (13)	17.6 (19)
Genotype p-value	0.986		1.000	
D Minor allele	38.6 (64)	39.1 (97)	39.9 (59)	39.8 (86)
Allele p-value	0.919		1.000	
HWE	0.192		0.360	

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Values are expressed as a frequency (%) with the number of participants (n) in parentheses. The maximum number (N) of participants in each group is also indicated. Significant p-values are indicated in bold.

[a] The genotyping method was unable to distinguish between the adjacent rs146776422 (C/T) and rs55748801 (G/A) variants. The CG wild-type allele of these adjacent variants, which contains a *Bst*UI restriction site, was designated as a W, while the three alternative nucleotide combinations CA, TG and TA were designated as an M. The *Bst*UI restriction site is destroyded in all three alternative sequence combinations.

[b] TT vs CT and CC.

[c] WW vs WM and MM.

HWE, Hardy-Weinberg equilibrium.

The rs71746744 variant was in linkage disequilibrium (LD) with rs13946 (CTS D'=0.612 and CON D'=0.764), rs146776422/rs55748801 (CTS D'=1.000 and CON D'=1.000) and rs12722 (CTS D'=0.941 and CON D'=0.761). Although rs146776422/rs55748801 and rs12722 were in LD (D'=1.000) within the CON group, they were not in LD (D'=0.267) within the CTS group. Similarly rs13946 was in LD with rs146776422/rs55748801 in the CTS group (D'=1.000) but not in the CON group (D'=0.000). The rs13946 variant was in linkage disequilibrium (LD) with rs12722 (CTS D'=0.735 and CON D'=0.822).

When the combined *COL5A1* rs146776422/rs55748801 and rs12722 genotypes (*Bst*UI RFLP) were analysed, there was a significant difference (p=0.026) in the combined genotypes distributions between the CTS and CON participants (Figure 5.6A). Similarly there were also significant difference (p=0.009) in the combined genotypes distributions when only the female participants were analysed (Figure 5.6B). Specifically the WW+CC (p=0.008, OR=0.45, 95% CI=0.26-0.80, Sensitivity=41.7%, Specificity=75.5%) and WW+CT (p=0.009, OR=2.0, 95% CI=1.2-3.4, Sensitivity=59.2%, Specificity=59.7%) genotypes were significantly over- and under-represented in the CON group respectively (Figure 5.6A). Similarly the WW+CC (p=0.014, OR=0.47, 95% CI=0.26-0.85) and WW+CT (p=0.001, OR=2.5, 95% CI=1.4-4.4) genotypes were significantly over- and under-represented in the

CON group respectively when the female participants were analysed separately (Figure 5.6B).

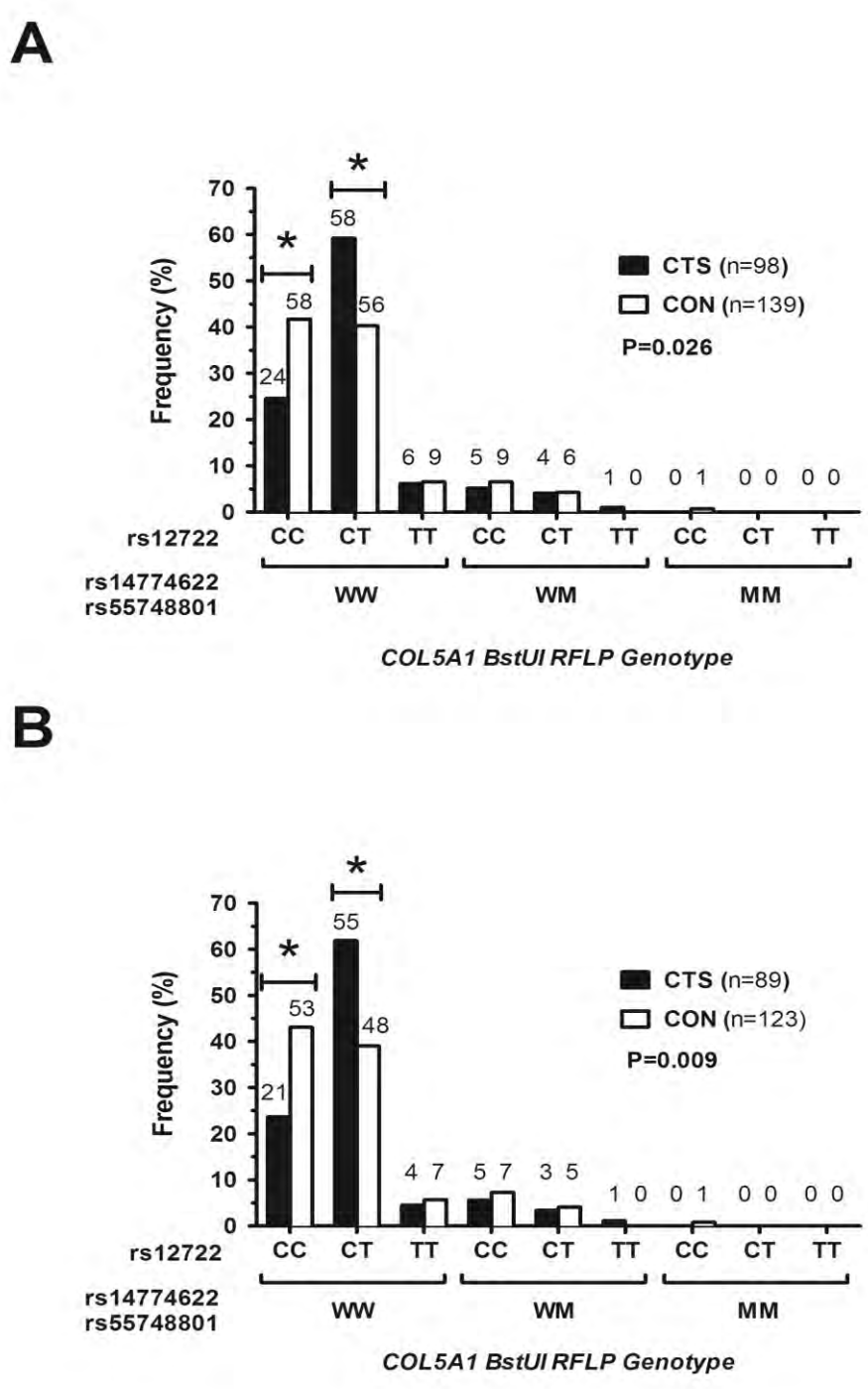

Figure 5.6 The combined genotype frequency distributions of the *COL5A1 Bst*UI restriction fragment length polymorphism (RFLP) for (A) all and (B) female participants in the carpal tunnel syndrome (CTS, solid bars) and control (CON, clear bars) groups.

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The *COL5A1 Bst*UI RFLP can genotype the rs12722 (C/T) variant and the adjacent rs146776422 (C/T) and rs55748801 (G/A) variants. Since the method is unable to distinguish between the adjacent variants, the CG wild-type allele of these adjacent variants, which contains a *Bst*UI restriction site, was designated as a W, while the three alternative nucleotide combinations, CA, TG and TA were designated as a M. The *Bst*UI restriction site is destroyed in all three alternative sequence combinations. The global p value and the significant differences between the groups for a specific genotype combination (asterisks) are indicated. The total number (n) of combined genotypes within the CON and CLS groups is also indicated in parenthesis on the graph. For all participants the allele phases of the WM and CT genotypes in the CTS and CON groups were 4 and 4 for WT/MC and 0 and 2 for MT/WC, respectively.

5.3.3 Inferred Haplotypes

Five of the inferred haplotypes constructed from the *COL5A1* rs13946 (T/C) rs146776422/rs5574880 (W/M) and rs12722 (C/T) variants out of a possible eight had a frequency greater than 2% (Figure 5.7A). The T-W-C inferred haplotype was significantly over-represented in the CON (51.2%) compared to the CTS (34.9%) group ($p<0.001$). The C-W-C inferred haplotype was significantly under-represented in the CON (15.9%) compared to the CTS (26.8%) group ($p=0.005$). Similarly, when inferred haplotypes were constructed from only the rs13946 (T/C) and rs12722 (C/T) variants, the T-C and C-C inferred haplotypes were significantly over- and under-represented (T-C $p<0.001$ and C-C $p=0.006$) in the CON (T-C 56.5% and C-C 15.9%) compared to the CTS (T-C 38.4% and C-C 27.1%) group respectively (Figure 5.7B). Although rare, the C-T inferred haplotype was also significantly under-represented ($p=0.034$) in the CON (0.8%) compared to the CTS (2.4%) group. No additional informative haplotypes were identified when rs71746744 (-/AGGG) was included in the analysis (data not shown).

Since it has previously been reported that *COL5A1* and *MMP3* variants interact to modulate risk for chronic Achilles tendinopathy[58], inferred pseudo-haplotypes were also constructed from the *COL5A1* rs12722 (C/T) and each of the four *MMP* variants (as described in Chapter 4), respectively. Only those pseudo-haplotypes inferred at a frequency greater than 2% were

included. There were no significant difference between the CTS and CON groups for the inferred pseudo-haplotypes constructed from *COL5A1* rs12722 (C/T) with *MMP10* rs486055 (C/T), *MMP1* rs1799750 (GG/GGGG) or *MMP12* rs2276109 (A/G) (Figure 5.8 A, B and D). The T-A inferred pseudo-haplotype constructed from *COL5A1* rs12722 (C/T) and *MMP3* rs679620 (A/G) was however over-represented in the CTS group (14.8%), compared to the CON group (4.6%) (p=0.006, Figure 5.8C).

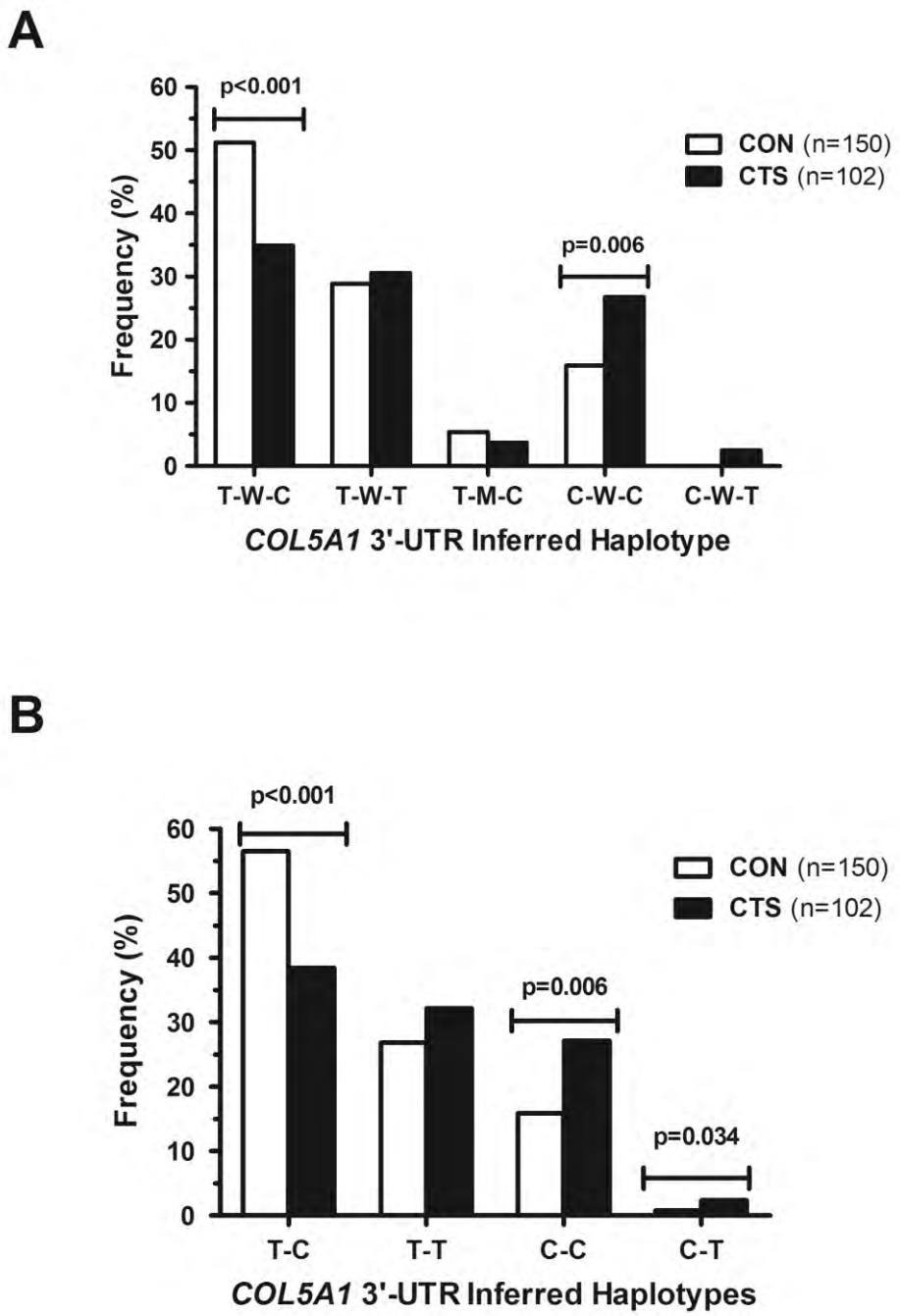

Figure 5.7 Inferred haplotype frequency distributions constructed from the *COL5A1* 3'-untranslated region (UTR) variants for the control (CON, clear bars) and carpal tunnel syndrome (CTS, solid bars) groups. **(A)** Inferred haplotypes constructed from (i) rs13946 (C/T), (ii) combined rs146776422 (C/T) and rs55748801 (G/A) (designated as W/M, where W=CG) and (iii) rs12722 (C/T). **(B)** Inferred haplotypes constructed from (i) rs13946 (C/T) and (ii) rs12722 (C/T). Significant differences between the groups are indicated with a solid line and the p value. The number (n) of subjects in each group is in parenthesis.

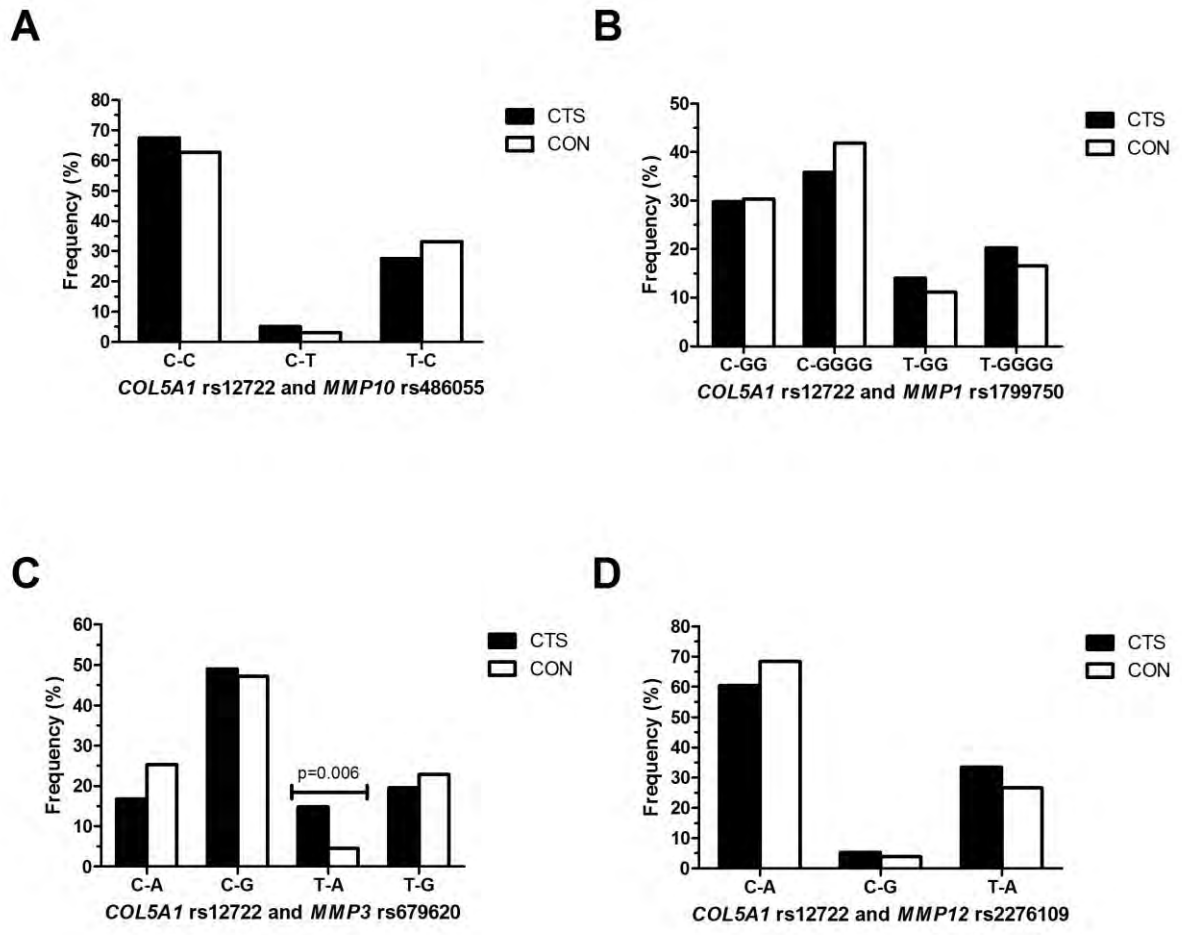

Figure 5.8 Inferred pseudo-haplotypes constructed from the *COL5A1* rs12722 (C/T) and **(A)** *MMP10* rs486055 (C/T), **(B)** *MMP1* rs1799750 (GG/GGGG), **(C)** *MMP3* rs679620 (A/G) and **(D)** *MMP12* rs2276109 (A/G) variants.

5.4 DISCUSSION

An association of common C/T (rs12722) and -/AGGG (rs71746744) variants within the functional *COL5A1* 3′-untranslated region (UTR) with chronic Achilles tendinopathy in Caucasian populations has previously been reported (Figure 5.1)[44,45,185,191]. Based on these previous findings, it was hypothesised that the CC genotype of rs12722 (C/T) will be under-represented in the CTS population, while the AGGG/AGGG genotype of rs71746744 (-/AGGG) will be over-represented.

The first main finding of this study was that although variants rs12722 and rs71746744 were not independently associated with CTS, the combined WW+CC genotypes of two additional adjacent variants, rs146776422 and rs55748801, with rs12722 were significantly under-represented in the CTS population (Figure 5.9). This study identified two additional informative variants within the population group investigated, rs146776422 and rs55748801, which were genotyped together with rs12722. The genotyping method (*Bst*UI RFLP) used in this study was unable to distinguish between rs146776422 (C/T) and rs55748801 (G/A), therefore the CG wild-type alleles of these variants was designated as a W, while the three alternative combinations, CA, TG and TA were designated as an M. To date we have only identified the CG (W) wild type alleles of these adjacent variants within the previously genotyped Caucasian populations (n>1700 participants)[45,46,186–189]. Therefore all the previously reported association of the CC genotype of rs12722 with chronic Achilles tendinopathy in Caucasian populations could also be reported as an association of the combined WW+CC genotypes of rs146776422/rs55748801 (W/M) with rs12722 (C/T). In this study, a 2.1-fold decrease risk for CTS was noted for individuals with a WW+CC genotype. This was similar to the previously reported 2.4- and 2.6-fold decrease risk of chronic Achilles tendinopathy noted in South African and Australian Caucasian populations with the same genotype[45]. The CC genotype of rs12722 was previously associated with decreased risk of

ACL ruptures in females[46], an age-related increase in lower limb range of motion measurements[189] and decreased risk of exercise-associated muscle cramps[186], while the TT genotype was associated with improved endurance running performance[187,188].

The *COL5A1* 3'-UTR variants rs12722 (C/T) and rs71746744 (-/AGGG) are believed to alter the stability of the *COL5A1* messenger RNA (mRNA)[185] . Specifically, we have previously shown that a 57 bp region of the 3′-UTR containing rs71746744 was functional and that it probably played an important role in the etiology of Achilles tendinopathy[191]. It has been reported that the T and I alleles of rs12722 and rs71746744, respectively, are associated with increased mRNA stability, which is hypothesized to result in increased α1(V) chain and type V collagen production, decreasing the fibril diameter and packing density, and thereby potentially altering the mechanical properties of tendons and other connective tissues[185,191] The significant under-representation of the combined WW+CC genotypes for rs146776422, rs55748801 and rs12722 in the CTS population is in agreement with this hypothesis. The lack of association of rs71746744 (-/AGGG) with CTS was however unexpected and probably further highlights the complex regulation of the *COL5A1* gene by the 3'-UTR. The lack of LD between the rs146776422/rs55748801 and rs12722 variants as well as the rs13946 and rs146776422/rs55748801 variants in the CON groups further highlights the complexity in the genomic architecture of this region.

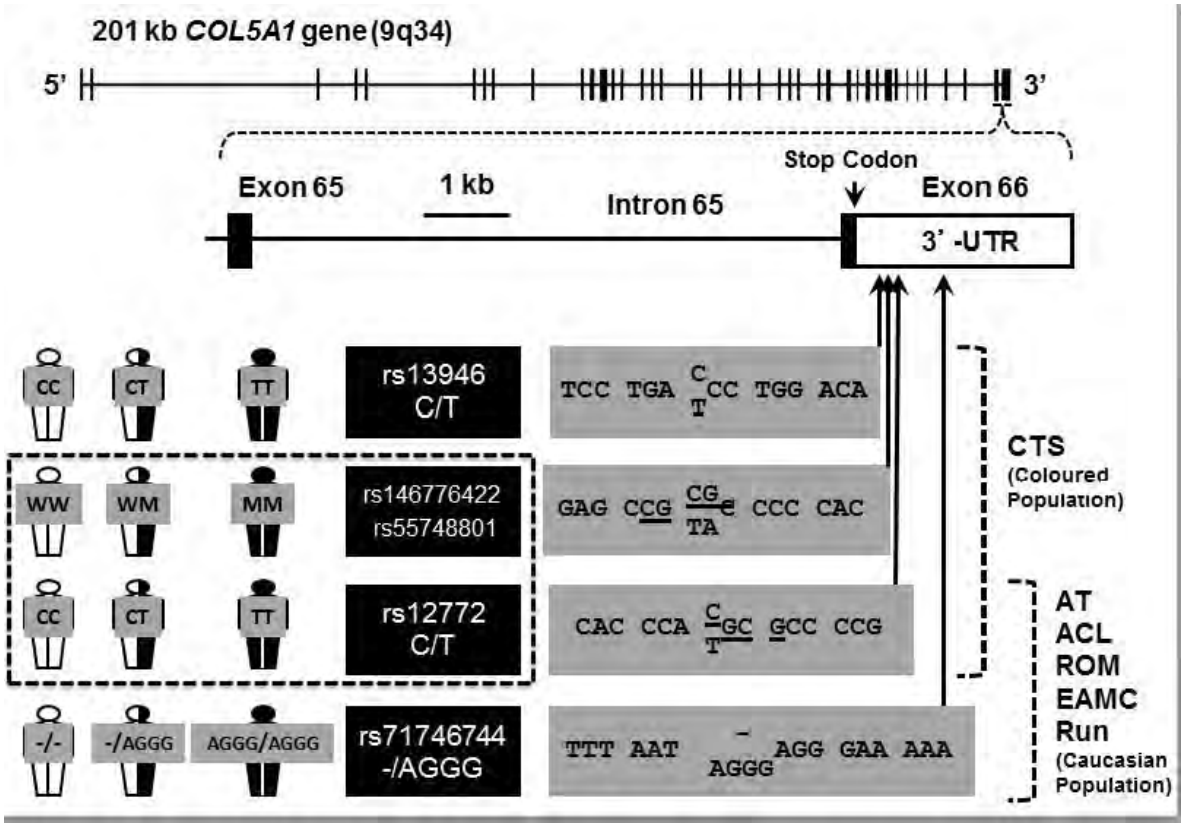

Figure 5.9 A schematic representation of the exon (vertical lines) and intron (horizontal lines) boundaries of the *COL5A1* gene located on chromosome 9q34. The 3'-end of the gene spanning exons 65 to 66 has been enlarged. Exon 66 encodes for the terminal amino acids of the α1(V) chain (black box), the stop codon and the 3'-untranslated region (UTR) (clear box). The three variants (indicated in black boxes), (i) rs13946 (C/T), (ii) rs12722 (C/T) and (iii) rs71746744 (-/AGGG), used in this study are all located within the 3'-UTR. The sequences flanking the variants and the polymorphic nucleotide changes, with the wild type nucleotide indicated on top, are indicated in the grey boxes. As illustrated in the figure, each variant produces three possible genotypes. Variant rs12722 (C/T) was genotyped by digesting a PCR amplified product with the restriction enzyme, *Bst*UI, which has the recognition sequence 5'-CGCG-3' (underlined in the grey boxes). Within the population group included in this study, the PCR products contain a second informative polymorphic *Bst*UI restriction site. The variants simultaneously genotypes by the *Bst*UI RFLP are boxed with a dashed line. The second upstream polymorphic *Bst*UI site contains two rare adjacent variants, rs146776422 (C/T) and rs55748801 (G/A). Since the genotyping method used in this study is unable to distinguish between the adjacent variants, the CG wild-type allele of these adjacent variants, which contains a *Bst*UI restriction site, was designated as a W, while the three alternative nucleotide combinations CA, TG and TA were designated as a M. The *Bst*UI restriction site is destroyed in all three alternative sequence combinations. The phenotypes, as well as the population groups investigated, shown to be associated with specific variants within the *COL5A1* 3'-UTR are indicated by dashed brackets.

The second and also unexpected main finding of this study was that the TT genotype of the downstream *COL5A1* 3'-UTR variant rs13946 (Figure 3.1, *Dpn*II RFLP) was significantly over-represented in the CON participants (p=0.007). This variant was previously not associated with chronic Achilles tendinopathy and ACL ruptures[44–46]. The T-C and C-C inferred haplotypes constructed from rs13946 (C/T) and rs12722 (C/T) were significantly over- and under-represented in the CON group respectively. These results highlight that a different functional region within the *COL5A1* 3'-UTR in close proximity to rs13946 is probably associated with CTS in this population group. Regulatory elements within the 3'-UTR of eukaryotic genes, such as poly (A) signals[195], protein[196] and microRNA (miRNA) binding sites[197,198] are emerging as important post-transcriptional regulators. MicroRNAs are a class of small (18–24 nucleotides) non-coding RNAs that can repressive translational by binding to specific sites of the mRNA[197,198]. Three putative miRNA binding sites, (i) hsa-miR-377*, (ii) hsa-miR-330-5p, (iii) Hsa-miR886-5p, have recently been identified within the region of the *COL5A1* 3'-UTR shown to be associated with CTS (Y Abrahams, personal communication). Interestingly, the binding site for Hsa-miR886-5p is polymorphic and contains variant rs1134114 (C/T). The function of these putative miRNAs in the regulation of *COL5A1* gene expression is currently unknown and warrants further work. Further work is also required to identify any important regulatory elements within this region of the 3'-UTR.

Another interesting finding was that the T-A inferred pseudo-haplotype, constructed from the *COL5A1* rs12722 (C/T) and *MMP3* rs679620 (A/G) variants, was over-represented in the CTS group. Similar findings were reported in a previous study, with the T-A inferred pseudo-haplotype being over-represented in female with ACL ruptures compared to an asymptomatic control group[59]. A different study reported that the T-G haplotype constructed from the same variants was associated with increased risk of chronic Achilles tendinopathy[58]. This study also reported that the GG genotype of *MMP3* rs679620 was independently associated with risk of chronic Achilles tendinopathy. Even though there were

no independent associations of MMP3 rs679620 reported in Chapter 4, this association of the *COL5A1* and *MMP3* T-A inferred pseudo-haplotype highlights the important role that gene-gene interactions may potentially play to modify the risk of CTS. This will be further investigated and discussed in the subsequent chapters of this thesis.

It is highly unlikely that the reported associations within this study were as a result of medical conditions believed to be associated with CTS, since (i) the CON and CTS groups were similarly exposed to these conditions (section 5.3.1) and (ii) similar *COL5A1* genotype distributions were reported when these associated medical conditions were excluded from the analysis. Further work is however required to confirm this finding.

In conclusion, this is the first study to report that variants within the 3'-UTR of the *COL5A1* gene are associated with the CTS. This study also implicated a possible interaction between the *COL5A1* and the *MMP3* genes in modifying the risk of CTS.

CHAPTER 6: PROTEOGLYCAN GENES AND CARPAL TUNNEL SYNDROME

The data presented in this chapter was published in the following peer-reviewed article: Marilize Burger, Hanli de Wet, Malcolm Collins. The BGN and ACAN genes and carpal tunnel syndrome **Gene** 2014; 551 (2):160 - 166.

6.1 INTRODUCTION

In the previous chapter, it was reported that DNA sequence variants within a functional region of the *COL5A1* 3'-untranslated region (UTR) are associated with altered risk of CTS. Briefly, the *COL5A1* encodes for the α1 chain of type V collagen, which is responsible for regulating fibrillogenesis[41]. Similarly, regions within the 3'-UTR of this gene have previously been associated with altered risk of Achilles tendinopathy (AT)[44,45] and Anterior cruciate ligament (ACL) rupture in females[46], while classic Ehlers-Danlos syndrome (EDS) is caused in most cases by rare mutations in *COL5A1*[42]

In addition to the collagens, the proteoglycans, such as biglycan and aggrecan, are also important structural components of tendons and other connective tissues[34] (Figure 6.1). Similar to type V collagen, biglycan, which is encoded by the X-linked *BGN* gene, is also an important regulator of fibrillogenesis[199]. Mice deficient in biglycan and three other small leucine-rich proteoglycans (SLRPs), decorin, fibromodulin and lumican, have a similar physical phenotype to humans with classic EDS[200,201]. In these mice, fibrillogenesis is compromised resulting in collagen fibrils of highly irregular diameters and abnormal organization[200,201]. Recently, the association of the common variants, rs1126499 (C/T) and rs1042103 (G/A), within the *BGN* gene with ACL ruptures was investigated (Figure 6.2)[56]. Although not significant, there was a trend for the rs1126499 T allele to be associated with decreased risk of ACL ruptures in females[56]. The *BGN* CG inferred haplotype constructed

from these two variants was however associated with decreased risk ACL ruptures in females.

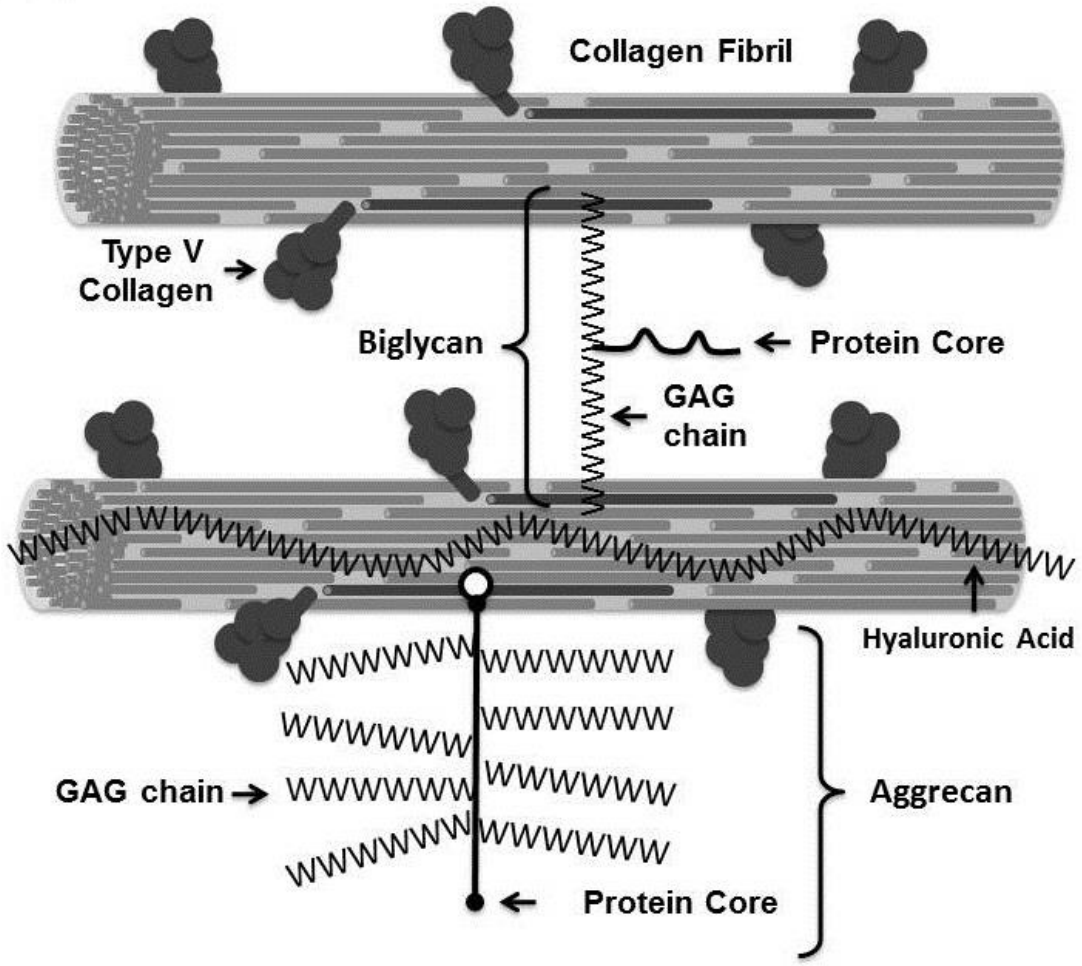

Figure 6.1 A schematic representation showing the interaction of the proteoglycans, biglycan and aggrecan, as well as the glycosaminoglycans (GAGs**),** with the collagen network. Multiple aggrecan core proteins bind hyaluronan via a link protein to form large aggregates; hyaluronan is able to directly interact with the collagen fibril. Figure adapted from Mannion et al. (2014)

The aggrecan (*ACAN*) gene rs1516797 (G/T) variant within exon 4 was previously associated with lumbar disk height narrowing[55] and altered risk of ACL injury[56] (Figure 6.2). Specifically, the G-allele of rs1516797 was significantly associated with increased risk of the ACL rupture. Furthermore, increased mRNA expression of both *ACAN* and *BGN* have been

reported in chronic tendinopathy[49,202] and higher levels of these proteoglycans are present in tissues that are subject to increased forces[49]. Considering the repetitive wrist flexion and extension in certain occupations and the forces created by these activities[3,203], is tempting to speculate that alterations in the proteoglycan content of the tendons within the carpal tunnel could be associated with predisposition to injury.

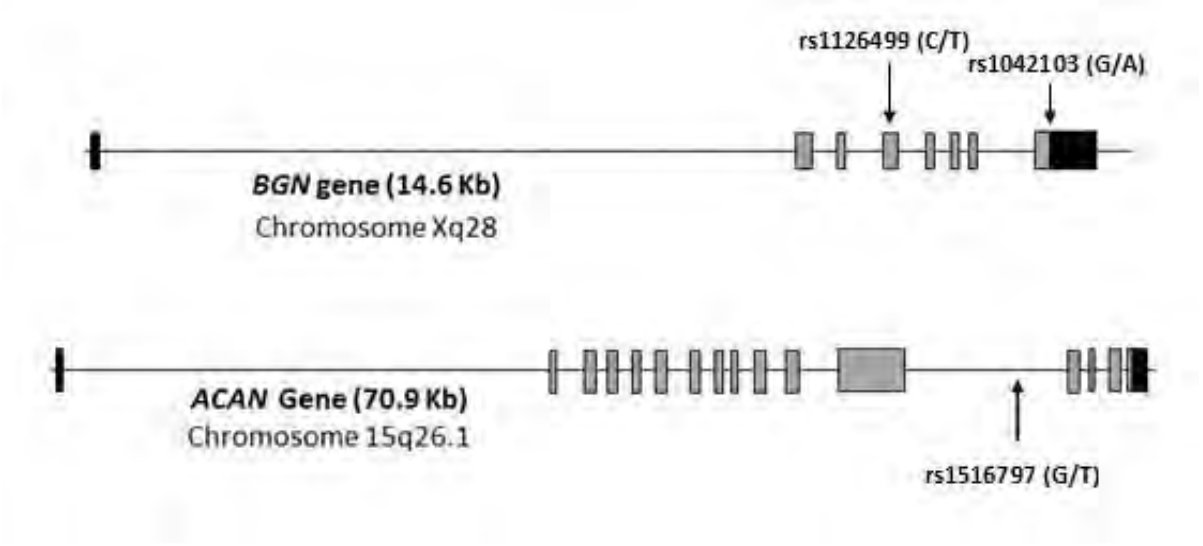

Figure 6.2 A schematic representation of the exon (rectangles) and intron (horizontal lines) structure of the *BGN* (chromosome Xq28) and *ACAN* (chromosome 15q26.1) genes. The translated exons are grey, while the untranslated regions are black. The variants of interest in this study, as well as another previously investigated variant rs1042103 (G/A), are indicated.

The aim of the study presented in this chapter was therefore to determine whether the *BGN* rs1126499 and *ACAN* rs1516797 variants, are associated with CTS. The interaction of these proteoglycan gene variants with the previously associated *COL5A1* variants in altering risk for CTS was also investigated.

6.2 METHODS

6.2.1 Participants & DNA Extraction

The same participants, described in section 4.2.1, were also analysed in this study. DNA extraction was performed as described in section 4.2.2.

6.2.2 Genotyping

All DNA samples (n=253) were genotyped for the *BGN* rs1126499 (C/T) and *ACAN* rs1516797 (G/T) variants, located on human chromosomes X and 15, respectively, at the MRC/UCT Research Unit for Exercise Science & Sports Medicine, University of Cape Town, South Africa, using a fluorescence-based Taqman® PCR assay (Applied Biosystems, Foster City, California, USA) (Assay ID, rs1126499: C___2617574_1_; rs.1516797: C____331789_10). Allele-specific primer and probe sets were used along with a pre-made PCR mastermix containing ampliTaq DNA polymerase Gold (Applied Biosystems, Foster City, California, USA) in a final reaction of 8 µl. The PCR reactions were carried out in the Applied Biosystems StepOnePlus real-time PCR System (Life Technologies, Applied Biosystems, Foster City, California, USA) following the manufacturer's recommended cycling conditions (Figure 6.2 and 6.2B). A number of positive controls of known genotype and DNA-free controls were randomly included on each PCR plate for quality control purposes. In addition, a subset of samples (approximately 10%) was genotyped twice using the same methodology to ensure genotyping was consistent. In order to avoid genotyping errors, samples that failed twice to amplify during PCR for a particular variant were considered to be unsuccessfully genotyped and no further attempts were made to genotype them at that specific locus. Ninety-two % (n=95 of 103) of the cases and 83% (n=125 of 150) of the controls were successfully genotyped for the *BGN* rs1126499 variant whereas 92% (n=95 of 103) and 84% (n=126 of 150) were successfully genotyped for the *ACAN* rs1516797 cases and controls, respectively.

6.2.3 Statistical Analysis

No allele frequency data was available for the Coloured, South African population in the public databases (http://www.ncbi.nlm.nih.gov/snp/). For this reason, the sample size for this study was calculated based on the range of the reported minor allele frequencies, 0.06 to 47.2% for *BGN* rs1126499 and 21.7 to 46.2% for *ACAN* rs1516797 previously described for populations in this public database (Table 6.1). Quanto V.1.2.4 was used to determine the statistical power for a given sample size and minor allele frequency. A sample size of approximately 100 cases and 150 controls was found to be adequate to detect a genetic effect size ranging from 2.05 to 2.80 at a power of 80% and a significance level of 5%, assuming minor allele frequencies ranging from 0.5 to 47.2%. Non-missing data was analysed using STATISTICA (version 11, StatSoft Inc., Tulsa, Oklahoma, USA) and Graphpad Prism (version 5, GraphPad Software, San Diego, CA, USA, http://www.graphpad.com). Since the *BGN* gene is located on the X chromosome, only female participants were analysed with regards to genotype and allele frequencies of the rs1126499 variant. A Pearson's chi-squared test or a Fisher's exact test was used to determine any significant differences between the genotype distributions or any other categorical data of the groups. An analysis of variance (ANOVA) was used to detect any significant differences between CTS and CON groups for continuous data. Statistical significance was accepted at $p<0.05$. Hardy-Weinberg equilibrium (HWE) was established using the program Genepop web version 4.0.10 (http://genepop.curtin.edu.au/)[169,170] Inferred haplotypes were constructed from *BGN* rs1126499 (C/T), *ACAN* rs1516797 and/or *COL5A1* rs12722 (C/T) (Chapter 5) using Chaplin (version 1.2.2, Emory University School of Medicine, Atlanta, Georgia, USA)[172,173]. No adjustments were made for multiple testing considering no obvious appropriate method currently exists[174,175]. The Bonferroni adjustment was considered too conservative since the statistical tests in this and following studies are all performed on the same group of participants[175]. Adjustment for multiple testing was also considered inappropriate since there is an *a priori* hypothesis that the gene variants investigated in this study are associated with the CTS phenotype[174].

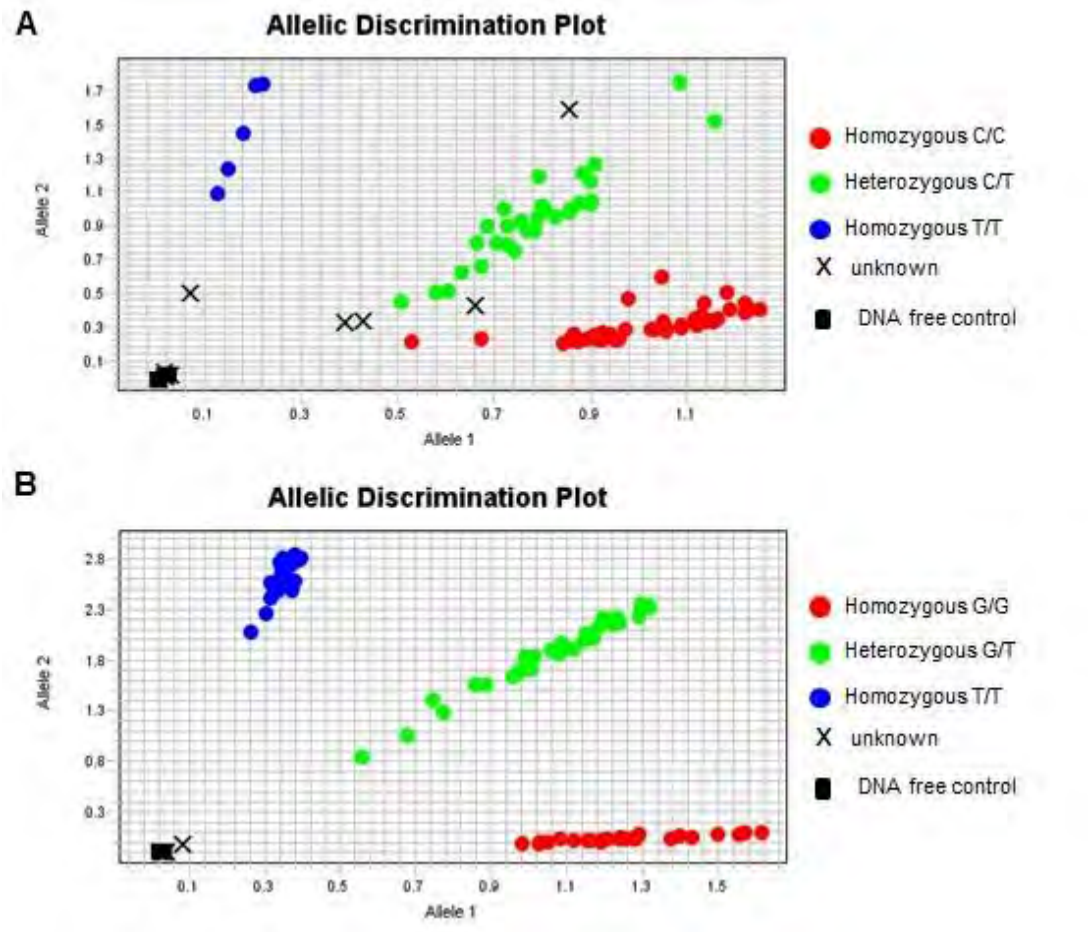

Figure 6.3 Typical allelic discrimination plots using the Taqman® Genotyping assay for **(A**) *BGN* rs1126499 (C/T) and **(B)** *ACAN* rs1516797 (G/T) on the StepOnePlus™ Real-time PCR System.

Table 6.1 Minor allele frequencies of the investigated polymorphisms in different populations.

Polymorphism	Population	Population size	Minor Allele	Minor allele frequency (%)
rs1126499 (C/T)	HAPMAP-YRI	224	T	31.4
	HAPMAP-GIH	176	T	40.3
	HAPMAP-LWK	180	T	0.06
	HAPMAP-ASW	98	T	23.5
	HAPMAP-MEX	100	T	45.0
	HAPMAP-MKK	286	T	19.9
	HAPMAP-TSI	176	C	47.2
	HAPMAP-CEU	226	T	42.9
rs1516797 (G/T)	HAPMAP-YRI	226	T	21.7
	HAPMAP-GIH	176	G	33.5
	HAPMAP-LWK	180	T	37.2
	HAPMAP-ASW	98	T	31.6
	HAPMAP-MEX	100	G	24.0
	HAPMAP-MKK	286	T	46.2
	HAPMAP-TSI	176	G	27.8
	HAPMAP-CEU	226	G	27.0

HAPMAP-YR, Yoruba in Ibadan Nigeria; HAPMAP-GIH, Gujarati Indians in Houston TX USA; HAPMAP-LWK, Luhya in Webuye Kenya; HAPMAP-ASW, African ancestry in Northwest USA; HAPMAP-MEX, Mexican ancestry in Los Angeles CA USA; HAPMAP-MKK, Maasai in Kinyawa Kenya; HAPMAP-TSI, Toscani in Italy; HAPMAP-CEU, Utah residents with Northern and Western European ancestry.

6.3 RESULTS

6.3.1 General characteristics

The general characteristics of the participants recruited for this thesis were previously reported in section 4.2.1. The general characteristics for all participants successfully genotyped for at least one of the investigated variants in this chapter are shown in Table 6.2 and were similar to all the participants recruited for this thesis (Table 4.1). The CTS and CON groups were matched for age of surgery (age of recruitment was used for the CON group), sex, height and country of birth (Table 6.2). Both groups were similarly matched for weight and BMI after adjusting for the significant difference ($p<0.001$) in age of recruitment between the CTS (45.6 ± 10.7, n=99) and CON (40.5 ± 9.9, n=135) groups.

Bilateral carpal tunnel release surgery was performed in 48 (53.3%) of the CTS participants successfully genotyped for at least one of the investigated variants, while only 31 (34.4%) had surgery on their dominant hand and 9 (10.0%) had surgery on their non-dominant hand. Two (2.2%) participants who reported being ambidextrous had carpal tunnel surgery on their right hand.

Participants within the CTS (24.2%, n=24) and CON (15.4%, n=21) groups self-reported similar histories (p=0.096) of medical condition(s), such as diabetes (9.1 % CTS, n=9 vs 8.1% CON, n=11), osteoarthritis (OA, 6.8 % CTS, n=7 vs 5.1% CON, n=7), rheumatoid arthritis (RA, 2.9 % CTS, n=3 vs 0.7% CON, n=1), thyroid disorders (1.0 % CTS, n=1 vs 0.7% CON, n=1), diabetes and OA 1.0 % CTS, n=1 vs 0.0% CON), RA and OA (0.0 % CTS vs 0.7% CON, n=1), diabetes and RA (1.0 % CTS, n=1 vs 0.0% CON), diabetes and thyroid disorder (1.0 % CTS, n=1 vs 0.0% CON), RA and systemic lupus erthematosus (1.0 % CTS, n=1 vs 0.0% CON), suggested to be associated with CTS.

Table 6.2 General characteristics of the carpal tunnel syndrome (CTS) and control (CON) groups successfully genotyped for at least one of the investigated variants.

	CTS (n=99)	**CON** (n=136)	**p-value**
Age of recruitment (yrs)	45.6 ± 10.7 (99)	40.5 ± 9.9 (135)	**<0.001**
Age of surgery (yrs)	42.1 ± 10.8 (88)	40.5 ± 9.9 (135)[a]	0.242
Sex (% Female)	90.9 (99)	88.2 (136)	0.758
Height (cm)	159.9 ± 7.7 (97)	160.5 ± 7.9 (135)	0.726
Weight (kg)	82.9 ± 18.2 (98)	78.4 ± 19.2 (134)	0.432[b]
BMI (kg/m^2)	32.5 ± 7.0 (97)	30.4 ± 6.7 (133)	0.343[b]
Country of birth (% SA)	99.0 (96)[c]	100.0 (127)	0.508

Values are expressed as a mean ± standard deviation or a frequency (%). The number of participants (n) with non-missing data is indicated in parentheses. The maximum number (n) of participants with non-missing data in each group is also indicated.
Significant p-values are indicated in bold.
[a] age at recruitment.
[b] co-varied for age at recruitment.
[c] One participant was born in Namibia.
yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, meter; SA, South Africa.

Sixty-three percent (n=62) of the CTS and 42.6 % (n=58) of the CON participants self-reported a history of one or more other medical conditions (p=0.002). These included hypertension (45.5% CTS, n=45 vs 27.1% CON, n=36), hypercholesterolemia (19.2% CTS, n=19 vs 3.8% CON, n=5), asthma (5.1% CTS, n=5 vs 3.0% CON, n=4), angina (7.1% CTS, n=7 vs 0.8% CON, n=1) and other conditions (5.1% CTS, n=5 vs 5.3% CON, n=7), which included hypotension, malignant disease, anaemia, aortic valve stenosis, epilepsy, kidney disease, spinal stenosis, Hirschsprung's disease and autonomic insufficiency.

The majority of the participants (29.3% CTS, n=29 and 52.9% CON, n=72) included in this chapter of the thesis with non-missing data were general poultry processing workers or general workers within other industries where repetitive action is performed with the upper limbs (Table 6.3). The other major self-reported occupations included administration (21.2% CTS, n=21 and 11.8% CON, n=16) and nursing (10.1% CTS, n=10 and 8.8% CON, n=12). As summarised in Table 6.3 the remaining participants were recruited from several high risk occupations which included work where the hands/wrists are used for a high percentage of the normal working day.

There was no significant difference (p=0.150) in the number of participants who reported spending their whole working day (100%) performing manual labour requiring the use of their hands between the CTS (79.2%, n=76 of 96) and CON (86.8%, n=118 of 136) groups. There was also no significant difference (p=0.067) between CTS (Median = 50%, interquartile range 10% - 100%) and CON (Median = 100%, interquartile range 10% - 100%) groups for the percentage time spent standing during a normal working day. There was no significant difference (p=0.419) in number of participants who reported repetitive leisure activities of the wrist (e.g. knitting/crocheting, gardening and kneading/rolling dough) within the CTS (50.5%, n=47 of 93) and CON (44.8%, n=60 of 134) groups.

Table 6.3 Occupations of the carpal tunnel syndrome (CTS) and control (CON) participants, successfully genotyped for at least one of the investigated variants.

Occupation	CTS	CON
	n=99	n=136
General worker		
Poultry processing	23.2 (23)	33.8 (46)
Other industries [a]	6.1 (6)	19.1 (26)
Administrator	21.2 (21)	11.8 (16)
Nurse	10.1 (10)	8.8 (12)
Packer	9.1 (9)	7.4 (10)
Seamstress	7.1 (7)	8.1 (11)
Operator	7.1 (7)	7.4 (10)
Food Handler	5.1 (5)	2.9 (4)
Other [b]	11.1 (11)	0.7 (1)

Values are expressed as a frequency (%) with the number of participants (n) in parentheses. The maximum number (N) of participants with non-missing data in each group is also indicated.

[a] includes bottle packer/cleaners, general workers in dairy industry, barrel makers and rotators, cleaners, fitters, wrappers.

[b] includes sanders, engineering clerks, security, cashiers, kitchen attendant, ironing clothes, fire fighters.

After adjusting for the effect of sex on BMI, there were no *ACAN* rs1516797 genotype effects on any of the other physiological characteristics of the participants (Supplementary table 6.1, Appendix B). Since the *BGN* gene is located on the X chromosome, only the female participants (90.0% of the participants) were analysed for the rs1126499 variant. There were no *BGN* genotype effects on age of recruitment, age of surgery, height, weight or BMI for the *BGN* rs1126499 variants in the female participants (Supplementary table 6.2, Appendix B).

6.3.2 Genotypes

There was no significant difference in the genotype or allele distributions between the CTS and CON groups for the *ACAN* rs1516797 variant (Table 6.4).

Table 6.4 Genotype frequency distributions of the *ACAN* rs1516797 (G/T) in carpal tunnel syndrome (CTS) and control (CON) groups for all participants (All) as well as the female participants (Female).

	All		Female	
	CTS	**CON**	**CTS**	**CON**
	n=95	n=126	n=86	n=110
GG genotype	30.5 (29)	32.5 (41)	27.9 (24)	30.9 (34)
GT genotype	47.4 (45)	42.9 (54)	47.7 (41)	42.7 (47)
TT genotype	22.1 (21)	24.6 (31)	24.4 (21)	26.4 (29)
Genotype p-value	0.795		0.786	
T allele	45.8 (87)	46.0 (116)	48.3 (83)	47.7 (105)
Allele p-value	0.960		0.917	
HWE	0.140		0.159	

Genotype and minor allele frequencies are expressed as a percentage with the number of participants (n) in parentheses. The maximum number (n) of participants with non-missing data in each group is also indicated.
HWE, Hardy-Weinberg equilibrium.

There was however a significant difference (CC vs CT+TT, p=0.0498) between the CON and CTS groups for *BGN* rs1126499 where CC was over-represented (OR=0.545, 95% CI=0.30-0.99; Sensitivity 0.581, 95% CI 0.471–0.687; Specificity 0.282, 95% CI 0.200-0.376) in the CON group (Figure 6.4). There was however no significant difference (p=0.205) n the rs1126499 allele frequency distribution between the CTS (132 C, 76.7% and 40 T, 23.3%) and CON (181 C, 82.3% and 39 T, 17.7%) groups. The *ACAN* rs1516797 variant was in Hardy-Weinberg equilibrium (HWE) in both groups (Table 6.4). Although the *BGN* rs1126499 was in HWE in the CTS group (p=0.277), the CON group was not in HWE (p=0.005). Similar genotype distributions were observed when participants with a history of a medical condition

believed to be associated with CTS were excluded from the analysis. However, there was no significant difference between the CTS and CON groups (CC vs CT+TT, p=0.418) (Supplementary table 6.3, Appendix B). Considering the low significant effect when all the participants were included and the low sample number, this is not a surprising finding. This, however, remains a limitation of the current study.

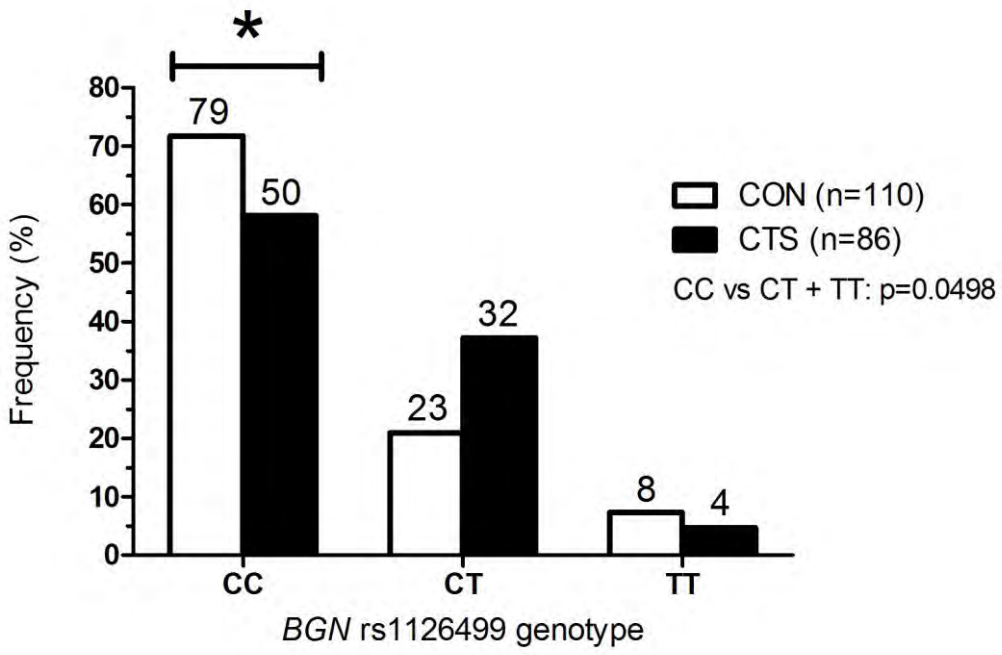

Figure 6.4 Genotype frequency distributions *BGN* rs1126499 (C/T) in carpal tunnel syndrome (CTS) and control (CON) groups for the female participants. Because of the small sample size of the TT genotype in the CTS and CON groups, the CC genotype was compared with the combined CT and TT genotypes. The number of participants in each group is indicated. Significant differences between the groups are indicated with a solid line and asterisk with the p-value shown.

6.3.3 Inferred Pseudo-Haplotypes

All four possible pseudo-haplotypes constructed from the *ACAN* rs1516797 (G/T) and the *BGN* rs1126499 (C/T) variants were inferred when the female participants were analysed (Figure 6.5). Although not significant, there was a trend for the T-T inferred pseudo-

haplotype to be over-represented in the CTS (13.3%) compared to the CON (7.5%) group (p=0.052).

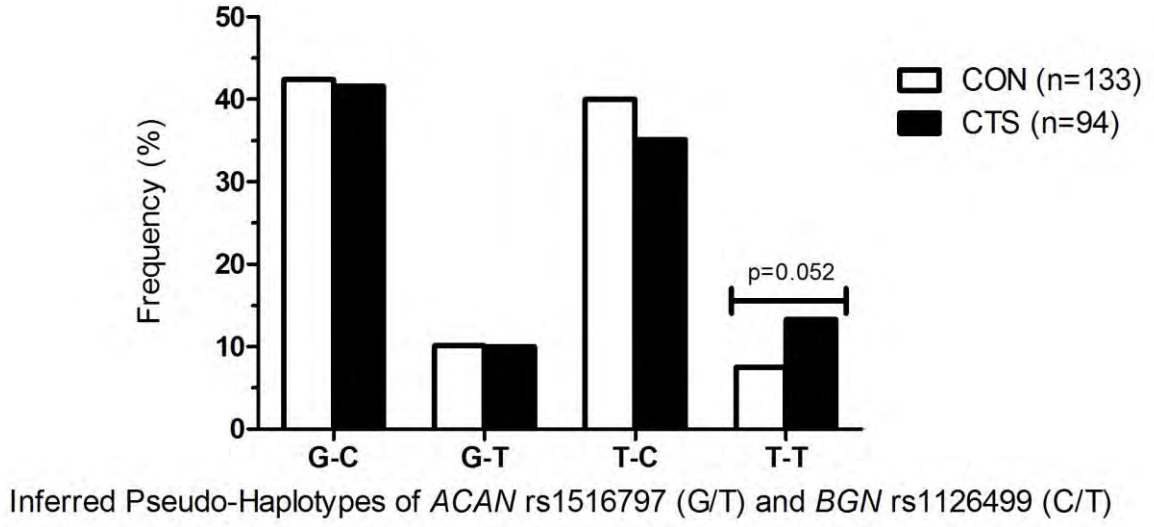

Figure 6.5 Inferred haplotypes constructed from the *ACAN* rs1516797 and *BGN* rs1126499 gene variants from female participants for carpal tunnel syndrome (solid bars) and controls (clear bars). Significant differences or trends between the groups are indicated with a solid line and the p-value.

Since the previously associated *COL5A1* gene (Chapter 5) is also involved in collagen fibrillogenesis, inferred pseudo-haplotypes were constructed in the female participants from the *COL5A1* rs13946 (T/C) and rs12722 (C/T) variants and *BGN* rs1126499 (C/T). Six of the possible eight inferred pseudo-haplotypes had a frequency greater than 2% (Figure 6.6). The C-C-C (24.9% CTS vs 12.3% CON) and T-T-T (11.2% CTS vs 7.6% CON) pseudo-haplotypes were significantly over-represented in the CTS group (p=0.001 and p=0.033 respectively) whilst the T-C-C (30.8% CTS vs 51.1% CON) pseudo-haplotype was significantly under-represented in the CTS group (p<0.001).

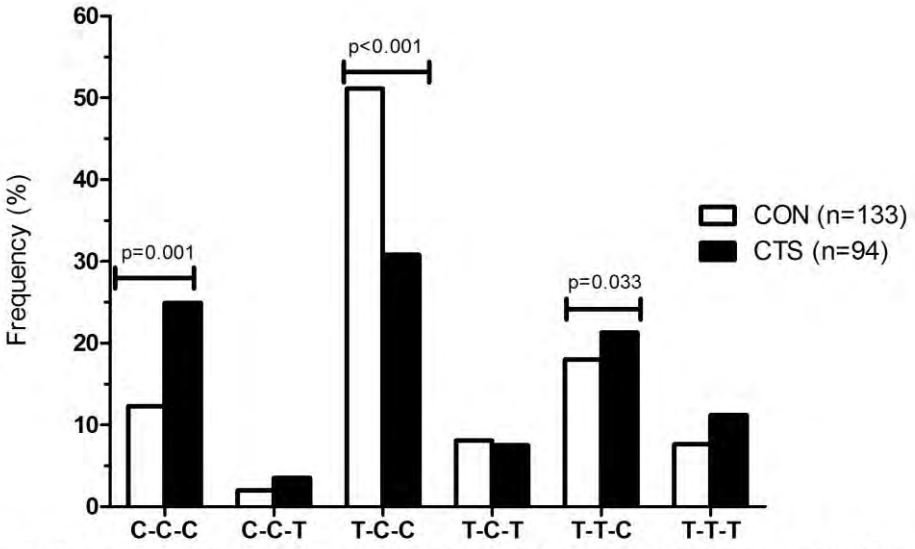

Figure 6.6 Inferred haplotypes constructed from the *COL5A1* rs13946 (C/T), *COL5A1* rs12722 (C/T) and *BGN* rs1126499 (C/T) gene variants from female participants for carpal tunnel syndrome (solid bars) and controls (clear bars). Significant differences between the groups are indicated with a solid line and the p-value.

6.3.4 *COL5A1* & *BGN* Protection Scores

Since, the TT *COL5A1* rs13946, WW+CC *COL5A1* rs146776422/rs55748801 + rs12722, and CC *BGN* rs1126499 genotypes were all independently associated with reduced risk of CTS (Chapter 5 and Figure 6.4), genotype-genotype interactions were investigated. Each of the individual "protective" genotypes contributed a score of 2 towards a participants′ genotype "protection" score. Participants with all the individual CTS "protective" genotypes therefore had a total score of 6, while those with none of the "protective" genotypes had a score of 0. Compared to the CTS group, the genotype "protection" score of 6 was significantly over-represented within the CON group ($p<0.001$, OR=0.19, 95% CI=0.07-0.51, sensitivity=0.07 (95% CI=0.02 – 0.16), specificity=0.71 (95% CI=0.61 – 0.80)) (Table 6.5 and Figure 6.7A), and the genotype "protection" score of ≤2 was significantly under-represented

within the CON group (p=0.002, OR=3.0, 95% CI=1.5-5.7, sensitivity=0.49 (95% CI=0.37 – 0.61), specificity=0.71 (95% CI=0.65 – 0.84) (Table 6.5 and Figure 6.7A). When the *BGN* rs1126499 genotypes was excluded from the analyses, the two *COL5A1* 3′-UTR "protective" genotypes were also significantly over-represented (p=0.009, OR=0.39, 95% CI=0.19-0.79, sensitivity=0.15 (95% CI=0.08 – 0.24), specificity=0.69 (95% CI=0.60 – 0.77) within the CON participants when compared to the CTS participants (Table 6.5 and Figure 6.7B). In contrast, the genotype "protection" score of 0 was significantly under-represented within the CON group (p=0.005, OR=3.3, 95% CI=1.4-7.4, sensitivity=0.23 (95% CI=0.15 – 0.33), specificity=0.92 (95% CI=0.85 – 0.96) (Table 6.5 and Figure 6.7B).

Table 6.5 Individual and combined *COL5A1* rs13946 (T/C), rs146776422rs55748801 (W/M) and rs12722 (C/T), as well as, *BGN* rs1126499 (C/T) genotypes which are associated with reduced (↓) or increased (↑) risk of carpal tunnel syndrome (CTS). Since the individual genotypes are independently associated with reduced risk of CTS, a "protection" score for CTS was calculated where the protective genotype of each variant contributed 2 points (rs13946 TT; rs146776422/rs55748801 WW + rs12722 CC; and rs1126499 CC) towards the "protection" score, while the non-protective genotypes contributed 0 points. The p-values, odds ratios (OR), sensitivity and the specificity, together with the respective 95% confidence intervals (CI) of the individual and combined genotypes are indicated.

COL5A1			*BGN*						
rs13946 (T/C)	rs146776422 rs55748801 (W/M) [a]	rs12722 (C/T) [a]	rs1126499 (C/T)	CTS	"Protection" Score	p-value	OR (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
			CC	↓	-	0.0498	0.55 (0.30 - 0.99)	0.58 (0.47 - 0.69)	0.28 (0.20 - 0.38)
TT				↓	-	0.007	0.45 (0.26 - 0.79)	0.51 (0.40 - 0.61)	0.31 (0.23 - 0.40)
	WW	CC		↓	-	0.008	0.45 (0.26 - 0.80)	0.25 (0.16 - 0.34)	0.58 (0.50 - 0.67)
TT	WW	CC		↓	4	0.009	0.39 (0.19 - 0.79)	0.15 (0.08 - 0.24)	0.69 (0.60 - 0.77)
TT	WW	CC	CC	↓	6	<0.001	0.19 (0.07 - 0.51)	0.07 (0.02 - 0.16)	0.71 (0.61 - 0.80)
YC	XX [b]	YT	YT	↑	0 or 2	0.002	3.00 (1.5 - 5.7)	0.49 (0.37 - 0.61)	0.75 (0.65 - 0.84)
YC	XX [b]	YT		↑	0	0.005	3.30 (1.4 - 7.4)	0.23 (0.15 - 0.33)	0.92 (0.85 - 0.96)
YC	XX [b]	YT	YT	↑	0	0.041	5.00 (1.0 - 24.8)	0.09 (0.04 - 0.19)	0.98 (0.92 - 1.00)

[a] The WW rs146776422/rs55748801 + CC rs12722 genotypes contributed a score of 2 towards the "protection" score, the other genotype combinations contributed a score of 0.

[b] XX = WM, MW or MM; Y= C or T

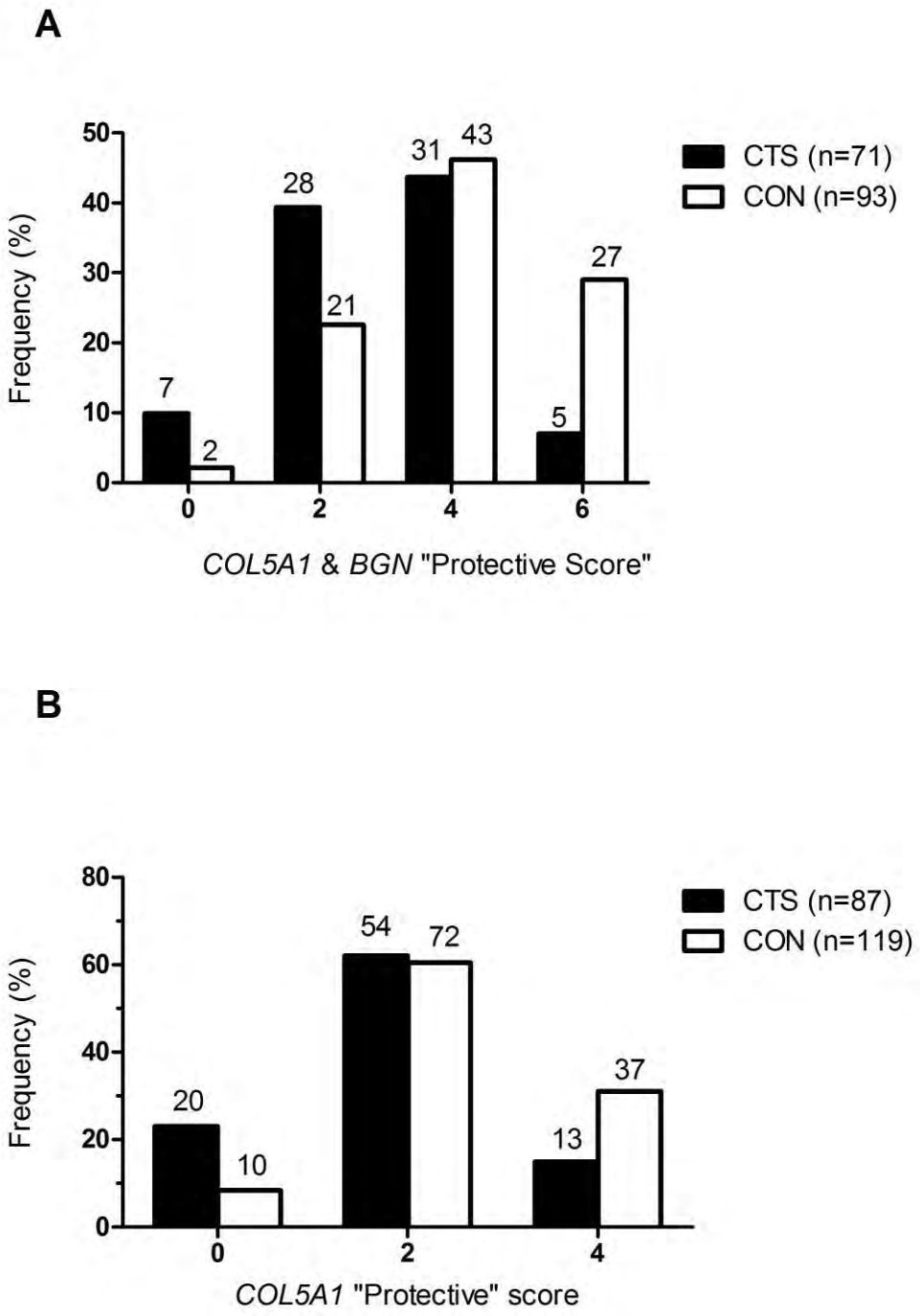

Figure 6.7 Genotype protective scores of the (A) *COL5A1* rs13946 (T/C), rs12722 (C/T) and *BGN* rs1126499 (C/T) and (B) *COL5A1* rs13946 (T/C) and rs12722 (C/T) variants for the carpal tunnel syndrome (CTS) (solid bars) and control (CON) (clear bars) groups, for female participants. Each of the three individual "protective" genotypes (rs13946, TT; rs12722, CC; rs11126499, CC) contributed a score of 2 towards a participants′ genotype "protection" score while the "risk" genotypes (rs13946, CC; rs12722, TT; rs11126499, TT) contributed 0. Participants with all three individual CTS "protective" genotypes therefore had a total score of 6 (A) or 4 (B), while those with none of the "protective" genotypes had a score of 0.

6.4 DISCUSSION

Proteoglycans, such as aggrecan and the small leucine rich proteoglycan, biglycan, plays important structural roles in connective tissue such as tendons. It is vital in the regulation of ECM remodeling as well as collagen fibrillogenesis, through the interactions between the proteoglycans and the major structural component of tendons, the collagen network[204,205]. Considering the previous associations of variants within genes involved in fibrillogenesis with risk of injury (including *COL5A1*, chapter 5) and the role of proteoglycans in this process, this study investigated two variants within two genes encoding proteoglycans (*ACAN* rs1516797 and *BGN* rs1126499) for its potential association with risk of carpal tunnel syndrome (CTS). The first main finding of this study was the independent association of *BGN* rs1126499 (C/T) with risk of CTS whilst there was no individual association of *ACAN* rs1516797 (G/T) variant with CTS.

Biglycan interacts with the collagen fibrils (Figure 6.1) and is able to regulate collagen fibrillogenesis which allows it to maintain the structure of the ECM[204,206], Considering the role of biglycan in the regulation of fibrillogenesis and the location of the *BGN* gene on the X-chromosome, together with the fact that CTS is more prevalent in females than males, it was considered an ideal gene to investigate for a possible role in altering risk for CTS. In this study, the CC genotype of the rs1126499 variant within this gene was significantly over-represented in the CON group (p=0.0498, Figure 6.4) indicating a possible protective effect against CTS. Furthermore, in a previous study, the C-allele was part of an inferred haplotype constructed from variants within the *BGN* gene shown to be over-represented in the control group compared to an ACL injury group[56]. This strengthens the evidence that the C-allele of rs1126499 having a protective role against injury. In addition, although not significant, there was a trend for the opposite rs1126499 T-allele to be associated with increased risk of ACL ruptures in the previous study[56]. In this study, there was also a trend

for the *BGN* T-allele within an inferred haplotype constructed from the *ACAN* rs1516797 and *BGN* rs1126499 variants to be over-represented in the CTS group (Figure 6.5). The two studies are therefore in agreement, indicating a protective role of the *BGN* C-allele and a trend towards the increase in risk with the opposite T-allele. The function, if any, of this synonymous variant (S180S) within exon 4 of *BGN* is currently unknown and remains to be determined. It is also possible that rs1126499 is tightly linked to another functional variant(s) within or around the *BGN* gene.

Aggrecan is a large structural proteoglycan with an extremely high fixed negative charge which creates an osmotic environment resulting in water retention, thereby stabilizing the collagen network[207].The interaction between aggrecan and the collagen fibril remains unclear, but it is known that this proteoglycan interacts with hyalouronan within the ECM to form large aggregates (Figure 6.1) thereby suggesting an indirect interaction[207]. Aggrecan is found to be most abundant at regions in tendon with increased compression[51] and considering the narrow space of the carpal tunnel and the increased forces within the wrist in maximum flexion and extension[208], it is likely that higher levels of proteoglycans, are present in the tissues, such as the flexor tendons within the wrist, that are subject to these increased forces[49]. The G-allele of the rs1516797 variant was previously associated with altered risk of ACL injury[56] and the same variant was also associated with lumbar disk height narrowing[55]. Although the *ACAN* rs1516797 variant investigated in this study was not independently associated with CTS, it does not exclude the possibility that additional variants within *ACAN* are associated. This is supported by the observation in this study that there was a tendency for inferred T-T haplotype constructed from the *BGN* and *ACAN* variants to be associated with increased risk of CTS. It is also likely that variants within other genes encoding for proteoglycans could be associated with risk of CTS. For example, a variant within the decorin gene (*DCN*) was independently associated and several inferred haplotypes

constructed from the *ACAN*, *BGN* and Lumican (*LUM*)-*DCN* variants were associated with altered risk of ACL injury[56].

The second main finding of this study was that inferred pseudo-haplotypes constructed from the *COL5A1* rs13946 (C/T), rs12722 (C/T) and the *BGN* rs1126499 (C/T) variants were significantly associated with altered risk of CTS. Both type V collagen, partly encoded by the *COL5A1* gene, and biglycan play an important role in fibrillogenesis. We have previously shown that the CC genotype of *COL5A1* rs13946 variant is independently associated with increased risk of CTS whereas the WW+CC genotype of the rs146776422/rs55748801 and rs12722 variants are independently associated with decreased risk of CTS (Chapter 5). As previously mentioned, in this study, the CC genotype of *BGN* rs1126499 was also independently associated with decreased risk of CTS. In agreement with these independent associations, the T-C-C pseudo-haplotype constructed from *COL5A1* rs13946 (C/T), *COL5A1* rs12722 (C/T) and *BGN* rs1126499 (C/T) was significantly over-represented in the CON group, suggesting a protective role against CTS. The C-C-C and T-T-C inferred pseudo-haplotypes were however significantly over-represented in the CTS group.

In addition to constructing inferred pseudo-haplotypes, a “protection” score for CTS was also calculated from the *COL5A1* and *BGN* genotypes. As expected, those with a low “protective” score (≤2), were at 3-fold increased risk of developing CTS while those with a “protection” score of 6 were at a 5-fold decreased risk. Similarly when only the *COL5A1* genotypes were included in the analysis, those with a low “protective” score of 0 were at a 3.3-fold increased risk of developing CTS while those with a “protection” score of 4 were at a 2.6-fold decreased risk. When analysed separately the “protective” genotypes of *COL5A1* (rs13946) and *BGN* had a low specificity ranging from 28 to 31%, with a sensitivity of ranging from 51 to 58%. The inclusion of all the *COL5A1* and *BGN* genotypes in the protection model increased the specificity to 71%. However the sensitivity significantly decreased to 7%.

Similarly the specificity of the at risk model including all the genotypes was 98% with a sensitivity of 9%. This supports the hypothesis that not a single, but rather many genetic variants are associated with risk of CTS.

Besides investigating only one variant within each of *ACAN* and *BGN* genes, another limitation of the study was the small sample size. This research should therefore be repeated in a larger sample.

In conclusion, the *BGN* rs1126499 variants was found to be individually associated with altered risk of CTS and inferred pseudo-haplotypes of variants within the *COL5A1* and *BGN* genes, both involved in collagen fibrillogenesis, are significantly associated with altered risk of this injury. Although the investigated *ACAN* variant was not associated with CTS, it does not exclude this and other *ACAN* variants to be important in the risk of CTS.

CHAPTER 7: GENE VARIANTS WITHIN THE CELL SIGNALLING PATHWAY AND RISK OF CARPAL TUNNEL SYNDROME

7.1 INTRODUCTION

In the previous chapters of this thesis, the association of DNA sequence variants within genes encoding for structural components of the collagen fibril, namely type V collagen (chapter 5), biglycan and aggrecan (chapter 6), and endopeptidases involved in the remodelling of the fibril, such as the MMPs (chapter 4), with modulating the risk of CTS has been investigated. The expression of structural proteins, such a type V collagen and MMPs, are regulated by signalling cascades in response to stimuli such as repetitive mechanical loading, which is often mentioned as a risk factor for CTS[1,209] and other occupational, overuse injuries[210].

Previous studies have shown altered expression of several signal transduction cytokines, such as interleukin-1β and interleukin-6, as well as genes/proteins involved in apoptosis and angiogenesis, in tendon and ligament injuries[37,41,65,66,211–214] (Figure 7.1). This chapter will therefore focus on the signalling pathways that (1) regulate the expression of ECM structural components, such as Type V collagen, and (2) lead to excessive tenocyte apoptosis[60] in response to repetitive loading.

The interleukin-1β protein, encoded by the *IL-1β* gene, is able to up-regulate its own expression and also that of other cytokines, such as interleukin-6, encoded for by the *IL-6* gene[212]. Furthermore, interleukin-1β, as well as vascular endothelial grown factor (VEGF), encoded by the *VEGF* gene, has been shown to up-regulate the matrix metalloproteinases (MMPs)[213]. Increased levels of interleukin-6 also leads to the up-regulation of transforming growth factor- β (TGF-β) which in turn indirectly regulates the MMPs[211,215] and thereby also

regulates type V collagen [60,215]. The proteoglycans, specifically biglycan, also stimulates the expression of IL-1β[216]. Furthermore, interleukin-6 induces tenocyte apoptosis causing the production of reactive oxygen species and subsequent activation of, amongst other proteins, caspase-8[65].

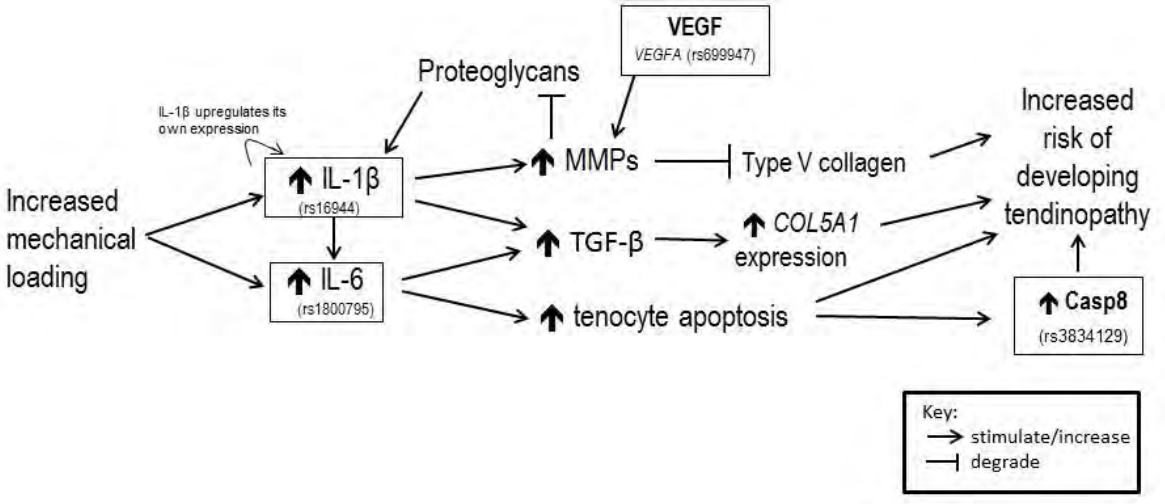

Figure 7.1 Proposed pathways through which increased mechanical loading induces expression of IL-1β and IL-6, which in turn may act to influence the expression of type V collagen thereby potentially modulating the risk of tendinopathy. IL-1β increases MMP expression by increasing expression of signal transduction mediators such as COX-2 and PGE_2. Proteoglycans, specifically biglycan, stimulates the expression IL-1β, and is degraded by the MMPs. IL-6 induces tenocyte apoptosis causing the production of reactive oxygen species and subsequent activation of caspase-8. Up (🡅) and down (🡇) arrows refer to increased or decreased expression, respectively. Molecules investigated in this study, are boxed, the variants are given in brackets and have previously been shown to affect the expression of the proteins as indicated by the arrows. IL-6, interleukin-6; IL-1β, interleukin-1β; MMP, matrix metalloproteinase; TGF- β, transforming growth factor β; VEGF, vascular endothelial growth factor; CASP8, Caspase-8. (Figure adapted from September et al., 2011)[60]

The functional variants within the *IL-1β*, *IL-6*, *IL-6R* (encodes for the IL-6 receptor), *VEGFA* and *CASP8* genes have all been associated with various multifactorial conditions. The *IL-1β* rs16944 variant has been associated with conditions such as oesophageal cancer[217] and keratoconus, a non-inflammatory corneal disorder characterized by progressive thinning of

the corneal tissue[218,219]. The G-allele of *IL-6* rs1800795 has, in turn, been associated with a 1.4 fold increase in risk of developing lung cancer[220]. Although *IL-1β* rs16944 and *IL-6* rs1800795 were not independently associated, inferred haplotypes constructed from these and other interleukin variants together with *COL5A1* rs12722 (Chapter 4) were significantly associated with altered risk of chronic Achilles tendinopathy (AT)[60]. This highlights that a pathway-based approach may be more informative to fully elucidate the role of genetic risk factors in multifactorial conditions, such as tendinopathy[60]. Although not included in the previously reported chronic Achilles tendinopathy study[60], a non-synonymous variant in the interleukin-6 receptor gene, *IL-6R* rs2228145, was considered a plausible candidate to be included in this study. The *IL-6R* gene has been investigated for its role in anterior cruciate ligament injuries (M Rahim, personal communication) and has also been implicated in various conditions with an inflammatory component including coronary heart disease, rheumatoid arthritis and asthma[221–223]. In addition, the functional variant within the promoter region, *VEGFA* rs699947, has been associated with altered risk of ACL ruptures[66] as well as risk of diabetic retinopathy[224] and the *CASP8* rs3834129 variant has been reported to independently associate with altered risk of Achilles tendinopathy[65].

The aim of this study was therefore to determine whether functional variants within several genes involved in the signal transduction, apoptosis and angiogenesis pathways, namely *IL-1β* rs16944 (C/T), *IL-6* rs1800795 (C/G), *IL-6R* rs2228145 (A/C), *CASP8* rs3834129 (I/D) and *VEGFA* rs699947 (C/A), are associated with altered risk of CTS.

7.2 METHODS

7.2.1 Participants & DNA Extraction

The same participants, described in section 4.2.1, were also analysed in this study. DNA extraction was performed as described in section 4.2.2.

7.2.2 Genotyping

All DNA samples (n=253) were genotyped for the *IL-1β* rs16944 (C/T), *IL-6* rs1800795 (C/G), *IL-6R* rs2228145 (A/C), *CASP8* rs3834129 (I/D) and *VEGFA* rs699947 (C/A) variants at the MRC/UCT Research Unit for Exercise Science & Sports Medicine, University of Cape Town, South Africa. At least 6 positive controls of known genotype and 4 DNA-free controls were randomly included on each PCR plate for quality control purposes. In addition, a subset of samples (approximately 10%) was genotyped twice using the same methodology to ensure genotyping was consistent. In order to avoid genotyping errors, samples that failed twice to amplify during PCR for a particular variant were considered to be unsuccessfully genotyped and no further attempts were made to genotype them at that specific locus.

7.2.2.1 IL-1β rs16944 (-511C/T)

The *IL-1β* rs16944 (C/T) was genotyped using the restriction fragment length polymorphism (RFLP) analysis. A 304 bp fragment containing the *Ava*I RFLP (SNP rs16944, C/T) was PCR amplified. The PCR reaction was performed in a final volume of 40µl containing at least 100ng DNA, 20pmol of the forward and reverse primers (Table 7.1), 2.0mM $MgCl_2$, 50mM KCl, 10mM Tris-HCl (pH 8.3), 200µmol of dNTPs (dATP, dTTP, dCTP and dGTP) and 1U of DNA Taq polymerase (New England Biolabs, Ipswich, Massachusetts, USA). The amplification was performed with an initial denaturation step of 94°C for 10 minutes, followed by 35 cycles of a denaturation step at 94°C for 25 seconds, annealing step at 54°C for 45 seconds and an extension step at 72°C for 30 seconds followed by a final extension step of

5 minutes at 72°C on a thermal cycler (Hybrid, PCR Express, Middle sex, UK). The 304 bp PCR product was digested with the restriction endonuclease, *Ava*I to produce 304 bp for the T allele and 190 bp and 114 bp for the C allele. The resultant fragments were separated together with a 100bp molecular weight marker and SYBER® *Gold* nucleic acid gel stain (Invitrogen Molecular Probes^{TM}, Oregon, USA) on a 2% agarose gel (Figure 5.2A). The gels were photographed under UV light using an Uvitec photodocumentation system (Uvitec Limited, Cambridge, UK) and genotypes were determined based on the resultant DNA fragment sizes. DNA from Ninety-seven % (n=100 of 103) of the cases and 85% (n=128 of 150) of the control participants were successfully genotyped for *IL-1β* rs16944.

Table 7.1 Primer and probe sequences used for genotyping

SNP	Primers (forward/reverse)/Probes	Restriction Enzyme	DNA fragments (bp)
IL-1β rs16944 (-511C/T)	5'- TGGCATTGATCTGGTTCATC-3'	*Ava*I	T - 304
	5'-GTTTAGGAATCTTCCCACT-3'		C- 190, 114
IL-6 rs1800795 (-174G/C)	5'-TTTTCTCTTTGTAAAACTTCGTGCATCACTT-3'	*Nla*III	G – 172, 56
	5'-TGGGGCTGATTGGAAACCTTATTAAF-3'		C – 123, 56, 49
IL-6R rs2228145 (C/A)	5'-GCTTGTCAAATGGCCTGTTG-3'	*Hind*III	A - 259
	5'-GCAATGCAGAGGAGCGTTC-3'		C – 181, 78
CASP8 rs3834129 (-652 6N del, I/D)	5'-TTGATTCTTTCAGACTTTTTCCTAGGCTT-3'	N/A	N/A
	5'-GGAAGGCACTGAGACGTTAAGTAA-3'		
	Insertion: 5'-VIC-TTGCTCTGCCACTTACT-3'		
	Deletion: 5'-FAM-CTCTGCCAAGCTGC-3'		
VEGFA rs699947 (-2578C/A)	5'-GCCTTAGGACACCATACCGATG-3'	*Bgl*II	C – 285
	5'-GCTGCCCCAGGGAACAAAGTTG-3'		A – 206, 79

Primer and probe (where applicable) sets were incorporated into a PCR mastermix and used as described in materials and methods. The restriction endonuclease and DNA fragment sizes are indicated where applicable.

SNP, single nucleotide polymorphism; bp, base pairs; N/A not applicable

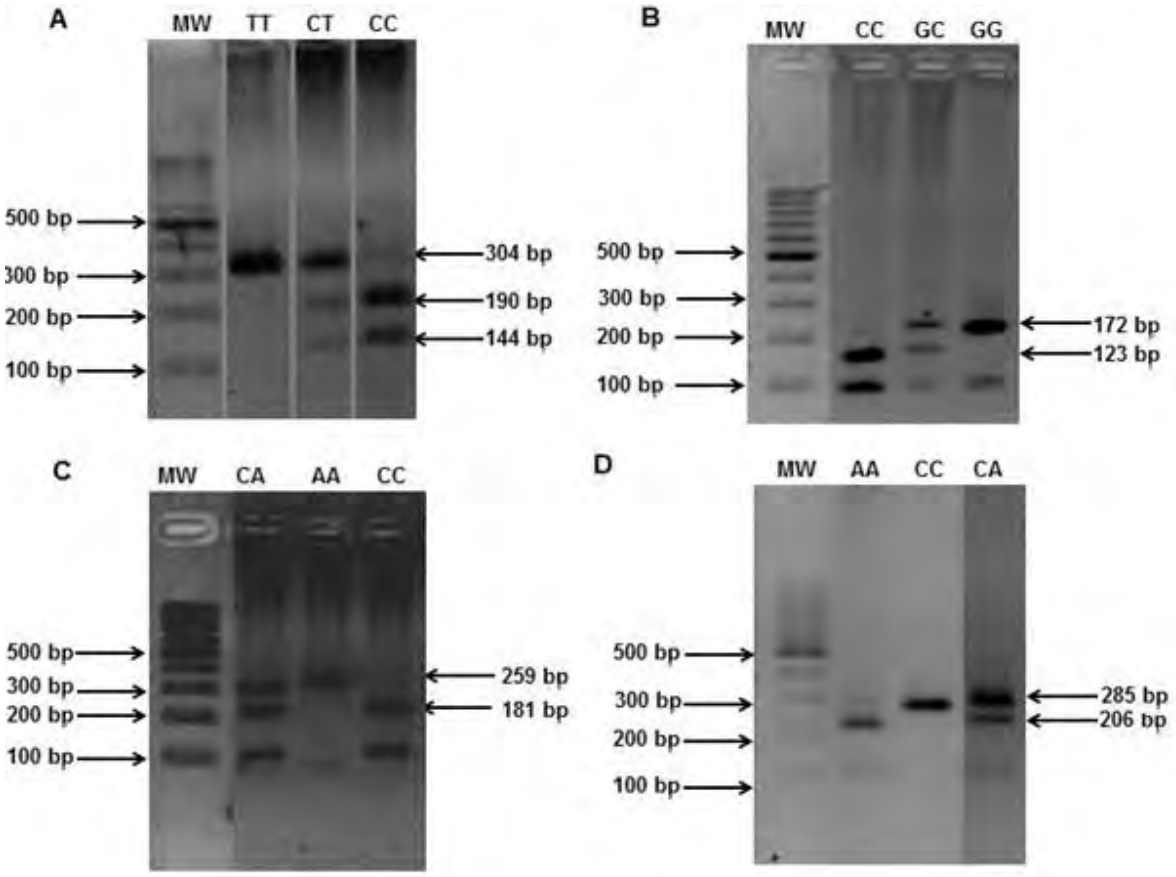

Figure 7.2 Typical 2% agarose gels showing the genotypes of the (A) *IL-1β* rs16944, (B) *IL-6* rs1800795, (C) *IL-6R* rs2228145 and (D) V*EGFA* rs699947 restriction fragment length polymorphisms (RFLPs). **(A)** Digestion of the 304 bp PCR product with *Ava*I produce a 304 bp fragment for the T-allele and (190 bp + 144 bp) fragments for the C-allele. **(B)** Digestion of the 228 bp PCR product with *Nla*III produce (172 bp + 56 bp) fragments for the G-allele and (123 bp + 56 bp + 49 bp) fragments for the C-allele. The 56 bp and 49 bp fragments migrated off the gel and are therefore not visible on the figure. **(C)** Digestion of the 259 bp PCR product with *Hind*III produce a 259 bp fragment for the A-allele and (181 bp + 78 bp) fragments for the C-allele. **(D)** Digestion of the 285 bp PCR product with *Bgl*II produce a 285 bp fragment for the C-allele and (206 bp + 79 bp) fragments for the A-allele.

7.2.2.2 IL-6 rs1800795 (-174G/C)

The *IL-6* rs1800795 (-174G/C) was genotyped by means of restriction fragment length polymorphism (RFLP) analyses. A 228 bp fragment containing the *Nla*III (SNP rs1800795, C/G) was amplified by means of PCR. The PCR reaction was performed in a final volume of 15µl containing at least 100ng DNA, 20pmol of the forward and reverse primers (Table 7.1), 2.0mM $MgCl_2$, 50mM KCl, 10mM Tris-HCl (pH 8.3), 200µmol of dNTPs (dATP, dTTP, dCTP and dGTP) and 1 unit of Taq DNA polymerase (New England Biolabs, Ipswich, Massachusetts, USA). The amplification was performed with an initial denaturation step of 94°C for 5 minutes, followed by 27 cycles of a denaturation step at 94°C for 30 seconds, annealing step at 56°C for 30 seconds and an extension step at 72°C for 40 seconds followed by a final extension step of 5 minutes at 72°C on a thermal cycler (Hybrid, PCR Express, Middle sex, UK). The 228 bp PCR amplicon contains two *Nla*III recognition sequences (CATG). The resultant digested products therefore included (172 bp + 56 bp) for the G allele and (123 bp + 56 bp + 49bp) for the C allele; which were resolved together with a 100bp molecular weight marker and SYBER® *Gold* nucleic acid gel stain (Invitrogen Molecular Probes™, Oregon, USA) on a 2% agarose gel (Figure 7.2B). The gels were photographed under UV light using a Uvitec photodocumentation system (Uvitec Limited, Cambridge, UK) and genotypes were determined based the DNA fragment sizes (Figure 7.2B). The 56 bp and 49 bp fragments were too small to be captured on the 2% agarose gel, however the diagnostic 172 bp and 123 bp fragments were sufficient to discriminate between the genotypes. Ninety-six % (n=99 of 103) of the cases and 97% (n=145 of 150) of the controls were successfully genotyped for *IL-6* rs1800795.

7.2.2.3 IL-6R rs2228145 (A/C)

The *IL-6R* rs2228145 (A/C) was genotyped by means of restriction fragment length polymorphism (RFLP) analyses. A 259 bp fragment containing the *Hind*III (SNP rs2228145, A/C) was amplified by means of PCR. The PCR reaction was performed in a final volume of

40µl containing at least 100ng DNA, 20pmol of the forward and reverse primers (Table 7.1), 2.0mM $MgCl_2$, 50mM KCl, 10mM Tris-HCl (pH 8.3), 200µmol of dNTPs (dATP, dTTP, dCTP and dGTP) and 1 unit of DNA Taq polymerase (New England Biolabs, Ipswich, Massachusetts, USA). The amplification was performed with an initial denaturation step of 92°C for 3 minutes, followed by 25 cycles of a denaturation step at 92°C for 30 seconds, annealing step at 55.5°C for 30 seconds and an extension step at 72°C for 45 seconds followed by a final extension step of 5 minutes at 72°C on a thermal cycler (Hybrid, PCR Express, Middle sex, UK). The 259 bp PCR product was digested with the restriction endonuclease, *Hind*III to produce 259 bp for the A allele and 181 bp and 78 bp for the C allele. The resultant fragments were separated together with a 100bp molecular weight marker and SYBER® *Gold* nucleic acid gel stain (Invitrogen Molecular Probes™, Oregon, USA) on a 2% agarose gel (Figure 7.2C). The gels were photographed under UV light using a Uvitec photodocumentation system (Uvitec Limited, Cambridge, UK) and genotypes were determined based the DNA fragment sizes. Eighty-three % (n=85 or 103) of the cases and 81% (n=122 of 150) of the controls were successfully genotyped for *IL-6R* rs2228145.

7.2.2.4 CASP8 rs3834129 (-652 6N del, I/D)

The *CASP8* rs3834129 (-652 6N del, -/CTTACT [I/D]) variant was genotyped using custom designed fluorescence-based Taqman® polymerase chain reaction (PCR) assays (Applied Biosystems, Foster City, California, USA). Allele-specific primer and probe sets (Table 7.1) were used along with a pre-made PCR mastermix containing ampliTaq DNA polymerase Gold (Applied Biosystems, Foster City, California, USA) in a final reaction of 8 µl following the manufacturer's recommended cycling conditions as described in section 4.2.3. The PCR reactions were carried out in the Applied Biosystems StepOnePlus real-time PCR System (Life Technologies, Applied Biosystems, Foster City, California, USA). Ninety-one % (n=94 of 103) of the cases and 88% (n=132 of 150) of the controls were successfully genotyped for *CASP8* rs3834129 (Figure 7.3).

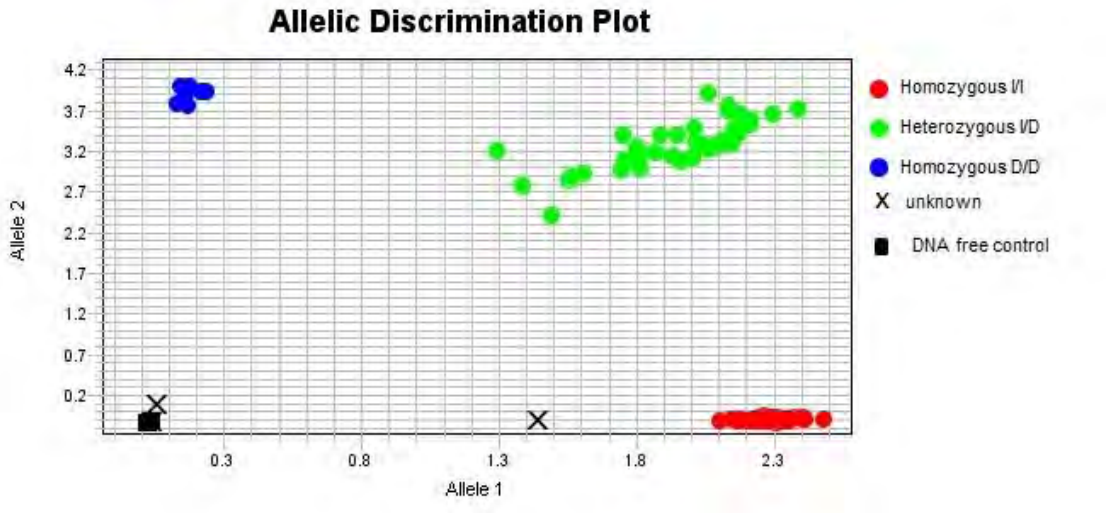

Figure 7.3 Typical allelic discrimination plots using the Taqman® Genotyping assay for *CASP8* rs3834129 (I/D).

7.2.2.5 VEGFA rs699947 (-2578C/A)

The *VEGFA* rs699947 (-2578C/A) was genotyped by means of restriction fragment length polymorphism (RFLP) analyses. A 285 bp fragment containing the *Bgl*II (SNP rs2228145, A/C) was amplified by means of PCR. The PCR reaction was performed in a final volume of 40µl containing at least 100ng DNA, 20pmol of the forward and reverse primers (Table 5.1), 2.0mM $MgCl_2$, 50mM KCl, 10mM Tris-HCl (pH 8.3), 200µmol of dNTPs (dATP, dTTP, dCTP and dGTP) and 1 unit of DNA Taq polymerase (New England Biolabs, Ipswich, Massachusetts, USA). The amplification was performed with an initial denaturation step of 95°C for 5 minutes, followed by 30 cycles of a denaturation step at 93°C for 25 seconds, annealing step at 54.4°C for 30 seconds and an extension step at 72°C for 45 seconds followed by a final extension step of 5 minutes at 72°C on a thermal cycler (Hybrid, PCR Express, Middle sex, UK). The 285 bp PCR product was digested with the restriction endonuclease, *Bgl*II to produce 285 bp for the C allele and 206 bp and 79 bp for the A allele. The resultant fragments were separated together with a 100bp molecular weight marker and SYBER® *Gold* nucleic acid gel stain (Invitrogen Molecular Probes™, Oregon, USA) on a 2%

agarose gel (Figure 7.2D). The gels were photographed under UV light using a Uvitec photodocumentation system (Uvitec Limited, Cambridge, UK) and genotypes were determined based the DNA fragment sizes. Eighty-two % (n=84 of 103) of the cases and 87% (n=131 of 150) of the controls were successfully genotyped for *VEGFA* rs699947.

7.2.3 Statistical Analysis

No allele frequency data was available for the Coloured, South African population in the public databases (http://www.ncbi.nlm.nih.gov/snp/). For this reason, the sample size for this study was calculated based on the range of the reported minor allele frequencies, 33.3 to 50.0% for *IL-1β* rs16944, 0.0 to 35.2% for *IL-6* rs1800795, 6.2 to 38.6% for *IL-6R* rs2228145, 21.7 to 50.0% for *CASP8* rs3834129 and 11.9 to 46.0% for *VEGFA* rs699947 previously described for populations in this public database (Table 7.2). Quanto V.1.2.4 was used to determine the statistical power for a given sample size and minor allele frequency[26]. A sample size of approximately 100 cases and 150 controls was found to be adequate to detect a genetic effect size ranging from 2.05 to 2.80 at a power of 80% and a significance level of 5%, assuming a minor allele frequencies ranging from 0.5 to 50.0%.

Data was analysed using STATISTICA (version 11, StatSoft Inc., Tulsa, Oklahoma, USA) and Graphpad Prism (version 5, GraphPad Software, San Diego, CA, USA, http://www.graphpad.com). A Pearson's chi-squared test or a Fisher's exact test was used to determine any significant differences between the genotype distributions or any other categorical data of the groups. An analysis of variance (ANOVA) was used to detect any significant differences between CTS and CON groups for continuous data. Where appropriate, values were adjusted for the effect of age at recruitment. A least squares difference (LSD) post-hoc test was used to identify specific differences when the overall F-value was found to be significant. Statistical significance was accepted at $p<0.05$. Hardy-Weinberg equilibrium (HWE) was established using the program Genepop web version

4.0.10 (http://genepop.curtin.edu.au/)[169,170]. Linkage disequilibrium (LD) was calculated using CubeX: cubic exact solution (www.oege.org/software/cubex/)[171]. Inferred pseudo-haplotypes were constructed from the *IL-1β* rs16944, *IL-6* rs1800795 and *IL-6R* rs2228145 and other variants investigated in previous chapters using Chaplin (version 1.2.2, Emory University School of Medicine, Atlanta, Georgia, USA)[172,173] and Hapstat software (version 3.0, University of North Carolina at Chapel Hill, North Carolina, USA)[194]. No adjustments were made for multiple testing considering no obvious appropriate method currently exists[174,175]. The Bonferroni adjustment was considered too conservative since the statistical tests in this and following studies are all performed on the same group of participants[175]. Adjustment for multiple testing was also considered inappropriate since there is an *a priori* hypothesis that the gene variants investigated in this study are associated with the CTS phenotype[174].

Table 7.2 Minor allele frequencies of the investigated polymorphisms in different populations.

Polymorphism	Population	Population size	Minor Allele	Minor allele frequency (%)
rs16944 (T/C)	HAPMAP-YRI	226	C	42.0
	HAPMAP-GIH	176	C	38.6
	HAPMAP-LWK	180	C	33.3
	HAPMAP-ASW	98	C	42.9
	HAPMAP-MEX	100	T/C	50.0
	HAPMAP-MKK	286	C	40.6
	HAPMAP-TSI	176	T	37.5
rs1800795 (G/C)	HAPMAP-YRI	120	C	0.0
	HAPMAP-GIH	176	C	12.5
	HAPMAP-ASW	98	C	9.2
	HAPMAP-MEX	100	C	16.0
	HAPMAP-MKK	284	C	4.9
	HAPMAP-TSI	176	C	35.2
rs2228145 (A/C)	HAPMAP-YRI	226	C	6.2
	HAPMAP-GIH	176	C	27.8
	HAPMAP-ASW	98	C	15.3
	HAPMAP-LWK	180	C	7.2
	HAPMAP-MKK	286	C	11.9
	HAPMAP-TSI	176	C	38.6
rs3834129 (I/D)	EGP_YORUB-PANEL	24	I/D	50.0
	EGP_HISP-PANEL	44	D	40.9
	EGP_CEPH-PANEL	42	I	45.2
	EGP_AD-PANEL	30	I	46.7
	EGP_ASIAN-PANEL	46	D	21.7

Table 7.2 Continued

Polymorphism	Population	Population size	Minor Allele	Minor allele frequency (%)
rs699947 (C/A)	HAPMAP-YRI	226	A	11.9
	HAPMAP-GIH	176	A	34.7
	HAPMAP-ASW	98	A	17.3
	HAPMAP-LWK	180	A	13.9
	HAPMAP-MEX	100	A	41.0
	HAPMAP-MKK	286	A	21.0
	HAPMAP-TSI	174	C	46.0

HAPMAP-YR, Yoruba in Ibadan Nigeria; HAPMAP-GIH, Gujarati Indians in Houston TX USA; HAPMAP-LWK, Luhya in Webuye Kenya; HAPMAP-ASW, African ancestry in Northwest USA; HAPMAP-MEX, Mexican ancestry in Los Angeles CA USA; HAPMAP-MKK, Maasai in Kinyawa Kenya; HAPMAP-TSI, Toscani in Italy; EGP_YORUB-PANEL, population of DNA available from the Coriell Cell Repository for Sub-saharan African population; EGP_HISP-PANEL, population of DNA available from the Coriell Cell Repository for Hispanic population; EGP_CEPH-PANEL, population of DNA available from the Coriell Cell Repository for European population; EGP_AD-PANEL, population of DNA available from the Coriell Cell Repository for African American population; EGP_ASIAN-PANEL, population of DNA available from the Coriell Cell Repository for Asian population.

7.3 RESULTS

7.3.1 General characteristics

The general characteristics of the participants recruited for this thesis were previously reported in section 4.2.1. The general characteristics for all participants successfully genotyped for at least one of the investigated variants in this chapter are shown in Table 7.2 and were similar to all the participants recruited for this thesis (refer to table 4.1). Briefly, both groups were matched for age of surgery (age of recruitment was used for the CON group), sex, height and country of birth (Table 7.3). Both groups were also matched for weight and BMI after adjusting for the significant difference ($p<0.001$) in age of recruitment between the CTS (45.6 ± 10.6, n=103) and CON (40.3 ± 9.7, n=148) groups. There were two related participants in both the CTS and the CON groups (mother and daughter). Similar results were obtained when the analyses were repeated after excluding one of the related participants.

Similar to the all the participants recruited for this thesis (section 4.2.1), bilateral carpal tunnel release surgery was performed in 50 (53.2%) of the CTS participants successfully genotyped for at least one of the investigated variants, while 33 (35.1%) only had surgery on their dominant hand and 9 (9.6%) had surgery on their non-dominant hand. Two (2.1%) participants who reported being ambidextrous had carpal tunnel surgery on their right hand.

Participants within the CTS (25.2%, n=26) and CON (15.3%, n=23) groups self-reported similar histories ($p=0.054$) of medical condition(s), such as diabetes (9.7 % CTS, n=10 vs 8.0% CON, n=12), osteoarthritis (OA, 6.8 % CTS, n=7 vs 5.3% CON, n=8), rheumatoid arthritis (RA, 2.9 % CTS, n=3 vs 0.7% CON, n=1), thyroid disorders (1.9 % CTS, n=2 vs 0.7% CON, n=1), diabetes and OA (1.0 % CTS, n=1 vs 0.0% CON), RA and OA (0.0 % CTS vs 0.7% CON, n=1), diabetes and RA (1.0 % CTS, n=1 vs 0.0% CON), diabetes and thyroid

disorder (1.0 % CTS, n=1 vs 0.0% CON), RA and systemic lupus erthematosus (1.0 % CTS, n=1 vs 0.0% CON), suggested to be associated with CTS

Table 7.3 General characteristics of the carpal tunnel syndrome (CTS) and control (CON) groups successfully genotyped for at least one of the investigated variants.

	CTS (n=103)	**CON** (n=149)	p-value
Age of recruitment (yrs)	45.6 ± 10.6 (103)	40.3 ± 9.7 (148)	**<0.001**
Age of surgery (yrs)	42.1 ± 10.7 (91)	40.3 ± 9.7 (148)[a]	0.197
Sex (% Female)	91.3 (103)	88.6 (149)	0.758
Height (cm)	159.9 ± 7.6 (101)	160.4 ± 7.7 (148)	0.632
Weight (kg)	82.8 ± 18.0 (102)	78.5 ± 19.1 (147)	0.198[b]
BMI (kg/m^2)	32.4 ± 6.9 (101)	30.4 ± 6.8 (146)	0.365[b]
Country of birth (% SA)	99.0 (99)[c]	100.0 (140)	0.486

Values are expressed as a mean ± standard deviation or a frequency (%). The number of participants (n) with non-missing data is indicated in parentheses. The maximum number (N) of participants in each group is also indicated.

Significant p-values are indicated in bold.

[a] age at recruitment.

[b] co-varied for age at recruitment.

Sixty-two percent (n=64) of the CTS and 42.2 % (n=62) of the CON participants self-reported a history of one or more other medical conditions (p=0.002). These included hypertension (46.6% CTS, n=48 vs 26.0% CON, n=39), hypercholesterolemia (18.4% CTS, n=19 vs 3.3% CON, n=5), asthma (4.9% CTS, n=5 vs 3.3% CON, n=5), angina (6.8% CTS, n=7 vs 0.7% CON, n=1) and other conditions (4.9% CTS, n=5 vs 4.7% CON, n=7), which included hypotension, malignant disease, anaemia, aortic valve stenosis, epilepsy, kidney disease, spinal stenosis, Hirschsprung's disease and autonomic insufficiency. This corresponds with the medical conditions of all participants recruited for this thesis (section 4.2.1).

Similar to all the participants recruited for this thesis (section 4.2.1, table 4.2), the majority of the participants (27.2% CTS, n=28 and 50.0% CON, n=75) who were successfully genotyped for at least one of the five investigated variants, with non-missing data were general poultry processing workers or general workers within other industries where repetitive action is performed with the upper limbs. The other major self-reported occupations included administration (22.3% CTS, n=23 and 11.3% CON, n=17) and nursing (10.7% CTS, n=11 and 8.0% CON, n=12).

Similar to all participants recruited for this thesis (section 4.2.1), there was also no significant difference (p=0.111) between the number of participants, successfully genotyped for at least one of the five investigated variants, who reported spending their whole working day (100%) performing manual labour requiring the use of their hands between the CTS (79.0%, n=79 of 100) and CON (87.8%, n=130 of 140). Similarly, there was a significant difference (p=0.015) between CTS (Median = 50%, interquartile range 5% - 100%) and CON (Median = 100%, interquartile range 10% - 100%) participants who were successfully genotyped, for the percentage time spent standing during a normal working day. There was no significant difference (p=0.29) in number of successfully genotyped participants who reported repetitive leisure activities of the wrist (e.g. knitting/crocheting, gardening and kneading/rolling dough) within the CTS (55.6%, n=49 of 95) and CON (32.9%, n=60 of 140) groups.

Participants with a TT genotype for *IL-1β* rs16944 had a significantly lower (p=0.028) BMI (29.3 ± 6.3 kg.m^2, n=68) than those with a TC (31.7 ± 7.5 kg.m^2, n=112) or CC (32.5 ± 5.4 kg.m^2, n=45) genotype (Supplementary table 7.1, Appendix B). Considering the low frequency of the CC genotype (n=2), the GC and CC genotypes were combined for *IL-6* rs1800795 (Supplementary table 7.2, Appendix B). Similarly, the AC and CC genotypes were also combined for *IL-6R* rs2228145 (Supplementary Table 7.3, Appendix B). Finally, there was also a significant difference (p=0.045) between the BMI of participants with I/I

(31.7 ± 6.8 kg.m^2, n=115), I/D (29.9 ± 6.4 kg.m^2, n=84) and D/D (33.6 ± 8.4 kg.m^2, n=22) genotypes for *Casp8* rs3834129. (Supplementary Table 7.4, Appendix B).There were no additional genotype effects on age of recruitment, age of surgery, height, weight or BMI for *IL-1β* rs16944, *IL-6* rs1800795, *IL-6R* rs2228145, *CASP8* rs3834129 and *VEGFA* rs699947 (Supplementary Tables 7.1 – 7.5, Appendix B).

7.3.2 Genotypes

There were no significant differences between in any of the genotypes distributions between the CTS and CON groups for the *IL-1β* rs16944 and *IL-6* rs1800795 variants (Table 7.4), nor the *CASP8* rs3834129 and *VEGFA* rs699947 variants (Table 7.5). There was however a significant difference between the CTS and CON groups for *IL-6R* rs2228145 where AA was over-represented in the CON group (p=0.005, OR=0.41, 95% CI 0.22-0.75; Sensitivity 0.59, 95% CI 0.48-0.69; Specificity 0.22, 95% CI 0.15-0.31) (Figure 7.4). All four variants were in Hardy-Weinberg equilibrium. Similar genotype distributions were observed when participants with a history of a medical condition believed to be associated with CTS were excluded from the analysis (Supplementary table 7.6, Appendix B).

Table 7.4 Genotype frequency distributions of the *IL-1β* rs16944 (C/T) and *IL-6* rs1800795 (C/G variants in carpal tunnel syndrome (CTS) and control (CON) groups for all participants (All) as well as the female participants (Female).

	All		**Female**	
	CTS	**CON**	**CTS**	**CON**
***IL-1β* rs16944**	n=100	n=128	n=91	n=113
TT	25.0 (25)	35.2 (45)	23.1 (21)	31.9 (36)
TC	57.0 (57)	43.8 (56)	57.1 (52)	45.1 (51)
CC	18.0 (18)	21.1 (27)	19.8 (18)	23.0 (26)
Genotype p-value	0.126		0.215	
C Minor allele	46.5 (93)	43.0 (110)	48.4 (88)	45.6 (103)
Allele p-value	0.506		0.618	
HWE	1.000		0.892	
***IL-6* rs1800795**	n=99	n=145	n=91	n=128
GG	81.8 (81)	81.4 (118)	84.6 (77)	81.3 (104)
CG	16.2 (16)	18.6 (27)	13.2 (12)	18.8 (24)
CC	2.0 (2)	0.0 (0)	2.2 (2)	0.0 (0)
Genotype p-value [a]	1.000		0.589	
C Minor allele	10.0 (20)	9.3 (27)	8.8 (16)	9.4 (24)
Allele p-value	0.758		0.868	
HWE	1.000		0.694	

Values are expressed as a frequency (%) with the number of participants (n) in parentheses. The maximum number (n) of participants in each group is also indicated. Significant p-values are indicated in bold.

HWE, Hardy-Weinberg equilibrium.

[a] GG vs GC + CC

Table 7.5 Genotype frequency distributions of the *CASP8* rs3834129 (I/D) and *VEGFA* rs699947 (C/A) variants in carpal tunnel syndrome (CTS) and control (CON) groups for all participants (All) as well as the female participants (Female).

	All		Female	
	CTS	**CON**	**CTS**	**CON**
***CASP8* rs3834129**	n=94	n=132	n=87	n=115
I/I	50.0 (47)	53.0 (70)	49.4 (43)	53.0 (61)
I/D	41.5 (39)	36.4 (48)	42.5 (37)	35.7 (41)
D/D	8.5 (8)	10.6 (14)	8.1 (7)	11.3 (13)
Genotype p-value	0.698		0.532	
D Minor allele	29.3 (55)	28.8 (76)	29.3 (51)	29.1 (67)
Allele p-value	0.917		1.000	
HWE	0.336		0.399	
***VEGFA* rs699947**	n=84	n=131	n=77	n=114
CC	51.2 (43)	61.8 (81)	52.0 (40)	60.5 (69)
CA	42.9 (36)	34.4 (45)	41.6 (32)	36.0 (41)
AA	6.0 (5)	3.8 (5)	6.5 (5)	3.5 (4)
Genotype p-value [b]	0.157		0.297	
A Minor allele	27.4 (46)	21.0 (55)	27.3 (42)	21.5 (49)
Allele p-value	0.131		0.221	
HWE	0.573		0.556	

Values are expressed as a frequency (%) with the number of participants (n) in parentheses. The maximum number (n) of participants in each group is also indicated. Significant p-values are indicated in bold.

HWE, Hardy-Weinberg equilibrium.

[a] GG vs GC + CC; [b] AA vs AC + CC; [b] CC vs CA + AA

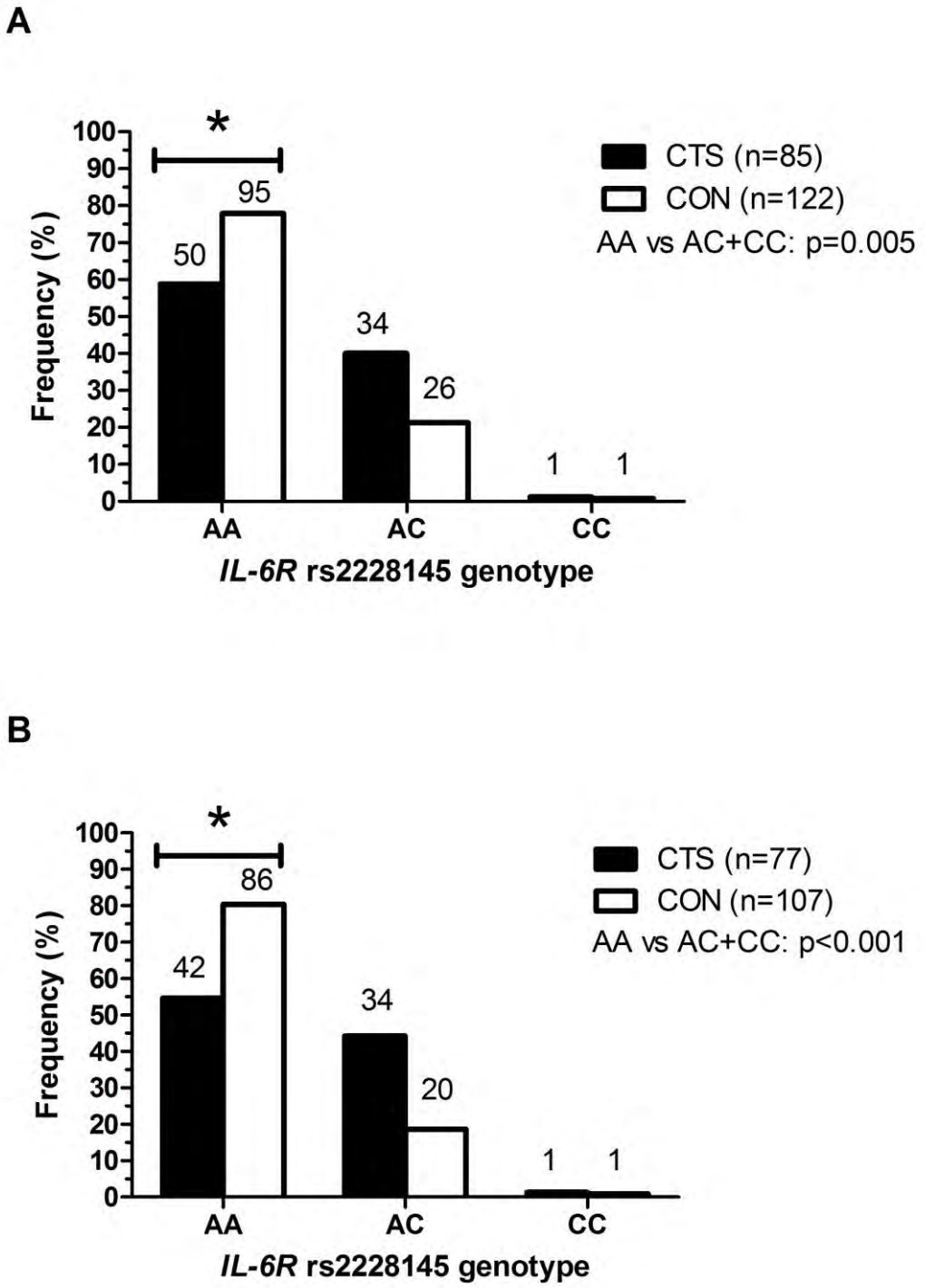

Figure 7.4 Genotype frequency distributions of *IL-6R* rs2228145 (A/C) in carpal tunnel syndrome (CTS) and control (CON) groups for **(A)** all and **(B)** female participants. Because of the small sample size of the CC genotype in the CTS and CON groups, the CC genotype was compared to the combined CT and TT genotypes. The number of participants in each group is indicated. Significant differences between the groups are indicated with a solid line and asterisk with the p-value shown

7.3.3 Inferred Pseudo-Haplotypes

Since a pathway-based approach was previously shown to be more informative[60], inferred pseudo-haplotypes were constructed from the IL-*6R* rs2228145 (A/C), *IL-1β* rs16944 (T/C) and *IL-6* rs1800795 (G/C) variants. Six of a possible eight pseudo-haplotypes were inferred at a frequency of greater than 2%. The C+C+C inferred pseudo-haplotype was significantly over-represented (p=0.002) in the CTS (12.2%) when compared to the CON (3.6%) group (Figure 7.5).

The *COL5A1* rs12722 and rs13946 variants were included in the inferred pseudo-haplotype analyses. However, the effect that was observed was that of *COL5A1* rather than the interleukin genes' influence and it was therefore not considered to add value to this chapter (Supplementary figure 7.1, Appendix B).

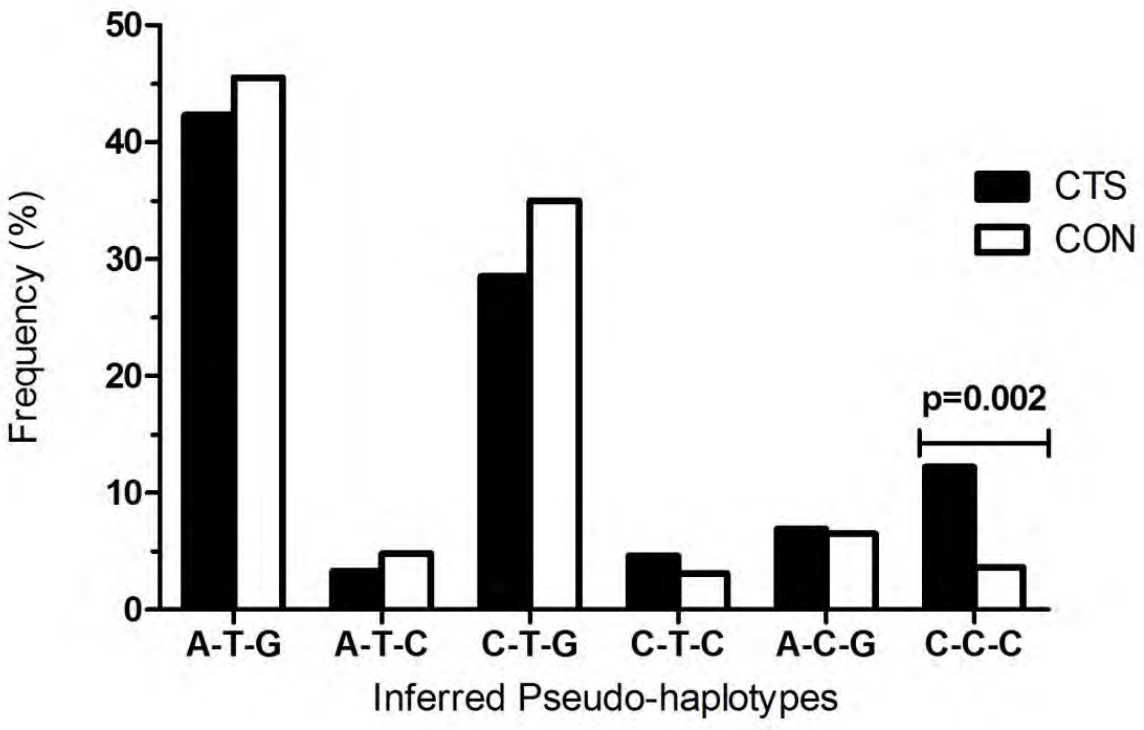

Figure 7.5 Inferred pseudo-haplotypes constructed from the *IL-6R* rs2228145, *IL-1β* rs16944 and *IL-6* rs1800795 variants**.** Significant differences are indicated with the p-value**.**

7.3.4 *COL5A1* & *IL-6R* Protection Scores

Since, the AA *IL-6R* rs2228145, TT *COL5A1* rs13946, WW+CC *COL5A1* rs146776422/rs55748801 + rs12722, genotypes were all independently associated with reduced risk of CTS (Chapter 5 and Figure 7.4), additional genotype-genotype interactions were investigated. Each of the individual "protective" genotypes contributed a score of 2 towards a participants′ genotype "protection" score. Participants with all the individual CTS "protective" genotypes therefore had a total score of 6, while those with none of the "protective" genotypes had a score of 0. Compared to the CTS group, the genotype "protection" score of 6 was significantly over-represented within the CON group ($p<0.001$, OR=0.08, 95% CI=0.02-0.39, sensitivity=0.03 (95% CI=0.00 – 0.10), specificity=0.75 (95% CI=0.66 – 0.83)) (Table 7.6 and Figure 7.6), and the genotype "protection" score of ≤2 was significantly under-represented within the CON group ($p<0.001$, OR=3.65, 95% CI=1.9-7.1, sensitivity=0.49 (95% CI=0.37 – 0.61), specificity=0.79 (95% CI=0.70 – 0.87) (Table 7.6 and Figure 7.6).

Table 7.6 Individual and combined *COL5A1* rs13946 (T/C), rs146776422rs55748801 (W/M) and rs12722 (C/T), as well as, *IL-6R* rs2228145 (A/C) genotypes which are associated with reduced (↓) or increased (↑) risk of carpal tunnel syndrome (CTS). Since the individual genotypes are independently associated with reduced risk of CTS, a "protection" score for CTS was calculated where the protective genotype of each variant contributed 2 points (rs13946 TT; rs146776422/rs55748801 WW + rs12722 CC; and rs2228145 AA) towards the "protection" score, while the non-protective genotypes contributed 0 points. The p-values, odds ratios (OR), sensitivity and the specificity, together with the respective 95% confidence intervals (CI) of the individual and combined genotypes are indicated.

COL5A1			***IL-6R***						
rs13946 (T/C)	rs146776422 rs55748801 (W/M) [a]	rs12722 (C/T) [a]	rs2228145 (A/C)	**CTS**	**"Protective" Score**	**p-value**	**OR (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
			AA	↓	-	0.005	0.41 (0.22 - 0.75)	0.59 (0.48 - 0.69)	0.22 (0.15 - 0.31)
TT	WW	CC	AA	↓	6	<0.001	0.08 (0.02 - 0.39)	0.03 (0.00 - 0.10)	0.75 (0.66 - 0.83)
YC	XX [b]	YT	MC	↑	0 or 2	<0.001	3.65 (1.9 – 7.1)	0.49 (0.37 - 0.61)	0.79 (0.70 - 0.87)
YC	XX [b]	YT	MC	↑	0	0.067	4.55 (0.89 – 23.21)	0.08 (0.03 - 0.17)	0.98 (0.93 - 1.00)

[a] The WW rs146776422/rs55748801 + CC rs12722 genotypes contributed a score of 2 towards the "protection" score, the other genotype combinations contributed a score of 0.

[b] XX = WM, MW or MM; Y= C or T; M = A or C

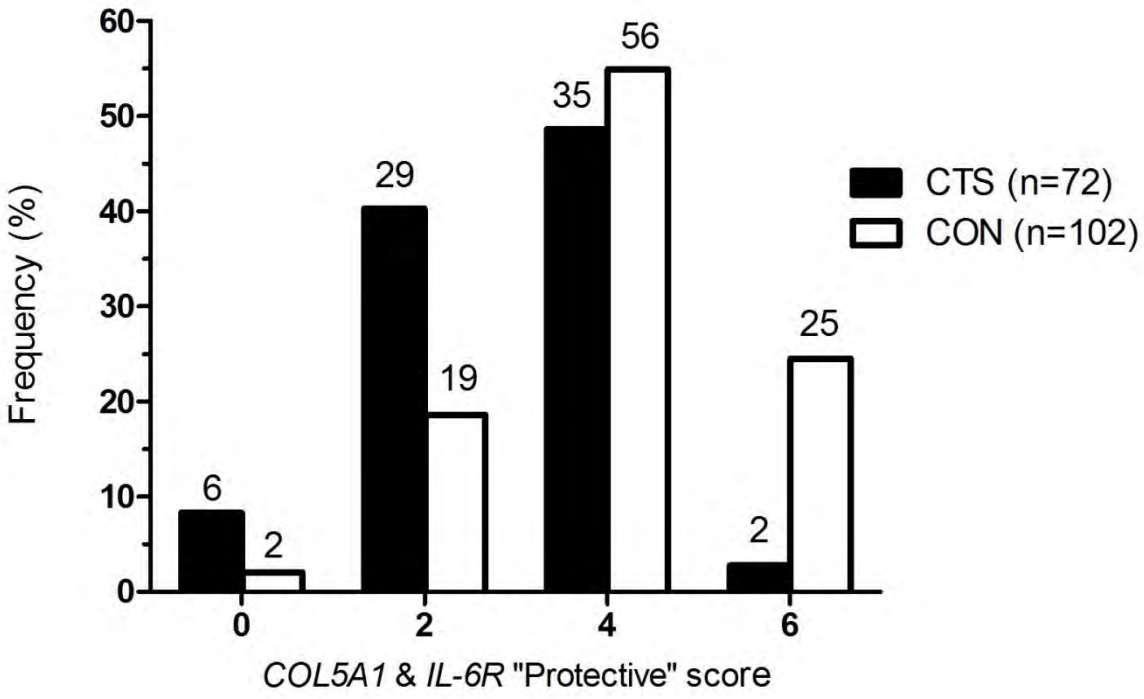

Figure 7.6 Genotype protective scores of the *COL5A1* rs13946 (T/C), rs12722 (C/T) and *IL-6R* rs2228145 (A/C) variants for the carpal tunnel syndrome (CTS) (solid bars) and control (CON) (clear bars) groups, Each of the three individual "protective" genotypes (rs13946, TT; rs12722, CC; rs2228145, AA) contributed a score of 2 towards a participants′ genotype "protection" score while the "risk" genotypes (rs13946, CC; rs12722, TT; rs11126499, TT) contributed 0. Participants with all three individual CTS "protective" genotypes therefore had a total score of 6 while those with none of the "protective" genotypes had a score of 0.

7.4 DISCUSSION

The expression of structural proteins, such as the collagens, proteoglycans and endopeptidases (refer to chapters 4-6), are regulated by signalling cascades in response to external stimuli (refer to Figure 7.1) and previous studies have shown altered expression of several of these regulatory genes, including genes involved in the signal transduction response as well as those involved in apoptosis and angiogenesis, in tendon and ligament injuries[37,41,65,66,211–214]. Considering previous associations as well as the previously reported interaction between two of these genes and *COL5A1*[60], this study investigated variants within five different genes (*IL-1β* rs16944, *IL-6* rs1800795, *IL-6R* rs2228145, *CASP8* rs3834129 and *VEGFA* rs699947) for its potential association with risk of carpal tunnel syndrome (CTS). The first main finding of this study was the independent association of *IL-6R* rs2228145 (A/C) with risk of CTS whilst there were no individual associations of any of the other variants with CTS. Specifically the AA genotype of rs2228145 was over-represented in the control group. Interestingly, there was a significant difference in BMI between the different genotype groups of *IL-1β* rs16944. This is, however, not surprising, since increased adiposity has been reported in, and thought to contribute to risk of tendinopathy[225,226]. It has also been hypothesized that tendinopathy might be mediated through the cytokines involved in signal transduction, that are associated with increased adiposity[60] .

The C-allele of the non-synonymous *IL-6R* rs2228145 variant causes a coding change from aspartic acid to alanine. This then leads to an increase in shedding of the interleukin-6 receptor (IL-6R) which, in turn, promotes IL-6 trans-signalling[221]. This particular variant is reported to be a modifier of lung function in participants with asthma[221] and to play a role in causal pathway of coronary heart disease[227]. Furthermore, IL-6 trans-signalling has been implicated in various inflammatory diseases[221]. In this chapter, the C-allele was over-

represented in the CTS group when compared to the asymptomatic control (CON) group. However, hypercholesterolemia, which is considered a proxy for coronary heart disease[228], was over-represented in the CTS group and this result should therefore be interpreted with caution.

Interestingly, no independent associations were found between the *IL-6* rs1800795 and *IL-1β* rs16944 variants and CTS. IL-6, which is activated by IL-6R, is produced during injury[229] and is thought to play a role in apoptosis[60]. Increased IL-6 levels have been measured in tenocyte apoptosis[214], typically seen in tendinopathy, and is also associated with other disease conditions[229]. IL-6 is also responsible for the up-regulation of transforming growth factor beta (TGF-β)[215], which in turn leads to an increase in *COL5A1* expression (Figure 7.1)[230]. Furthermore, increased levels of IL-6 it thought to lead to increased tenocyte apoptosis which in turn activated the apoptotic mediator caspase-8[214] and the result of this pathway is ultimately an increase in the risk of tenocyte apoptosis[60]. These pathways are hypothesized to both ultimately lead to an increased risk of developing tendinopathy. Similar to IL-6, Interleukin-1β, (IL-1β, encoded by *IL-1β*) is also responsible for the up-regulation of TGF-β[215]. In addition, IL-1β up-regulates its own, as well as the expression of IL-6[212]. Considering the close interaction between IL-1β and IL-6 and by implication, IL-6R, the variants within the genes encoding for these cytokines were investigated in the form of an inferred pseudo-haplotype constructed from the *IL-1β* rs16944, *IL-6* rs1800795 and *IL-6R* rs2228145 variants. It is interesting to note that the C-C-C inferred haplotype was over-represented in the CTS group. The C-allele of *IL-1β* rs16944 leads to increased expression of *IL-1β* mRNA which in turns increases ECM degradation and is therefore proposed to associated with increased risk of injury. Similarly, previous research has implicated the C-allele as part of the GC genotype of *IL-6* rs1800795 to be associated with increased risk of non-contact ACL ruptures in females whereas the GG genotype was protective (S Mannion, personal communication). Finally, the AA genotype of *IL-6R* rs2228145 was found to be

protective against CTS in this study. Therefore, the C-C-C inferred pseudo-haplotype contains the potential risk allele of all three variants. This finding highlights the role of gene-gene interactions in the aetiology of CTS.

Similarly, no independent associations were found between the apoptosis and angiogenesis gene variants, *CASP8* rs3834129 and *VEGFA* rs699947. These variants have been previously associated with risk of chronic Achilles tendinopathy[65] and ACL ruptures[66], respectively. Considering the role of caspase-8 in apoptosis, with increased levels being present in tendinopathy[65], and VEGF in the up-regulation of the matrix metalloproteinases (MMPs)[213], other variants within these and other genes within the cell signalling pathway should be investigated in the future.

Although there are a large number of inferred pseudo-haplotypes that could be constructed from the various variants investigated in chapters 4-7, it is important to keep in mind that the results presented in this thesis were hypothesis-driven. Therefore only previously found associations or newly identified, independent associations were investigated as part of inferred pseudo-haplotype investigations. It is very likely that there are several other pseudo-haplotype associations between various other variants, however the investigation of those interactions does not fall within the scope of this thesis.

The second main finding of this thesis was that those participants with a low a “protection” score for CTS “protective” score (≤2), calculated from the *IL-6R* and *COL5A1*genotypes, were at 3.7-fold increased risk of developing CTS while those with a score of 6 were at 12.5-fold decreased risk. When analysed separately the “protective” genotype of *IL-6R* rs2228145 had a low specificity of 22% with a sensitivity of 59%. The inclusion of all the *IL-6R* and *COL5A1* genotypes in the protection model increased the specificity to 75% whilst the sensitivity decreased significantly to 3%. Similarly, the specificity of the ‘at risk’ model, which

includes all the risk genotypes, was 98% with a sensitivity of 8%. These findings support the hypothesis that many different genetic variants interact to modify the risk of CTS.

A limitation of this study was the small sample size. Future research should aim to investigate these variants, as well as other variants within important genes in the cell signalling pathway in a larger sample.

In conclusion, this study has shown that the *IL-6R* rs2228145 variant is independently associated with altered risk of CTS and that those with a low “protective” score, which was calculated using the *IL-6R* genotype data, were at reduced risk whereas those with a high score were at increased risk of CTS. This highlights the cell signalling pathway as a target for future investigations into causal pathways for soft tissue injuries.

CHAPTER 8: CONCLUSION

CTS has been described as a condition resulting from the compression of the median nerve within the wrist and is commonly referred to as an occupational injury[3]. The exact aetiology of this condition is still unclear[135] and it is considered to be multifactorial[3]. There are several risk factors thought to be important in the aetiology of this condition and these include[3,8,26]:

- Non-occupational risk factors (including age, sex, obesity, increased wrist ratio, genetic factors and specific medical conditions such as diabetes, thyroid disorders and arthritis)
- Occupational risk factors (including repetitive action of the wrist/hand, weight-bearing activity and extreme flexion and extension).
- Psychosocial risk factors (including poor psychological well-being and having little job control)

However, as discussed in the systematic reviews presented in chapters 2 and 3, the current level of certainty for these and other risk factors is currently low, except for one. The available evidence within the scientific literature is insufficient to facilitate reliable assessment or risk estimation[70] because of (i) the limited number of studies investigating particular risk factors, ii) the sample sizes included in the published studies and (ii) inconsistency of the reported findings across individual studies[70]. Only sex was found to have a moderate certainty to modify the risk of CTS. Specifically, current research suggests that females are at increased risk of CTS, however the exact biological mechanisms underpinning this hypothesis has not been unravelled. Furthermore, it was noted that various non-occupational and occupational factors, widely believed to alter risk, had insufficient evidence to support them as risk factors for CTS. In contrast, blue-collar workers, which comprises occupations often associated with repetitive work, seemed to be at increased risk of CTS. One can hypothesise that in many cases the low level of certainty may not necessarily be reflecting a lack of a true association but is rather a consequence of the

absence of good quality, prospective studies investigating these factors in the literature. Future research investigating risk factors for CTS should therefore focus on improving the research study designs.

The current scientific literature suggests that the level of certainty for a family history and a genetic predisposition as a modulator CTS risk is low, however, they are both often mentioned as risk factors[8]. To date, only one study has investigated the association of specific genetic variants with CTS aetiology. This study looked at variants within the proteasome modulator 9 gene (*PSMD9*), which is considered a 'risk gene' for type 2 diabetes and also found it to be associated with risk of CTS[156]. It is interesting to note that research has focused on the compression of the median nerve in the aetiology of CTS, however, the involvement of the flexor tendons and the subsynovial connective tissue (SSCT) in the aetiology of CTS cannot be excluded. In support of this, tendinopathy and tenosynovitis have both been mentioned as being comorbid conditions or a precursor of CTS[12]. Furthermore, flexor tenosynovitis can lead to an increase in carpal tunnel pressure[3] and it has also been suggested that fibrosis of the SSCT could be a cause of CTS[13]. It is therefore reasonable to hypothesise that alterations in the properties of the connective tissue structures and/or tendons within the carpal tunnel structure may contribute to the pathogenesis of CTS. With this in mind, the specific candidate genes investigated in this thesis were selected based on their structural and biological function within tendons. In addition these genes have all previously been associated with tendon and/or ligament injuries as a result of participation in physical activity. The hypothesis tested in this thesis is that common genetic polymorphisms previously implicated with risk of exercise-associated musculoskeletal soft tissue injuries could also be associated with occupational overuse injuries, such as CTS.

The first aim of this thesis was therefore to determine whether DNA sequence variants within genes encoding matrix building blocks (*COL5A1*, *ACAN*, *BGN*), matrix enzymes (*MMP10*, *MMP1*, *MMP3*, *MMP12*) and signalling factors (*IL-1β, IL-6*, *CASP8* and *VEGFA*), were associated with CTS. A secondary aim of the thesis was to investigate hypothesis-driven interactions between the investigated variants.

8.1 NOVEL FINDINGS

The novel findings of this thesis highlight the possible role that various genetic factors, which play vital roles in tendon biology and function, and thus by implication pathology within the flexor tendons and other connective tissue structures in the carpal tunnel, have, at least in part, in the aetiology of CTS.

This thesis investigated a self-reported South African Coloured study sample and found:

- The novel association of four specific variants within three genetic loci to be independently associated with CTS (Table 8.1). The independent associations include the (1) *COL5A1* rs13946 (T/C) and rs146776422 (C/T) /rs55748801 (G/A) (W/M where W=CG) + rs12722 (C/T) variants (2) *BGN* rs1126499 (C/T) variant (3) *IL-6R* rs2228145 (A/C) variant (Chapters 5, 6 and 7)
- An interaction between the independent associations between *COL5A1* and *BGN* as well as *COL5A1* and *IL-6R* to modulate the risk of CTS (Chapters 6 and 7).
- Variants within the *MMP* genes (Chapter 4), the *ACAN* gene (Chapter 6) and *IL-1β*, *IL-6*, *CASP8* and *VEGFA* genes (Chapter 7) were not independently associated with CTS (Table 8.1).

This is to our knowledge the first research to investigate common polymorphisms, previously associated with musculoskeletal soft tissue injury risk, in a South African case-control study sample with CTS from the Western Cape Province of South Africa of self-reported Coloured ancestry.

8.1.1 Independent associations to modify CTS risk

As described in chapter 1, type V collagen plays a vital role in collagen fibril formation[41]. Considering the hypothesised involvement of the flexor tendons and tendon-structures in the aetiology of CTS, it was interesting to find that the combined WW+CC genotypes of rs146776422/rs55748801 + rs12722 was associated with reduced risk of CTS in a study sample of participants of self-reported Coloured ancestry[231]. The 3'-UTR of the *COL5A1* gene has been implicated in musculoskeletal injuries[44–46] as well as other exercise-associated phenotypes[187–191,232,233], in participants of self-reported Caucasian ancestry. Specifically, the CC genotype of rs12722 has been associated with decreased risk of chronic Achilles tendinopathy[44,45] and ACL injury in females[46]. The genotypes of the rs146776422/rs55748801 variants were not reported in the previously published studies because these variants are extremely rare in Caucasian population (minor allele frequency in the South African Caucasian population of 1.2%, M. Posthumus, personal communication) suggesting that the same WW+CC genotypes were also associated with reduced risk of chronic Achilles tendinopathy[44,45] and ACL ruptures in females[46]. The *COL5A1* rs146776422 (C/T) and rs55748801 (G/A) variants, although not informative in Caucasian populations, should be genotyped together with rs12722 (C/T) in future studies investigating the association of *COL5A1* 3'-UTR variants with CTS and other musculoskeletal soft tissue injuries in non-Caucasian populations.

In contrast to the previous musculoskeletal soft tissue injury genetic association findings[44–46], the downstream *COL5A1* 3'-UTR rs13946 (C/T) variant was independently associated with CTS, while the upstream rs71746744 (−/AGGG) variant was not associated (Figure 8.1). One can hypothesise that these observations suggest that collectively all the studies are implicating a region within the *COL5A1* 3'-UTR region but that the functional genetic motifs underpinning the injury susceptibility within this defined region is possibly injury specific/

unique. These observations therefore further highlight the complexities underlying the efforts to define an “at risk” profile for these complex multifactorial phenotypes

Table 8.1 Summary of all the investigated variants and their associations (if any) with carpal tunnel syndrome (CTS), chronic Achilles tendinopathy (AT) and anterior cruciate ligament (ACL) injury.

	Gene	Polymorphism	CTS	AT	ACL
Matrix Building Blocks	*COL5A1*	rs13946 (C/T)	TT	-[44]	-[46]
	COL5A1	rs146776422/rs55748801 (W/M)[a]	WW + CC	(WW)[44,b]	(WW)[46,b]
	COL5A1	rs12722 (C/T)		CC[44]	CC (F)[46]
	COL5A1	rs71746744 (-/AGGG)	-	AGGG/AGGG[44]	n.d.
	ACAN	rs1516797 (G/T)	-	n.d.	G-allele[56]
	BGN	rs1126499 (C/T)	CC	n.d.	C-allele in a *BGN* haplotype[56]
Matrix Enzymes	*MMP1*	rs1799750 (GG/GGGG)	-	n.d.	-[59]
	MMP3	rs679620 (G/A)	-	GG[58]	-[59]
	MMP10	rs486055 (C/T)	-	n.d.	-[59]
	MMP12	rs2276109 (A/G)	-	n.d.	G-allele[59]
Signalling Factors	*IL-1β*	rs16944 (C/T)	-	T- and C-alleles in a *IL-1β -IL-6* haplotype[60]	n.d.
	IL-6	rs1800795 (G/C)	-		GG[c]
	IL-6R	rs2228145 (C/A)	AA	n.d.	-[d]
	CASP8	rs2228145 (I/D)	-	D/D[65]	n.d.
	VEGFA	rs699947 (C/A)	-	n.d.	CC[66]

Independent associations are indicated with the genotype/allele. Protective associations are indicated with green, increased risk associations are indicated in red and no associations (-) indicated in blue. White blocks (n.d.) indicate that no investigation has been performed. The citation to all investigations in AT and ACL are indicated.

AT, Achilles tendinopathy; ACL, anterior cruciate ligament; n.d., not determined; F, female

[a] rs146776422 (C/T)/rs55748801 (G/A) (W/M where W=CG)

[b] Rare variants in Caucasian populations, with the WW genotype been present in the majority (99%) of individuals.

[c] S. Mannion, personal communication

[d] M. Rahim, personal communication

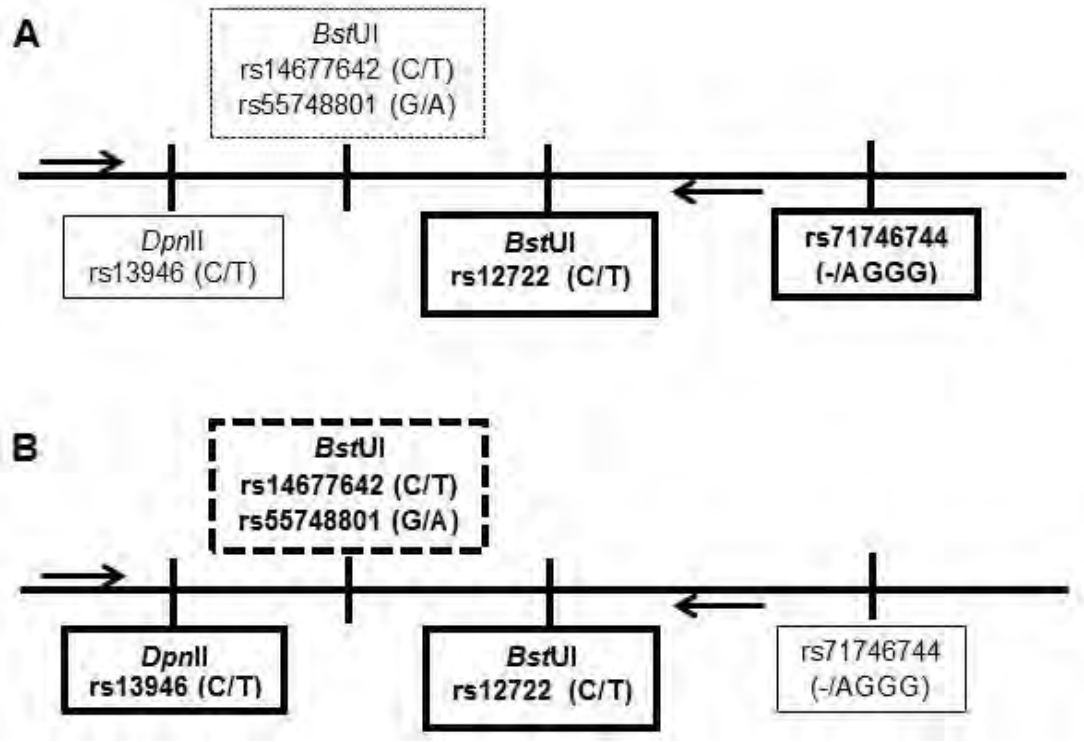

Figure 8.1 Schematic representation of the *COL5A1* gene with the regions associated with **(A)** chronic Achilles tendinopathy and **(B)** Carpal tunnel syndrome indicated in bold. The arrows indicate the position of the forward and reverse primers for the region amplified for the *Dpn*II and *Bst*UI RFLPs (not on scale).

Similar to type V collagen, biglycan is also an important regulator of fibrillogenesis. Biglycan and type V collagen interact to regulate collagen fibrillogenesis which allows it to maintain the structure of the ECM[204,206]. Mice deficient in the small, leucine-rich proteoglycans (SLRPs), which include biglycan, decorin, fibromodulin and lumican, present with similar physical phenotype as humans with classic Ehlers-danlos syndrome (EDS)[199–201], emphasizing the important role it plays in the maintenance of ECM structure. As previously mentioned in chapter 5, the majority of the classic forms of EDS is caused by rare mutations with *COL5A1*[42]. The findings in chapter 6, where the CC genotype of the rs1126499 variant within the *BGN* gene (which encodes biglycan) played a protective role against CTS, are in agreement with previously published results where there was a trend for the T-allele to be

associated with increased risk of ACL injury[56]. In addition, the CG inferred haplotype constructed from rs1126499 and a second *BGN* variant, rs1042103 (G/A) was associated with a decreased risk of ACL injury in female participants[56], suggesting that the C-allele of rs1126499, as found in chapter 6, contributes to a protective role of *BGN* for some musculoskeletal soft tissue injuries. Future studies should investigate the association of rs1042103 (G/A) and other *BGN* variants with CTS.

It is important to keep in mind that there are signalling pathways that regulate the expression of the genes that encode structural components of the ECM, such as type V collagen and the proteoglycans, in response to a specific stimulus, such as mechanical loading. Disturbances in these pathways can therefore potentially influence risk of injury and previous studies have shown altered expression of several proteins involved in cell signalling, in tendon and ligament injuries[37,41,65,66,211–214]. A single functional variant that plays an important role in the cell signalling pathway, *IL-6R* rs2228145 was found to independently associate with CTS risk. Specifically, the AA genotype of this variant within the gene encoding the interleukin-6 receptor, was found to be associated with decreased risk of CTS.

8.1.2 Gene-gene interactions modify risk of CTS

The second novel findings of this thesis were the identification of gene–gene interactions between the independently associated variants, to collectively contribute to risk of CTS. As previously mentioned, biglycan and type V collagen interact to regulate collagen fibrillogenesis facilitating the structural integrity of the ECM[204,206]. Considering their roles in fibrillogenesis, it was not surprising to observe allele-allele combinations between *COL5A1* and *BGN* variants to cumulatively be associated with altered CTS risk profiles between the cases and control participants (Chapter 6). The (i) independent associations of the *COL5A1* 3'-UTR and the *BGN* variant as well as (ii) the combined interactions between

COL5A1 and *BGN* variants collectively provide further evidence towards the theory that tendon pathology might play a significant role in the aetiology of CTS.

Similarly, considering the effect of *IL-6* and by implication, *IL-6R* which encodes the interleukin-6 receptor, on regulating type V collagen expression (Chapter 7), the interaction between the *COL5A1* and *IL-6R* variants was also investigated. The observations of allele-allele pairs between *COL5A1* and *IL-6R* variants to be associated with modulating CTS risk were noted. These gene-gene interactions are highlighting the biological importance and the complexity of the cell signalling pathways contribution to modulating risk in a multifactorial condition.

8.1.3 No associations with risk of CTS

The third novel finding of this thesis was that several gene variants investigated in this thesis revealed no independent associations with CTS (Chapters 4-7). The selection of these candidate genetic loci was hypothesis-driven and based primarily on the biological function of gene products within the tendon as well as their previous associations with multifactorial conditions and injuries. The candidate genes included: *MMP*, *ACAN, IL-1β*, *IL-6*, *CASP8* and *VEGFA* genes. However, these proteins still remain biologically relevant in tendon biology and future studies should include exploring additional functional variants within these same genes in the assessment of the aetiology of CTS.

Interestingly, a previous study noted the collective contribution between variants in both the *COL5A1* and *MMP3* genes to modulate the risk of common musculoskeletal soft tissue injuries[58,59]. Considering the independent association of the combined *COL5A1* rs146776422/rs55748801 and rs12722 variants with CTS, it was reasonable that this thesis explored the cumulative effects between the *COL5A1* rs12722 and *MMP3* rs679620 genetic loci in this CTS risk model. Although there was no independent association with *MMP3*

rs679620, an inferred pseudo-haplotype constructed from the *COL5A1* rs12722 (T/C) and *MMP3* rs679620 (G/A) variants were associated with increased risk of CTS (Chapter 5). These findings were in agreement with one previous study where the association between these two variants were observed in female participants with ACL injuries [59] but in contrast to another study where the alternate inferred pseudo-haplotype was associated with risk of chronic Achilles tendinopathy[58]. This contrast is interesting and the biological significance of these difference are yet unknown. Posthumus et al. (2011) has however speculated that the difference in the risk factor profiles and the distinct difference between the injuries, could partially account for these different findings[59]. Further research is however required to elucidate the extent of these differences and the biological impact of *MMPs* on the pathogenesis of musculoskeletal injuries.

Similarly, although no independent associations were noted between the *IL-1β* and *IL-6* variants, an inferred pseudo-haplotype constructed from these variants together with the independently associated *IL-6R* variant was significantly associated with increased risk of CTS (Chapter 7). A previous study also found inferred pseudo-haplotypes constructed from the *IL-1β* and *IL-6* variants, which were also not independently associated, together with other interleukin variants and the *COL5A1* rs12722 variant to collectively contribute to risk using a Achilles tendinopathy risk model[60]. This highlights that a pathway-based approach may be more informative to fully elucidate the role of genetic risk factors in multifactorial conditions, such as CTS[60,65] and that the investigated gene variants might also additively contribute to risk of multifactorial musculoskeletal injuries.

8.1.4 Biological mechanisms implicated by this thesis and future directions

The independent associations together with the gene-gene interactions noted in this thesis support the hypothesis proposing tendon involvement in CTS pathology. In addition, these

collective associations are potentially highlighting specific biological pathways that may partially contribute to CTS injury pathogenesis.

The independent association of variants within *COL5A1* with CTS risk noted in this study, although differences were observed in comparison to previous publications, are in alignment with the proposed "type V collagen genotype and exercise-related phenotype hypothesis"[184]. All the associated *COL5A1* variants are located within a functional region of the 3'-UTR, previously proposed to alter *COL5A1* messenger RNA (mRNA) stability within the cytoplasm of the tenocyte[191]. Irrespective of the specific injury, all the reported "at risk" genotypes or alleles, as well as the inferred haplotypes constructed from these variants, within this and previous studies, are believed to be associated with increased *COL5A1* mRNA stability. In support of this Laguette et al. (personal communication) has shown a significant increase in *COL5A1* mRNA levels in primary skin fibroblasts with a *COL5A1* rs12722 TT genotype when compared to those with a CC genotype. The increase in mRNA stability has been proposed to result in increased type V collagen production which potentially impacts on collagen fibril architecture, structure and its biomechanical properties and hereby, susceptibility to musculoskeletal soft tissue injuries (Figure 8.2).

The 3'-UTR of eukaryotic genes contain many elements, such as microRNA (miRNA) binding sites, which are emerging as important post-transcriptional regulators[197,198,234,235]. MicroRNAs are a class of small (18–24 nucleotides) non-coding RNAs that can in animal cells induce translational repression[197,198] and are directly involved in disease aetiology[236]. Several putative miRNA binding sites have recently been identified within the functional region of the *COL5A1* 3'-UTR (Y. Abrahams, personal communication). Future research is required to determine whether any of these miRNA are directly or indirectly involved in the aetiology of CTS and other musculoskeletal soft tissue injuries. It is also theoretically possible that these *COL5A1* 3'-UTR binding miRNAs could also be responsible for any possible injury-specific differences in the mechanisms of injury.

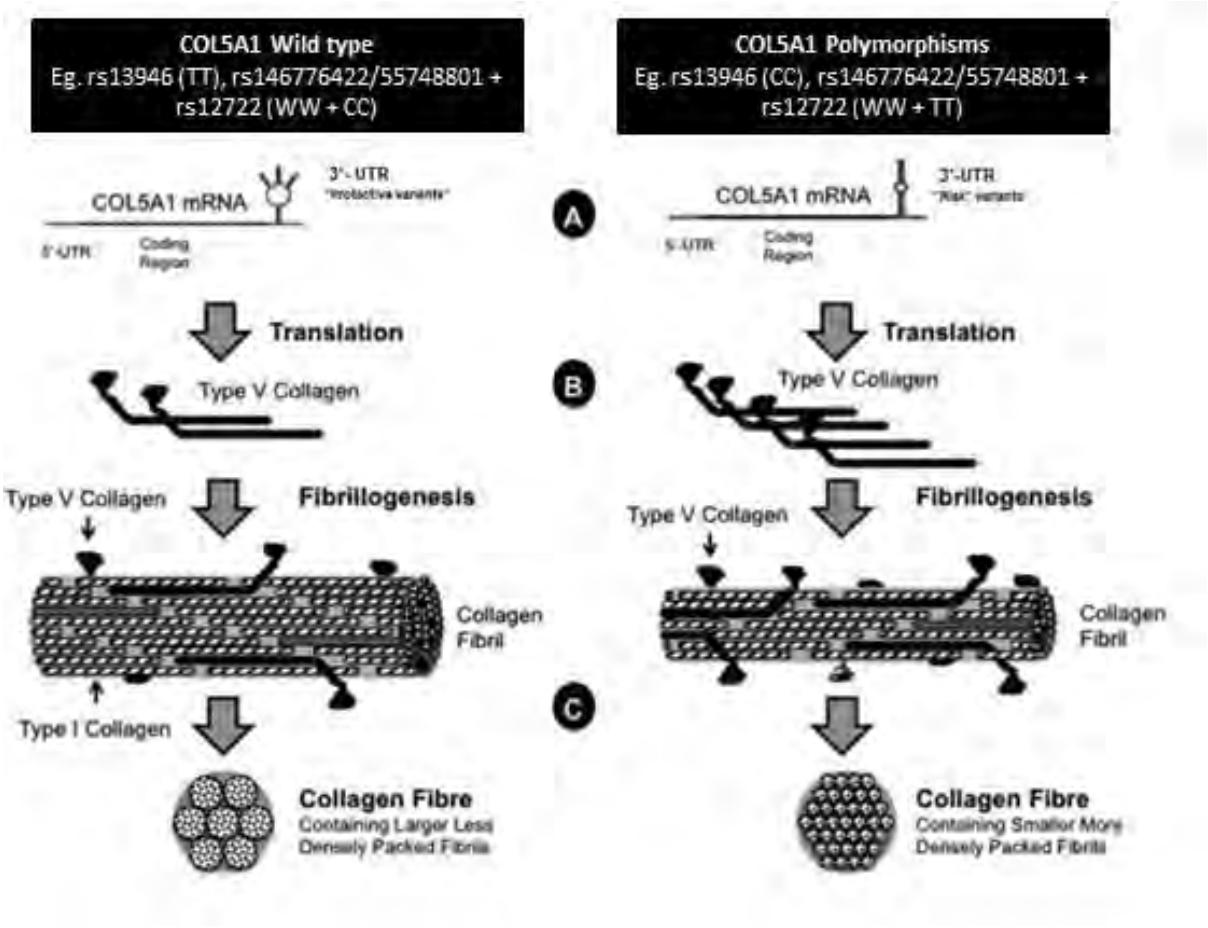

Figure 8.2 A schematic diagram illustrating the proposed mechanism of how polymorphisms within COL5A1 potentially affect fibrillogenesis. (A) The associated *COL5A1* variants are associated with increased risk of CTS and are located within a functional region of the 3'-UTR, which has been proposed to alter *COL5A1* messenger RNA (mRNA) stability within the cytoplasm of the tenocyte. Increased mRNA degradation is indicated in the left panel while decreased mRNA degradation is indicated in the right panel. (B) The altered mRNA stability associated with these polymorphisms is believed to result in an altered α1(V) chain and leads to altered type V collagen production (decreased in the left and increased in the right panel). (C) Type V collagen regulate collagen fibrillogenesis and therefore the mechanical properties of the tendon. There is an inverse relationship between type V collagen and the content of the fibril and its diameter. Thinner, more densely packed collagen fibrils are produced due to increased type V collagen production (right panel). It has previously been proposed that thinner fibrils are associated with chronic Achilles tendinopathy. Figure adapted from Collins & Posthumus (2011)[184] and Hay et al. (2013)[237].

Interestingly, type XI collagen share structural and functional homology with type V collagen[238] and also plays an important role in regulating fibrillogenesis[238,239]. Although type XI collagen is predominately expressed in cartilage[238], it is also expressed in developing tendons[240]. The protein is a heterotrimer, consisting of α1 (XI), α2(XI) and α3(XI) chains encoded by the *COL11A1*, *COL11A2* and *COL2A1* genes, respectively[241]. Rare mutations in all three type XI collagen encoding genes have been implicated in various inherited connective tissue disorders[242], while common variants have also been associated with multifactorial musculoskeletal injuries and connective tissue disorders[55,243–245]. Although not independently associated, *COL11A1* and *COL11A2* variants interacted to modulate the risk with chronic Achilles tendinopapthy[237]. The type XI and *COL5A1* genes were also shown to interact with one another to collectively modulate the risk for Achilles tendinopapthy[237]. It is therefore, reasonable to propose that future studies should investigate the possible interaction of types XI and V collagen genes in modulating the risk of CTS.

Similar to types V and XI collagen, there are several other proteoglycans that also play important roles in the regulation of fibrillogenesis and, as mentioned earlier, murine models deficient in these proteoglycans exhibit symptoms similar to EDS in humans[199–201]. Variants within the genes encoding these proteoglycans have been investigated for their potential association in ligament injuries and a variant within the gene (*DCN*) that encodes decorin was found to independently modify risk of injury[56]. Considering the function in fibrillogenesis and its previous association, as well as the findings of this thesis where a variant within *BGN* was independently associated with altered risk of CTS, it is therefore tempting to speculate that variants within the other SLRP genes could also potentially modify risk of CTS both independently and in combination with other related genes. Future studies should therefore investigate the effect of the other SLRP genes and their interaction with type V collagen.

Similarities and differences in the genetic profile of specific recreational musculoskeletal soft tissue injuries have previously been reported between males and females[46,59], ligament and tendon injuries[44,46,58,59], as well as, acute and chronic injuries[40,44]. It is therefore expected that there will be important differences between the genetic profile of occupational and recreational musculoskeletal soft tissue injuries. The four experimental chapters in this thesis have therefore showed that this admix population has either shifted the genomic interval to include additional markers (Chapter 5), revealed no association where previous associations were found (Chapter 4, 6 and 7) or revealed similar variants to be associated with risk of injury in an occupational setting (Chapters 5 and 7). Furthermore, the various interactions between different genes that were observed require further investigation to increase the understanding of the underlying mechanisms of these interactions.

The findings of this thesis therefore provide information about the regions containing associated variants which can now be further interrogated within this population to assist in identifying the “causal risk alleles”.

8.2 STRENGTHS AND LIMITATIONS

There are several strengths to this study design. One of the strengths is that the injury population consisted of a group of participants with confirmed diagnosis of an extreme form of CTS who were required to undergo carpal tunnel release surgery. Furthermore, these participants are from the occupational sector, therefore conservative treatments are required, typically lasting at least one year, before surgery can be considered should the conservative treatment, including NSAIDS, ice packs and physiotherapy, fail (personal communication, Dr Hanli de Wet). This further ensures an extreme phenotype of CTS. An additional strength is that the asymptomatic control group have worked in similar industries, performing similar jobs for equivalent periods of time. Since only Coloured participants were

recruited, it is of note that the control participants were recruited from the same companies and therefore the same geographical area as the cases, reducing the possible effect of migration in this population.

Although there was little evidence to support ethnicity as risk factor for CTS (refer to chapter 2), this thesis investigated a South African Coloured population, since this population group is seemingly the most affected ethnic group in the Western Cape area of South Africa (H. de Wet, personal communication). Idiopathic CTS is, on the other hand, reported to be rarely seen in Black South African populations[5,6]. Admixed populations, like the Coloured population, are not ideally suitable for investigation of new variants in disease/injury risk. However the genetic loci investigated in this thesis have, as previously been mentioned, been implicated in independent populations and more importantly, it has been implicated in a tendinopathy and/or ligament rupture risk model. This study sample investigated is of Coloured ancestry representative of the indigenous populations of South Africa and thereby is predicted to potentially contain more genetic variation compared to the South African populations of Caucasian ancestry[246]. For this reason, this admixed population can be considered ideal to use in the characterisation and refining of the genetic interval containing functional genomic motifs that are relevant to both the common occupational and recreational, multifactorial soft tissue injuries[246]. The novel investigations presented in this thesis did therefore not aim to identify novel polymorphisms that are associated with soft tissue injury, but rather to (i) add to the proof of concept that already exists about the different investigated genetic loci that have previously been associated with other, multifactorial musculoskeletal conditions, (ii) to redefine the genetic intervals harbouring potential “at risk” genetic elements for future targeted sequencing projects, (iii) characterising the underlying involvement of these previously implicated genetic loci in the pathogenesis of CTS and (iv) to provide evidence for the proposed hypothesis of CTS to have tendon involvement[3,12,176].

The variants investigated in this thesis (chapters 4-7) were not previously described in a Coloured population; therefore, the minor allele frequencies of each of each of the variants were unknown at the start of this investigation. An assumption was made that the frequencies will fall within the range of previously reported populations (refer to tables 4.4, 5.3, 6.1 and 7.1). The minor allele frequencies for this study sample were therefore only calculated retrospectively (Supplementary table 8.1, Appendix B). With the exception of *COL5A1* rs12722 and rs71746744, all the minor allele frequencies fell within the predicted range of previously published frequencies reported for Caucasian populations and therefore the power calculation for each of these individual variants was reasonable. The subsequent calculated genetic effect size of rs12722 and rs7174677 (section 5.2.3), was also not influenced by the difference in minor allele frequency and still fell within the suspected genetic effect size range.

Several limitations to this research were noted. One limitation of the thesis is that nerve conduction studies, the current gold standard in the diagnosis of CTS, was not performed on all CTS participants since this is not a requirement of the Commissioner for Workman's compensation in South Africa. In addition, CTS is a syndrome which is believed to occur co-morbidly with several medical conditions, such as diabetes, osteoarthritis and rheumatoid arthritis, even though there is limited evidence to support this belief (Chapter 2). It is highly unlikely that the reported associations within this study were as a result of these other medical conditions, since (i) the CTS and CON groups were similarly exposed to these conditions in all studies (ii) similar genotype distributions for CTS and CON groups were reported when these co-morbid medical conditions were excluded from the analysis. In chapters 4, 5 and 7 the exclusion of associated medical conditions had no effects on the findings of the study. However, in chapter 6 excluding those participants with a medical condition thought to be co-morbid with CTS, resulted in the loss of the independent association between *BGN* rs1126499 and CTS and therefore this association should be

interpreted with caution. However, considering the low positive association (p=0.0498) and the small sample size, this is not surprising but this highlights the necessity of this work being repeated in another, preferably larger cohort to confirm the findings made in this study. It was noted that the prevalence of other medical conditions, such as hypertension, hypercholesterolemia, asthma and angina, that are not suspected to be co-morbid with CTS, were on average, more prevalent in the CTS than the CON group. A possible explanation for this may be that the CTS group was significantly older at time of recruitment than the CON group, as some of these conditions, such as hypertension and angina, are more prevalent with increased age[247,248]. However, DNA sequence variants investigated in this thesis for their potential involvement in musculoskeletal injuries have also been investigated in other conditions, such as coronary artery disease and asthma[221,249]. Therefore, the observation of seemingly unrelated medical conditions to be more prevalent in the CTS than the CON group should be explored in future research.

Further limitations in this thesis include the small sample size of the entire cohort as well as the fact that all samples could not be successfully genotyped for all the investigated variants reducing the total sample size further (Supplementary table 8.2). A possible explanation for the reduced genotype call rate could be the presence of other, unknown polymorphisms within the primer binding sites of the investigated polymorphisms. Evidence on this hypothesis was noted in the exploration of the *COL5A1* 3'-UTR investigation chapter (Chapter 5) regarding the polymorphism within the restriction enzyme cutting site (refer to Figure 5.9) where two additional, informative variants within *COL5A1*, rs146776422 and rs55748801, were genotyped together with rs12722[231]. The South African Coloured population has a different ancestral lineage, as described in section 4.2.1, than other, more regularly studied populations. The variants investigated in this thesis have mostly been investigated in Caucasian participants and, considering the difference in ancestry, it is possible that there are differences in sequence homology, which could affect primer pair

binding in the DNA of this population group. It would be reasonable to sequence a proportion of this specific population group in order to identify novel variants and a similar sequencing study is currently underway.

Finally, it is important to understand the limitations of genetic association studies. The associations in this thesis do not form part of a cause-effect relationship and it is suggested that genetic factors is one of various intrinsic risk factors that, together with environmental exposure, work together to alter the risk of CTS (Figure 8.2). It is also essential to remember that susceptibility to injury does not equal predetermination; it merely describes the relative risk of developing the injury. The associations made in this thesis should be evaluated in other, independent populations and, as mentioned, future research should also consider other variants, besides the ones investigated in these studies, since it is likely that various variants contribute collectively to alter the risk of CTS. Proposed molecular mechanisms from genetic association studies should then be tested using molecular biology techniques, which can assist in determining a cause-effect relationship between the different risk factors and CTS. Further research is also required to determine to what extent the non-genetic risk factors commonly believed to alter risk of CTS (as shown in grey in Figure 8.2) do in fact contribute to CTS aetiology.

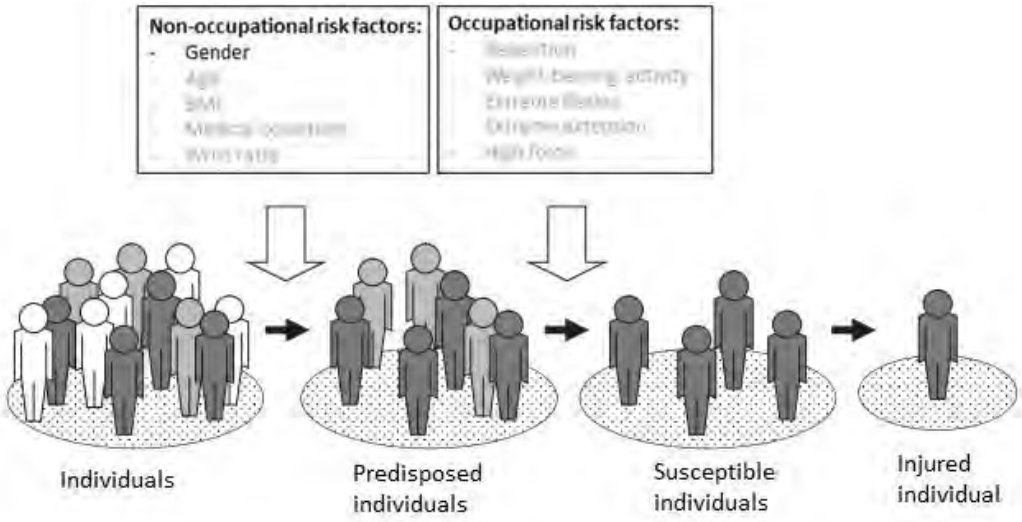

Figure 8.2 Schematic diagram indicating the relationship between non-occupational and occupational risk factors to alter the risk of developing CTS. Risk factors commonly believed to be associated with CTS, but with little evidence to support this, are indicated in grey whereas the risk factor with more supporting evidence (gender) is indicated in black. Since there is usually no particular inciting event causing CTS, further research should investigate possible reasons why only certain susceptible individuals eventually develop the injury.

In summary, this thesis investigated CTS, a common entrapment neuropathy, from a genetic context by exploring sequence variants that have previously been associated with specific musculoskeletal injuries, as potential modifiers of CTS risk. Variants within the *COL5A1*, *BGN* and *IL-6R* genes were shown for the first time to be independently associated with altered risk for CTS. Specifically, within the *COL5A1* 3'-UTR, (1) the rs13946 TT genotype and the combined rs146774622/rs55748801 and rs12722 WW+CC genotypes, as well as, (2) the *BGN* rs1126499 CC genotype and (3) the *IL-6R* rs2228145 AA genotype were significantly over-represented in the CON group compared to the CTS group. In addition, CTS risk was also modulated by multiple gene-gene interactions. Finally, no independent associations were identified with CTS for the *MMP10*, *MMP1*, *MMP3*, *MMP12*, *ACAN*, *IL-1β*, *IL-6*, *CASP8* and *VEGFA* variants, suggesting that although there were similarities, there were also differences in the genetic profile of CTS when compared to other common specific musculoskeletal injuries. The novel findings of this thesis therefore highlight the possible

important role that multiple genetic factors, and by implication pathology within the flexor tendons and other connective tissue structures in the carpal tunnel in part may be contributing to the aetiology of CTS.

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SUPPLEMENTARY MATERIAL

(A) ETHICAL APPROVAL & RECRUITMENT FORMS

1. **Approval letter from Human Ethics Research Committee**
2. **Recruitment information sheet**
3. **Informed Consent form**
4. **Participant questionnaire**

(B) SUPPLEMENTARY RESULTS

SUPPLEMENTARY MATERIAL

ETHICAL APPROVAL & RECRUITMENT FORMS

Approval letter from Human Research Ethics Committee

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences
Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6626 • Facsimile [021] 406 6411
e-mail: shuretta.thomas@uct.ac.za

28 April 2011

Sent via internal mail

HREC REF: 158/2011

A/PROF M COLLINS,
HUMAN BIOLOGY
SPORT SCIENCE INSTITUTE
3RD FLOOR

Dear A/PROF COLLINS,

PROJECT TITLE: GENETIC RISK FACTORS FOR CARPAL TUNNEL SYNDROME.

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

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

Approval is granted until 30 April 2012

Please submit an annual progress report (FHS016) if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close our file.

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

Please quote the HREC. REF in all your correspondence.

Yours sincerely

Signed by candidate

A/PROF MARC BLOCKMAN
CHAIRPERSON, FHS HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

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) and Declaration of Helsinki 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.

Recruitment information sheet

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GENETIC RISK FACTORS FOR CARPAL TUNNEL SYNDROME

INFORMATION SHEET

Although there is a high incidence of Carpal Tunnel Syndrome (CTS) as a result of overuse of the wrist and hand, the cause(s) of these injuries are poorly understood. Some researchers have suggested that there is a genetic component to CTS. In an attempt to determine whether there is a genetic basis for CTS, we are interested in studying whether certain genes are associated with this injury. This project is being conducted by Ms Marilize Burger, a PhD student, from the UCT/MRC Research Unit for Exercise Science and Sports Medicine, Department of Human Biology at the University of Cape Town.

Participation in this study should take no longer than 30 minutes. You will be asked to donate 5 ml (1 teaspoon) of a blood sample for DNA analysis. You will also be asked to complete personal particulars, occupational history, personal and family medical history questionnaires. Confirmation of your diagnosis and history of CTS and other relevant medical information will be obtained from your medical records at the occupational health clinic.

All the information retrieved from this study will be treated with the strictest confidentiality and will be used only for scientific research purposes. Your name and personal particulars will not be released under any circumstances and all data will be analysed anonymously. Your DNA sample will be destroyed on completion of the study on the genetic risk factors associated with CTS. You are also free to request that your DNA sample be destroyed before the completion of the study.

You will not be reimbursed or compensated if you participated in this study. In addition you will not receive personal genetic results.

The University of Cape Town (UCT) has an appropriate insurance policy to cover payment for any trial-related injury.

We will keep you informed about the outcomes of this study and look forward to working together with you. If you have any questions about this study, please feel free to contact us at:-

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Informed Consent form

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GENETIC RISK FACTORS FOR CARPAL TUNNEL SYNDROME
INFORMED CONSENT

I, the undersigned, have been fully informed about the UCT/MRC Research Unit for Exercise Science and Sports Medicine within the Department of Human Biology of the University of Cape Town's study to identify genetic risk factors associated with Carpal Tunnel Syndrome (CTS). I have agreed to donate five millilitres of venous blood or a Buccal mouthwash/swab sample, which will be used for the extraction and analysis of genetic material (DNA). I agree that the blood sample will be taken by a nurse, physician or phlebotomist. I have also agreed to complete personal particulars, personal and family medical history, occupational history questionnaires and understand that all the information that is collected during the study will be treated with the strictest confidentiality and will only be used for scientific research purposes. I also understand that my name and personal particulars will be not released under any circumstances and that all data will be analysed anonymously. I give permission that the study investigators may access my medical records (doctor/physiotherapist) in order to confirm my diagnosis.

I agree to participate in the study and I have been informed that I will be free to withdraw from the study at any time if I so wish. I understand that withdrawal will not negatively affect the normal standard clinical treatment I currently receive or will receive in the future. I understand that my DNA sample will be destroyed on completion of the study to identify genetic risk factors associated with CTS. I also understand that I will be free to request that my DNA sample be destroyed before the completion of the study.

The potential risks associated with blood collection technique from the ante-cubital veins are: infection, delayed healing, haematoma, physical pain, mental discomfort and injury to a nerve or a vessel. These risks are small and will be minimized by the use of trained phlebotomists, use of sterile techniques and the use of disposable, single use materials.

I understand that the DNA will be genotyped (analysed) for variations (polymorphisms) within selected candidate genes. I understand that whilst there is no direct benefit to myself, if a

genetic predisposition for CTS can be established, then future generations will be able to establish their risk for this condition. This may allow better prevention and treatment options in the future. I have read (or where appropriate, have had read to me) and understand the information about this study, and any questions I have asked have been answered to my satisfaction. I agree to participate in the study, realizing that I have the right to request that my DNA sample be destroyed at any time. I agree that research data provided by me or with my permission during the project may be included in a thesis, presented at conferences and published in journals on the condition that neither my name nor any other identifying information is used.

Any questions regarding this project may be directed to the Principle Investigator: **Ms Marilize Burger** on telephone number **0820462614** or e-mail **marilize.burger@uct.ac.za**.

This study has obtained ethical approval from the UCT Faculty of Health Sciences Research Ethic Committee (FHS REC). If you have any complaints or queries that the investigator has not been able to answer to your satisfaction, you may contact **Prof Marc Blockman** from the FHS REC on telephone number **021 406 6452**.

FULL NAME OF PARTICIPANT: _____

PARTICIPANT'S SIGNATURE: _____

DATE: _____

INVESTIGATOR: _____

INVESTIGATOR'S SIGNATURE: _____

Participant questionnaire

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Genetic Risk Factors for Carpal Tunnel Syndrome

Instructions

Please answer each question by filling in the details in the allocated space or checking one or more of the option boxes.

Please complete all six sections A to F

Version 1
(March 2010)

Subject No: _____

Section A: Personal details			
Surname			
First Name			
Postal Address		Postal/ Zip Code	
E-mail address			
Phone (day time)	code number	Date of birth	yyyy-mm-dd
Cell (mobile)		Sex	Male ☐ Female ☐
Height	cm	Weight	
BMI	kg	Age	yrs
Ethnic group (Only Required and Used for Research Purposes)	Black/African ☐ White ☐ Indian ☐ Mixed Ancestry (Coloured) ☐ Asian ☐ Other ☐		
Ancestry: Tribal or national background (eg Xhosa, Dutch, Zulu, German, Italian)	Father: Unknown ☐ Mother: Unknown ☐		
Country of Birth			
Dominant Hand	Left ☐ Right ☐ Both ☐	Dominant Leg	Left ☐ Right ☐ Both ☐
Current Occupation			
Amount of years in current occupation			
What **percentage** of your **working** day is spent in the following activities?	Sitting: _____ % Standing: _____ % Walking (Lower body activity) _____ % Manual Labour (upper and body activity) _____ %		

Subject No: _____

Section B: Lifestyle and habits history			
Please indicate your smoking status	Current smoker ☐	Ex smoker ☐	Never smoked ☐
If you answered yes, (past or current smoker) please complete the section on the right	Number of years of smoking:	If stopped, how many years ago:	
	What is (was) the average number of cigarettes per day:		
On average, how much alcohol do you drink per week (tots, glasses) of spirits, wine or beer?		_____ glasses beer/cider per week _____ glasses wine per week _____ tots of spirits per week	
Do your hobbies include anything with repetitive hand/wrist movements? (eg. Knitting, crochet etc.)		Yes ☐ No ☐	

Subject No: _____

Section C: General Personal Medical History

Do you currently suffer from any of these medical conditions:

☐ High Blood Pressure	☐ Angina/Heart Attack	☐ Asthma
☐ Emphysema	☐ Rheumatoid arthritis	☐ Osteoarthritis (wear & tear)
☐ Malignant disease (cancer)	☐ Elevated Blood Cholesterol	☐ Adrenal disorders
If Yes, what type?	☐ Diabetes mellitus	☐ Thyroid disorders
_____	☐ Renal disease	☐ Amyloidosis

Do you currently suffer from any other Connective Tissue, Rheumatological Or Muscle Diseases & Disorders?	Yes ☐ No ☐	If Yes, please select from the list below

List of some Connective Tissue and/or Rheumatic Diseases and Disorders

☐ Ankylosing Spondylitis	☐ Lipid Storage Diseases	☐ Pseudogout
☐ Aspartylglycosaminuria (AGU)	☐ Marfan Syndrome	☐ Reactive Arthritis
☐ Behcet's Syndrome	☐ Menkes Kinky Hair Syndrome	☐ Reiter's Syndrome
☐ Crohn's Disease	☐ Mucopolysaccharidoses	☐ Relapsing Polychrondritis
☐ Discoid Lupus Erythematosus	☐ Myopathies and Dystrophies	☐ Scleroderma
☐ Ehlers-Danlos syndrome (EDS)	☐ Ochronosis (Homocystinuria)	☐ Sjogren's Syndrome
☐ Eosinophilic Fascitis	☐ Osteogenesis imperfecta (OI)	☐ Systemic Lupus Erythematosus (SLE)
☐ Giant Cell (Temporal) Arthritis	☐ Polyarteritis Nodosa	☐ Systemic Sclerosis
☐ Gout	☐ Polymyalgia Rheumatica	☐ Wegener's Granulomatosis
☐ Hypersentive Vasulatis	☐ Polymyositis & Dermatomyositis	☐ Rhabdomyolysis
☐ Muscular dystrophy	☐ Myopathy	☐ Other_____

What surgical operations have you had? (please list and give dates)	Operation	Date

Were you pregnant when your CTS symptoms started?	☐ Yes ☐ No
Indicate which hand is affected	☐ Left ☐ Right ☐ Both
If female:	
At what age did you start menstruating? (years)	
Are you currently using any type of contraception?	☐ Yes ☐ No
If Yes, what type of contraception are you using?	☐ Pill ☐ Injection ☐ IUD

Subject No: _____

Are you currently?	☐ Pre-menopausal (±12 cycles per year at intervals of 23– 33 days & bleeding lasts 3-7 days) ☐ Menopausal (cycles are irregular and less frequent) ☐ Post-menopausal (no longer menstruating)

Section D: Family Medical History

Have any of your blood (biological) relatives ever had the following?

Please tick yes or no. If yes, please tick the relationship of that person to you (You may tick more than one of the relationship blocks).

Description		If Yes, please indicate the relationship
Any Carpal Tunnel Syndrome	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Any tendon injury	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Any ligament injury	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Do any other members of your family suffer from elevated blood cholesterol?	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Is there any history of arthritis in your family?	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Heart Disease	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Diabetes	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother

Subject No: _____

Section E: History of Medication Use			
What medication, if any, are you currently using? (please list)	Name of medication		Years taken
Have you ever used oral corticosteroids (cortisone tablets)? (If **yes**, how long ago?)	Yes ☐ No ☐	☐ 3 months ☐ 12 months	☐ 6 months ☐ 24 or more months
Have you ever been given an injection with corticosteroids? (If **yes**, how long ago?)	Yes ☐ No ☐	☐ 3 months ☐ 12 months	☐ 6 months ☐ 24 or more months
Have you ever used fluoroquinoline antibiotics? (refer to the following list)	Yes ☐ No ☐	☐ 3 months ☐ 12 months	☐ 6 months ☐ 24 or more months

List of some fluoroquinoline antibiotics (may be used in treatment of chlamydia, pneumonia, acute bronchitis, urinary tract infections, skin and soft tissue infection):		
ADCO-CIPRIN	CIPROBAY	SANDOZ CIPROFLOXACIN
AVELON	CIPROGEN	TAFLOC
BACTIDRON	CPL ALLIANCE CIPROFLOXACIN	TARIVID
CIFLOC	DYNAFLOC	TAVANIC
CIFRAN	FACTIVE	TEQUIN
CIPLA-CIPROFLOXACIN	FLOXIN	UNIQUIN
CIPLOXX	MAXAQUIN	UTIN-400
CIPRO-HEXAL	NOROXIN	ZANOCIN
	ORPIC	

Subject No: _____

Section F: Details of Any Other Chronic (Longstanding) Current Injury

Please complete this section for each injury. If you have had more than one past injury additional forms will be available.

What was the approximate date when you first became aware of the injury?	Month Year
Please indicate which side of your body is injured (if applicable)	☐ Right ☐ Left
Please indicate which anatomical area is currently injured	☐ Head ☐ Neck ☐ Face ☐ Front chest ☐ Back chest ☐ Shoulder ☐ Upper arm ☐ Elbow ☐ Forearm ☐ Wrist ☐ Finger ☐ Lower back ☐ Hip ☐ Thigh ☐ Hamstring ☐ Quadriceps ☐ Knee ☐ Shin ☐ Achilles ☐ Ankle ☐ Foot Other (Specify: _____)
Please indicate the type of structure that was injured	☐ Muscle ☐ Tendon ☐ Bone ☐ Ligament ☐ Joint Other (Specify): _____)
Please indicate how your injury was treated to date (you can tick more than one)?	☐ Rest ☐ Stretches ☐ Physiotherapy ☐ Surgery ☐ Strengthening exercises ☐ Equipment change ☐ Tablets ☐ Cortisone injection ☐ Other injection ☐ Orthotics Other (Specify: _____)

(B) SUPPLEMENTARY RESULTS

Supplementary table 4.1 General characteristics for the *MMP10* rs486055 (C/T) genotype groups for all participants as well as the carpal tunnel syndrome (CTS) and the control (CON) participants.

	Age of recruitment (yrs)	**Age of surgery** (yrs)	**Height** (cm)	**Weight** (kg)	**BMI** (kg/m^2)
All	n=227	-	n=225	n=225	n=223
CC	42.6 ± 10.5 (206)	-	160.7 ± 7.8 (204)	80.4 ± 17.3 (204)	31.2 ± 6.9 (202)
CT	43.9 ± 9.6 (21)	-	157.3 ± 8.6 (21)	78.2 ± 22.6 (21)	31.9 ± 6.9 (21)
p-value	0.571	-	0.064	0.592	0.702
CTS	n=97	n=89	n=95	n=96	n=95
CC	45.8 ± 10.3 (89)	42.0 ± 10.2 (81)	160.4 ± 7.9 (87)	83.1 ± 18.3 (88)	32.4 7.1 (87)
CT	42.6 ± 11.5 (8)	38.1 ± 11.1 (8)	156.9 ± 5.5 (8)	78.1 ± 17.2 (8)	31.9 ± 6.4 (8)
p-value	0.402	0.314	0.223	0.459	0.843
CON	n=130	-	n=130	n=129	n=128
CC	40.1 ± 10.0 (117)	-	160.9 ± 7.7 (117)	78.3 ± 16.2 (116)	30.4 ± 6.7 (115)
CT	44.7 ± 8.6 (13)	-	157.6 ± 10.3 (13)	78.2 ± 26.0 (13)	31.8 ± 7.5 (13)
p-value	0.109	-	0.160	0.990	0.463

Values are expressed as a mean ± standard deviation with the number of participants with non-missing data is indicated in parentheses. The maximum number (n) of participants in each group is also indicated.

yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, meter.

Supplementary table 4.2 General characteristics for the *MMP1* rs1799750 (G/GG) genotype groups for all participants as well as the carpal tunnel syndrome (CTS) and the control (CON) participants.

	Age of recruitment (yrs)	**Age of surgery** (yrs)	**Height** (cm)	**Weight** (kg)	**BMI** (kg/m^2)
All	n=216	-	n=215	n=216	n=214
GG	38.9 ± 10.7 (43)	-	159.8 ± 8.5 (43)	74.7 ± 20.4 (43)	29.6 ± 7.7 (43)
GGG	43.7 ± 11.3 (98)	-	160.3 ± 8.0 (98)	81.8 ± 19.9 (97)	31.4 ± 6.5 (97)
GGGG	42.4 ± 9.0 (75)	-	160.7 ± 7.6 (74)	81.3 ± 17.0 (76)	31.8 ± 7.1 (74)
p-value	0.048	-	0.857	0.108	0.246
CTS	n=88	n=78	n=87	n=88	n=87
GG	39.2 ± 10.7 (20)	33.8 ± 10.3 (16)	158.9 ± 7.8 (20)	75.4 ± 20.1 (20)	30.1 ± 8.8 (20)
GGG	48.1 ± 11.4 (37)	45.5 ± 10.9 (34)	160.1 ± 9.0 (37)	85.0 ± 17.4 (37)	33.1 ± 6.1 (37)
GGGG	44.7 ± 8.4 (31)	41.3 ± 7.7 (28)	160.6 ± 6.6 (30)	85.0 ± 17.5 (31)	33.2 ± 6.5 (30)
p-value	0.010	<0.001	0.750	0.118	0.224
CON	n=128	-	n=128	n=128	n=127
GG	38.7 ± 11.1 (23)	-	160.7 ± 9.1 (23)	74.2 ± 21.1 (23)	29.2 ± 6.8 (23)
GGG	40.9 ± 10.4 (61)	-	160.4 ± 7.4 (61)	79.9 ± 21.2 (60)	30.3 ± 6.5 (60)
GGGG	40.8 ± 9.1 (44)	-	160.7 ± 8.3 (44)	78.7 ± 16.3 (45)	30.8 ± 7.3 (44)
p-value	0.639	-	0.981	0.495	0.664

Values are expressed as a mean ± standard deviation with the number of participants with non-missing data is indicated in parentheses. The maximum number (n) of participants in each group is also indicated.

yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, meter.

Supplementary table 4.3 General characteristics for the *MMP3* rs679620 (G/A) genotype groups for all participants as well as the carpal tunnel syndrome (CTS) and the control (CON) participants.

	Age of recruitment (yrs)	**Age of surgery** (yrs)	**Height** (cm)	**Weight** (kg)	**BMI** (kg/m^2)
All	n=221	-	n=220	n=221	n=219
GG	42.9 ± 10.2 (107)	-	160.9 ± 7.8 (107)	81.5 ± 18.1 (108)	31.6 ± 7.2 (107)
GA	42.8 ± 10.5 (92)	-	159.8 ± 7.8 (91)	79.7 ± 18.4 (91)	31.4 ± 6.9 (90)
AA	41.1 ± 11.7 (22)	-	160.2 ± 8.2 (22)	76.6 ± 15.2 (22)	30.0 ± 6.4 (22)
p-value	0.755	-	0.656	0.481	0.599
CTS	n=95	n=84	n=94	n=95	n=94
GG	45.8 ± 10.7 (43)	42.6 ± 10.2 (38)	160.5 ± 7.5 (42)	85.7 ± 19.0 (43)	33.4 ± 7.4 (42)
GA	45.9 ± 10.3 (44)	42.7 ± 11.2 (38)	160.0 ± 8.2 (44)	84.0 ± 16.9 (44)	32.9 ± 6.5 (44)
AA	43.5 ± 13.2 (8)	40.3 ± 11.7 (8)	157.4 ± 7.5 (8)	70.5 ± 13.3 (8)	28.5 ± 5.6 (8)
p-value	0.837	0.834	0.585	0.087	0.185
CON	n=126	-	n=126	n=126	n=125
GG	40.9 ± 9.4 (64)	-	161.1 ± 8.1 (65)	78.7 ± 17.0 (65)	30.4 ± 6.9 (65)
GA	40.0 ± 10.0 (48)	-	159.7 ± 7.5 (47)	75.6 ± 19.0 (47)	30.1 ± 7.0 (46)
AA	39.7 ± 11.1 (14)	-	161.9 ± 8.4 (14)	80.1 ± 15.6 (14)	30.8 ± 6.9 (14)
p-value	0.850	-	0.557	0.576	0.936

Values are expressed as a mean ± standard deviation with the number of participants with non-missing data is indicated in parentheses. The maximum number (n) of participants in each group is also indicated.

yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, meter.

Supplementary table 4.4 General characteristics for the *MMP12* rs2276109 (A/G) genotype groups for all participants as well as the carpal tunnel syndrome (CTS) and the control (CON) participants.

	Age of recruitment (yrs)	**Age of surgery** (yrs)	**Height** (cm)	**Weight** (kg)	**BMI** (kg/m^2)
All	n=226	-	n=224	n=224	n=222
AA	42.3 ± 10.4 (201)	-	160.6 ± 8.0 (199)	80.0 ± 18.0 (199)	31.2 ± 6.9 (197)
AG	44.8 ± 12.4 (25)	-	158.7 ± 6.6 (25)	76.4 ± 13.1 (25)	30.4 ± 5.9 (25)
GG	N/A	-	N/A	N/A	N/A
p-value	0.271	-	0.264	0.331	0.598
CTS	n=96	n=86	n=94	n=95	n=94
AA	45.3 ± 10.6 (84)	41.3 ± 10.5 (74)	160.0 ± 8.1 (82)	82.9 ± 18.4 (83)	32.5 ± 7.2 (82)
AG	47.4 ± 12.5 (12)	45.4 ± 13.0 (12)	158.8 ± 5.6 (12)	78.4 ± 12.6 (12)	31.0 ± 4.5 (12)
GG	N/A	N/A	N/A	N/A	N/A
p-value	0.531	0.227	0.596	0.416	0.483
CON	n=130	-	n=130	n=129	n=128
AA	40.2 ± 9.8 (117)	-	160.9 ± 8.0 (117)	77.9 ± 17.6 (116)	30.3 ± 6.6 (115)
AG	42.5 ± 12.2 (13)	-	158.6 ± 7.6 (13)	74.5 ± 13.7 (13)	29.9 ± 7.1 (13)
GG	N/A	-	N/A	N/A	N/A
p-value	0.470	-	0.325	0.497	0.855

Values are expressed as a mean ± standard deviation with the number of participants with non-missing data is indicated in parentheses. The maximum number (n) of participants in each group is also indicated.

yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, meter.

Supplementary table 4.5 Genotype frequency distributions of the *MMP10* rs486055, *MMP1* rs1799750, *MMP3* rs679620 and *MMP12* rs2276109 variants in carpal tunnel syndrome (CTS) and control (CON) groups for all participants (All) as well as the female participants (Female), excluding medical conditions that are suggested to be associated with CTS.

	All		**Female**	
	CTS	**CON**	**CTS**	**CON**
***MMP10* rs486055**	n=72	n=112	n=65	n=96
CC	91.7 (66)	90.2 (101)	90.8 (59)	88.5 (85)
CT	8.3 (6)	9.8 (11)	9.2 (6)	11.5 (11)
TT	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Genotype p-value	0.734		0.652	
T minor allele	4.2 (6)	4.9 (11)	4.6 (6)	5.7 (11)
Allele p-value	0.740		0.661	
***MMP1* rs1799750**	n=66	n=109	n=59	n=94
1G1G	25.8 (17)	18.4 (17)	28.8 (17)	20.2 (19)
1G2G	37.9 (25)	48.6 (53)	35.6 (21)	50.0 (47)
2G2G	36.4 (24)	33.0 (36)	35.6(21)	29.8 (28)
Genotype p-value	0.322		0.200	
1G minor allele	44.7 (59)	41.0 (87)	46.6 (55)	45.2 (85)
Allele p-value	0.504		0.811	
***MMP3* rs679620**	n=70	n=108	n=63	n=92
GG	42.9 (30)	51.9 (56)	42.9 (27)	47.8 (44)
GA	48.6 (34)	38.0 (41)	47.6 (30)	41.3 (38)
AA	8.6 (6)	10.2 (11)	9.5 (6)	9.5 (10)
Genotype p-value	0.375		0.738	
A minor allele	32.9 (46)	29.2 (63)	32.9 (42)	31.5 (58)
Allele p-value	0.461		0.738	
***MMP12* rs2276109**	n=72	n=112	n=65	n=96
AA	88.9 (64)	88.4 (99)	89.2 (58)	86.5 (83)
AG	11.1 (8)	11.6 (13)	10.8 (7)	13.6 (13)
GG	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Genotype p-value	0.918		0.601	
G minor allele	5.6 (8)	5.8 (13)	5.4 (7)	6.8 (13)
p-value	0.920		0.613	

Values are expressed as a frequency (%) with the number of participants (n) in parentheses. The maximum number (n) of participants in each group is also indicated. Significant p-values are indicated in bold.HWE, Hardy-Weinberg equilibrium.

Supplementary table 5.1 General characteristics for the three *COL5A1* rs13946 (C/T) genotype groups for all participants as well as the carpal tunnel syndrome (CTS) and the control (CON) participants.

	Age of recruitment (yrs)	**Age of surgery** (yrs)	**Height** (cm)	**Weight** (kg)	**BMI** (kg/m^2)
All	n=218	-	n=216	n=216	n=214
CC	41.3 ± 9.6 (12)	-	162.1 ± 9.4 (12)	82.1 ± 19.0 (12)	31.5 ± 7.8 (12)
CT	42.2 ± 11.0 (73)	-	161.0 ± 8.4 (71)	82.5 ± 20.2 (71)	32.0 ± 7.7 (70)
TT	42.7 ± 10.5 (133)	-	159.6 ± 7.4 (133)	79.6 ± 18.7 (133)	31.1 ± 6.7 (132)
p-value	0.893	-	0.332	0.570	0.674
CTS	n=91	n=81	n=89	n=90	n=89
CC	42.3 ± 11.3 (8)	39.0 ± 11.9 (8)	161.5 ± 9.3 (8)	83.0 ± 21.1 (8)	31.9 ± 8.3 (8)
CT	46.5 ± 9.8 (37)	42.3 ± 9.9 (31)	159.2 ± 7.5 (35)	85.9 ± 20.2 (36)	34.1 ± 7.7 (35)
TT	45.5 ± 11.8 (46)	42.8 ± 11.8 (42)	160.1 ± 7.8 (46)	82.0 ± 15.9 (46)	32.0 ± 6.1 (46)
p-value	0.604	0.671	0.714	0.628	0.365
CON	n=127	-	n=127	n=126	n=125
CC	39.5 ± 5.8 (4)	-	163.3 ± 11.0 (4)	80.3 ± 16.5 (4)	30.8 ± 7.9 (4)
CT	37.8 ± 10.5 (36)	-	162.7 ± 8.9 (36)	79.1 ± 19.8 (35)	29.9 ± 7.2 (35)
TT	41.2 ± 9.5 (87)	-	159.3 ± 7.3 (87)	78.4 ± 20.0 (87)	30.6 ± 6.9 (86)
p-value	0.220	-	0.070	0.971	0.880

Values are expressed as a mean ± standard deviation with the number of participants (n) with non-missing data is indicated in parentheses. The maximum number (n) of participants in each group is also indicated.

yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, meter.

Supplementary table 5.2 General characteristics for the three *COL5A1* rs146776422/rs55748801 (W/M) genotype groups for all participants as well as the carpal tunnel syndrome (CTS) and the control (CON) participants.

rs146776422 rs55748801	**Age of recruitment** (yrs)	**Age of surgery** (yrs)	**Height** (cm)	**Weight** (kg)	**BMI** (kg/m^2)
All	n=236	-	n=234	n=234	n=232
WW	42.4 ± 10.4 (210)	-	160.4 ± 7.6 (209)	81.0 ± 18.7 (208)	31.5 ± 6.8 (207)
WM	43.1 ± 12.2 (25)	-	158.4 ± 6.2 (24)	74.8 ± 16.6 (25)	29.9 ± 6.5 (24)
MM	35.0 ± 0.0 (1)	-	165.0 ± 0.0 (1)	76.0 ± 0.0 (1)	28.0 ± 0.0 (1)
p-value	0.742	-	0.379	0.277	0.499
CTS	n=98	n=86	n=96	n=97	n=96
WW	45.4 ± 10.2 (88)	41.8 ± 10.2 (78)	160.1 ± 7.6 (86)	82.7 ± 16.9 (87)	32.4 ± 6.7 (86)
WM	47.0 ± 15.9 (10)	43.6 ± 17.2 (8)	158.6 ± 8.6 (10)	74.0 ± 18.1 (10)	29.4 ± 6.3 (10)
MM	N/A	N/A	N/A	N/A	N/A
p-value	0.668	0.652	0.127	0.569	0.181
CON	n=138	-	n=138	n=137	n=136
WW	40.1 ± 9.9 (122)	-	160.6 ± 7.6 (123)	79.8 ± 19.9 (121)	30.8 ± 6.8 (121)
WM	40.5 ± 8.5 (15)	-	158.2 ± 3.6 (14)	75.3 ± 16.1 (15)	30.2 ± 6.9 (14)
MM	35.0 ± 0.0 (1)	-	165.0 ± 0.0 (1)	76.0 ± 0.0 (1)	28.0 ± 0.0 (1)
p-value	0.865	-	0.429	0.697	0.888

Values are expressed as a mean ± standard deviation with the number of participants (n) with non-missing data is indicated in parentheses. The maximum number (n) of participants in each group is also indicated. The wild-type (W) bases of both rs146776422 (C/T) and rs55748801 (G/A) are located in a *Bst*UI restriction site. The restriction site is destroyed in all three of the remaining sequence combinations (M).

yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, meter.

Supplementary table 5.3 General characteristics for the three *COL5A1* rs12722 (C/T) genotype groups for all participants as well as the carpal tunnel syndrome (CTS) and the control (CON) participants.

rs12722	**Age of recruitment** (yrs)	**Age of surgery** (yrs)	**Height** (cm)	**Weight** (kg)	**BMI** (kg/m^2)
All	n=239	-	n=237	n=237	n=235
CC	41.7 ± 10.0 (116)	-	160.4 ± 7.7 (114)	80.4 ± 17.4 (114)	31.4 ± 7.0 (113)
CT	41.8 ± 10.3 (102)	-	160.0 ± 7.1 (101)	80.8 ± 20.5 (101)	31.3 ± 6.9 (100)
TT	48.2 ± 12.3 (21)	-	160.7 ± 8.2 (22)	78.2 ± 15.0 (22)	30.3 ± 5.3 (22)
p-value	**0.025**	-	0.882	0.838	0.776
CTS	n=99	n=87	n=97	n=98	n=97
CC	45.7 ± 10.0 (43)	42.0 ± 10.2 (39)	160.5 ± 7.0 (41)	82.3 ± 16.2 (42)	32.1 ± 6.6 (41)
CT	44.4 ± 9.9 (44)	39.8 ± 9.8 (37)	159.3 ± 8.0 (44)	82.4 ± 19.7 (44)	32.5 ± 7.2 (44)
TT	49.3 ± 15.7 (12)	48.7 ± 14.4 (11)	160.5 ± 9.02 (12)	80.5 ± 17.3 (12)	31.2 ± 5.6 (12)
p-value	0.368	0.055	0.753	0.941	0.827
CON	n=140	-	n=140	n=139	n=138
CC	39.4 ± 9.4 (73)	-	160.3 ± 8.1 (73)	79.3 ± 18.2 (72)	31.0 ± 7.2 (72)
CT	39.8 ± 10.3 (58)	-	160.5 ± 6.4 (57)	79.5 ± 21.8 (57)	30.4 ± 6.6 (56)
TT	46.8 ± 5.8 (9)	-	160.9 ± 7.5 (10)	75.4 ± 12.1 (10)	29.2 ± 5.0 (10)
p-value	0.096	-	0.972	0.821	0.710

Values are expressed as a mean ± standard deviation with the number of participants (n) with non-missing data is indicated in parentheses. The maximum number (n) of participants in each group is also indicated. Significant p-values are indicated in bold.

yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, meter.

Supplementary table 5.4 General characteristics for the three *COL5A1* rs71746744 (-/AGGG) genotype groups for all participants as well as the carpal tunnel syndrome (CTS) and the control (CON) participants.

rs71746744	**Age of recruitment** (yrs)	**Age of surgery** (yrs)	**Height** (cm)	**Weight** (kg)	**BMI** (kg/m^2)
All	n=206	-	n=205	n=205	n=203
-/-	41.4 ± 10.8 (36)	-	159.4 ± 7.1 (35)	79.1 ± 17.7 (35)	31.5 ± 7.6 (34)
-/AGGG	42.0 ± 9.5 (88)	-	160.1 ± 8.8 (89)	79.9 ± 19.6 (89)	31.2 ± 7.3 (89)
AGGG/AGGG	43.0 ± 10.9 (82)	-	160.6 ± 7.6 (81)	81.8 ± 19.9 (81)	31.4 ± 6.7 (80)
p-value	0.702	-	0.760	0.741	0.973
CTS	n=83	n=72	n=82	n=83	n=82
-/-	46.1 ± 7.8 (14)	42.0 ± 9.2 (13)	158.9 ± 5.9 (13)	83.2 ± 17.2 (14)	33.5 ± 7.4 (13)
-/AGGG	43.8 ± 9.2 (36)	40.7 ± 9.5 (30)	159.7 ± 8.3 (36)	84.2 ± 20.3 (36)	33.0 ± 7.9 (36)
AGGG/AGGG	46.0 ± 11.9 (33)	43.1 ± 11.9 (29)	160.5 ± 8.7 (33)	83.3 ± 16.7 (33)	32.3 ± 5.9 (33)
p-value	0.620	0.674	0.834	0.976	0.859
CON	n=123	-	n=123	n=122	n=121
-/-	38.5 ± 11.6 (22)	-	159.6 ± 7.9 (22)	76.4 ± 17.8 (21)	30.3 ± 7.6 (21)
-/AGGG	40.8 ± 9.6 (52)	-	160.3 ± 9.2 (53)	77.0 ± 18.8 (53)	30.0 ± 6.7 (53)
AGGG/AGGG	41.0 ± 9.8 (49)	-	160.6 ±6.8 (48)	80.7 ± 22.0 (48)	30.7 ± 7.1 (47)
p-value	0.598	-	0.889	0.575	0.882

Values are expressed as a mean ± standard deviation with the number of participants (n) with non-missing data is indicated in parentheses. The maximum number (n) of participants in each group is also indicated.

yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, meter.

Supplementary table 5.5 General characteristics for the *COL5A1* BstUI RFLP, containing rs146776422/rs55748801 (W/M) and rs12722 (T/C), genotype groups for all participants as well as the carpal tunnel syndrome (CTS) and the control (CON) participants.

rs146776422 rs55748801	**rs12722**	**Age of recruitment** (yrs)	**Age of surgery** (yrs)	**Height** (cm)	**Weight** (kg)	**BMI** (kg/m^2)
All		n=236	-	n=234	n=234	n=232
WW	CC	40.4 ± 9.9 (82)	-	160.4 ± 7.9 (81)	81.1 ± 17.3 (80)	31.7 ± 7.1 (80)
WW	CT	43.6 ± 10.8 (114)	-	160.1 ± 7.2 (113)	81.4 ± 19.9 (113)	31.6 ± 6.8 (112)
WW	TT	43.6 ± 8.1 (14)	-	162.3 ± 8.4 (15)	77.6 ± 17.4 (15)	29.3 ± 5.4 (15)
WM, MM	CC, CT, TT	42.8 ± 12.0 (26)	-	158.6 ± 6.2 (25)	74.8 ± 16.2 (26)	29.8 ± 6.4 (25)
p-value		0.213	-	0.497	0.372	0.407
CTS		n=98	n=86	n=96	n=97	n=96
WW	CC	45.2 ± 8.8 (24)	42.0 ± 8.9 (22)	161.2 ± 6.7 (23)	83.0 ± 14.5 (23)	32.1 ± 6.7 (23)
WW	CT	46.1 ± 10.7 (58)	41.8 ± 11.0 (51)	159.1 ± 7.6 (57)	82.8 ± 17.3 (58)	32.9 ± 6.9 (57)
WW	TT	40.0 ± 9.5 (6)	40.2 ± 8.0 (5)	164.7 ± 9.3 (6)	81.2 ± 23.8 (6)	29.5 ± 6.0 (6)
WM, MM	CC, CT, TT	47.0 ± 15.9 (10)	43.6 ± 17.2 (8)	158.6 ± 8.9 (10)	74.0 ± 18.1 (10)	29.4 ±6.3 (10)
p-value		0.589	0.958	0.287	0.502	0.361
CON		n=138	-	n=138	n=137	n=136
WW	CC	38.5 ± 937 (58)	-	160.0 ± 8.3 (58)	80.3 ± 18.3 (57)	31.5 ± 7.3 (57)
WW	CT	40.9 ± 10.3 (56)	-	161.1 ± 6.8 (56)	80.0 ± 22.4 (55)	30.3 ± 6.6 (55)
WW	TT	46.4 ± 6.0 (8)	-	160.8 ± 7.9 (9)	75.2 ± 12.8 (9)	29.2 ± 5.3 (9)
WM, MM	CC, CT, TT	40.1 ± 8.3 (16)	-	158.7 ± 3.9 (15)	75.4 ± 15.5 (16)	30.1 ±6.6 (15)
p-value		0.148	-	0.695	0.738	0.664

Values are expressed as a mean ± standard deviation with the number of participants (n) with non-missing data is indicated in parentheses. The maximum number (n) of participants in each group is also indicated.

yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, meter.

Supplementary table 5.6 Genotype frequency distributions of the *COL5A1* 3'-untranslated region (UTR) rs13946 (C/T), rs12722 (C/T) and rs71746744 (-/AGGG), as well as the adjacent rs146776422 (C/T) and rs55748801 (G/A), variants in carpal tunnel syndrome (CTS) and control (CON) groups for all participants (All) as well as the female participants (Female), excluding medical conditions suspected to be associated with Carpal tunnel syndrome.

***COL5A1* Genotype**	**All**		**Female**	
	CTS	**CON**	**CTS**	**CON**
rs13946	n=64	n=107	n=58	n=91
TT	46.9 (30)	69.16 (74)	46.6 (27)	72.5 (66)
CT	40.6 (26)	27.1 (29)	43.1 (25)	24.2 (22)
CC	12.5 (8)	3.7 (4)	10.3 (6)	3.3 (3)
Genotype p-value [b]	**0.006**		**0.002**	
C minor allele	32.8 (42)	17.3 (37)	31.9 (37)	15.4 (28)
Allele p-value	**0.001**		**<0.001**	
rs146776422/rs55748801	n=72	n=118	n=65	n=103
WW (CG/CG) [a]	89.0 (64)	86.4 (102)	89.4 (58)	85.4 (88)
WM	11.1 (8)	12.7 (15)	10.8 (7)	13.6 (14)
MM	0.0 (0)	0.9 (1)	0.0 (0)	1.0 (1)
Genotype p-value [c]	0.661		0.640	
M minor allele	5.9 (8)	7.2 (17)	5.4 (7)	7.8 (16)
Allele p-value	0.530		0.400	
rs12722	n=74	n=120	n=67	n=105
CC	45.21 (33)	53.3 (64)	45.5 (30)	55.2 (58)
CT	42.5 (31)	40.8 (49)	43.9 (29)	40.0 (42)
TT	12.3 (9)	5.8 (7)	10.6 (7)	4.8 (5)
Genotype p-value	0.230		0.237	
T minor allele	33.6 (49)	26.3 (63)	32.6 (43)	24.8 (52)
Allele p-value	0.125		0.116	
rs71746744	n=62	n=105	n=55	n=90
AGGG/AGGG	39.3 (24)	41.0 (43)	35.2 (19)	38.9 (35)

Supplementary table 5.6 Continued

-/AGGG	41.0 (25)	41.0 (43)	44.4 (24)	43.3 (39)
-/-	19.7 (12)	18.1 (19)	20.4 (11)	17.8 (16)
Genotype p-value	0.963		0.881	
- minor allele	40.2 (49)	38.6 (81)	42.6 (46)	49.0 (71)
Allele p-value	0.774		0.599	

Values are expressed as a frequency (%) with the number of participants (n) in parentheses. The maximum number (N) of participants in each group is also indicated. Significant p-values are indicated in bold.

[a] The genotyping method was unable to distinguish between the adjacent rs146776422 (C/T) and rs55748801 (G/A) variants. The CG wild-type allele of these adjacent variants, which contains a *Bst*UI restriction site, was designated as a W, while the three alternative nucleotide combinations CA, TG and TA were designated as an M. The *Bst*UI restriction site is destroyed in all three alternative sequence combinations.

[b] TT vs CT and CC.

[c] WW vs WM and MM.

HWE, Hardy-Weinberg equilibrium.

Supplementary table 6.1 General characteristics for the three *ACAN* rs1516797 (C/T) genotype groups for all participants as well as the carpal tunnel syndrome (CTS) and the control (CON) participants.

	Age of recruitment (yrs)	**Age of surgery** (yrs)	**Height** (cm)	**Weight** (kg)	**BMI** (kg/m^2)
All	n=220		n=218	n=218	n=216
G/G	42.4 ± 9.8 (69)		161.3 ± 8.1 (70)	80.6 ± 18.2 (68)	31.3 ± 7.3 (68)
G/T	42.7 ± 11.5 (99)		160.3 ± 8.0 (97)	80.1 ± 20.4 (98)	30.8 ± 6.6 (97)
T/T	43.6 ± 8.9 (52)		159.6 ± 7.6 (51)	80.5 ± 16.4 (52)	32.0 ± 6.5 (51)
p-value	0.817		0.501	0.983	0.578
CTS	n=95	n=84	n=93	n=94	n=93
G/G	46.2 ± 9.6 (29)	42.3 ± 9.6 (27)	162.6 ± 7.4 (29)	83.5 ± 18.1 (29)	31.6 ± 7.1 (29)
G/T	45.3 ± 11.5 (45)	41.7 ± 11.5 (41)	158.8 ± 7.8 (43)	79.8 ± 18.6 (44)	31.6 ± 6.8 (43)
T/T	45.5 ± 10.0 (21)	43.3 ± 10.6 (16)	159.1 ± 8.1 (21)	90.0 ± 15.1 (21)	35.9 ± 6.3 (21)
p-value	0.932	0.874	0.107	0.100	0.062[a]
CON	n=125		n=125	n=124	n=123
G/G	39.6 ± 9.0 (40)		160.3 ± 8.6 (41)	78.4 ±18.3 (39)	30.8 ± 7.5 (39)
G/T	40.5 ± 11.1 (54)		161.6 ± 8.0 (54)	80.3 ± 22.0 (54)	30.1 ± 6.4 (54)
T/T	42.3 ± 8.0 (31)		159.9 ± 7.4 (30)	74.1 ± 14.2 (31)	29.3 ± 5.2 (30)
p-value	0.52		0.623	0.353	0.651

Values are expressed as a mean ± standard deviation with the number of participants (n) with non-missing data is indicated in parentheses. The maximum number (N) of participants in each group is also indicated.

[a] co-varied for sex

yrs, years; cm, centimeters; kg, kilograms; BMI, body mass index; m, meter.

Supplementary table 6.2 General characteristics for the three *BGN* rs1126499 (C/T) genotype groups for all female participants as well as the female carpal tunnel syndrome (CTS) and control (CON) participants.

	Age of recruitment (yrs)	**Age of surgery** (yrs)	**Height** (cm)	**Weight** (kg)	**BMI** (kg/m^2)
All	n=195		n=193	n=194	n=192
C/C	43.0 ± 10.2 (128)		159.1 ± 6.6 (126)	79.5 ± 18.2 (127)	31.6 ± 7.1 (125)
C/T	42.9 ± 11.1 (55)		158.3 ± 7.7 (55)	79.9 ± 16.8 (55)	31.9 ± 6.5 (55)
T/T	43.7 ± 12.1 (12)		159.7 ± 8.8 (12)	80.2 ± 23.7 (12)	31.2 ± 7.0 (12)
p-value	0.971		0.716	0.987	0.926
CTS	n=86	n=76	n=84	n=85	n=84
C/C	46.3 ± 9.3 (50)	42.8 ± 10.2 (42)	159.1 ± 5.2 (48)	81.8 ± 17.8 (49)	32.5 ± 7.2 (48)
C/T	43.5 ± 12.9 (32)	39.7 ± 12.3 (31)	157.8 ± 8.7 (32)	81.4 ± 18.4 (32)	32.6 ± 6.7 (32)
T/T	47.3 ± 16.2 (4)	48.0 ± 8.5 (3)	158.0 ± 7.4 (4)	77.5 ± 15.3 (4)	31.3 ± 7.1 (4)
p-value	0.503	0.300	0.690	0.898	0.939
CON	n=109		n=109	n=109	n=108
C/C	40.8 ± 10.3 (78)		159.0 ± 7.4 (78)	78.1 ± 18.4 (78)	31.1 ± 7.0 (77)
C/T	41.9 ± 8.1 (23)		158.9 ± 6.3 (23)	78.0 ± 14.4 (23)	31.1 ±6.27 (23)
T/T	41.9 ± 10.3 (8)		160.5 ± 9.7 (8)	81.5 ± 27.9 (8)	31.1 ± 7.4 (8)
p-value	0.875		0.853	0.880	1.000

Values are expressed as a mean ± standard deviation with the number of participants (n) with non-missing data is indicated in parentheses. The maximum number (N) of participants in each group is also indicated.

yrs, years; cm, centimeters; kg, kilograms; BMI, body mass index; m, meter

Supplementary table 6.3 Genotype frequency distributions of the *ACAN* rs1516797 and *BGN* rs1126499 variants in carpal tunnel syndrome (CTS) and control (CON) groups for all participants (All) as well as the female participants (Female), excluding medical conditions suspected to be associated with Carpal tunnel syndrome.

	All		**Female**	
	CTS	**CON**	**CTS**	**CON**
***ACAN* rs1516797**	n=71	n=107	n=64	n=92
GG genotype	33.8 (24)	29.9 (32)	31.3 (20)	28.3 (26)
GT genotype	45.1 (32)	43.9 (47)	45.3 (29)	43.5 (40)
TT genotype	21.1 (15)	26.2 (28)	23.4 (15)	28.3 (26)
Genotype p-value	0.716		0.788	
T minor allele	43.6 (62)	48.1 (103)	46.1 (59)	50.0 (92)
Allele p-value	0.408		0.497	
***BGN* rs1126499**	-	-	n=63	n=92
CC genotype	-	-	60.3 (38)	71.7 (66)
CT genotype	-	-	34.9 (22)	19.6 (18)
TT genotype	-	-	4.8 (3)	8.7 (8)
Genotype p-value [a]	-		0.165	
T minor allele	-	-	22.2 (28)	18.5 (34)
Allele p-value	-		0.418	

Values are expressed as a frequency (%) with the number of participants (n) in parentheses. The maximum number (N) of participants in each group is also indicated. Significant p-values are indicated in bold.

[a] CC vs CT + TT

HWE, Hardy-Weinberg equilibrium.

Supplementary table 7.1 General characteristics for the *IL-1β* rs16944 genotype groups for all participants as well as the carpal tunnel syndrome (CTS) and the control (CON) participants.

	Age of recruitment (yrs)	**Age of surgery** (yrs)	**Height** (cm)	**Weight** (kg)	**BMI** (kg/m^2)
All	n=227	-	n=225	n=227	n=225
CC	43.8 ± 12.3 (45)	-	159.1 ± 5.9 (45)	82.0 ± 13.6 (45)	32.5 ± 5.4 (45)[a]
CT	42.6 ± 10.4 (112)	-	160.3 ± 7.6 (112)	81.1 ± 18.8 (113)	31.7 ± 7.5 (112)[b]
TT	41.3 ± 10.2 (70)	-	160.9 ± 8.7 (68)	75.4 ± 17.1 (69)	29.3 ± 6.3 (68)[a,b]
p-value	0.466	-	0.476	0.056	**0.028**
CTS	n=100	n=90	n=98	n=99	n=98
CC	49.7 ± 12.1 (18)	45.5 ± 12.4 (17)	160.1 ± 5.1 (18)	87.7 ± 13.7 (18)	34.1 ± 4.8 (18)
CT	45.6 ± 10.6 (57)	42.0 ± 10.6 (53)	159.5 ± 7.7 (57)	83.4 ± 18.8 (57)	32.9 ± 7.5 (57)
TT	43.5 ± 9.3 (25)	39.4 ± 9.3 (20)	161.4 ± 9.0 (23)	78.3 ± 18.2 (24)	30.2 ± 6.6 (23)
p-value	0.168	0.227	0.612	0.235	0.157
CON	n=127	-	n=127	n=128	n=127
CC	40.0 ± 10.9 (27)	-	158.4 ± 6.5 (27)	78.3 ± 12.4 (27)	31.4 ± 5.6 (27)
CT	39.6 ± 9.4 (55)	-	161.2 ± 7.6 (55)	78.8 ± 18.8 (56)	30.5 ± 7.3 (55)
TT	40.2 ± 10.5 (45)	-	160.6 ± 8.7 (45)	73.8 ± 16.5 (45)	28.9 ± 6.2 (45)
p-value	0.96	-	0.317	0.297	0.274

Values are expressed as a mean ± standard deviation with the number of participants with non-missing data is indicated in parentheses. The maximum number (n) of participants in each group is also indicated.

yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, meter.

[a] CC vs TT (p=0.017)

[b] CT vs TT (p=0.025)

Supplementary table 7.2 General characteristics for the *IL-6* rs1800795 genotype groups for all participants as well as the carpal tunnel syndrome (CTS) and the control (CON) participants.

	Age of recruitment (yrs)	**Age of surgery** (yrs)	**Height** (cm)	**Weight** (kg)	**BMI** (kg/m^2)
All	n=243	-	n=241	n=241	n=239
GG	42.0 ± 10.8 (198)	-	160.3 ± 7.9 (196)	79.6 ± 17.8 (196)	31.1 ± 6.9 (194)
GC+CC[a]	44.5 ± 8.8 (45)	-	159.6 ± 7.5 (45)	82.7 ± 23.0 (45)	31.6 ± 7.0 (45)
p-value	0.155	-	0.588	0.319	0.678
CTS	n=98	n=87	n=96	n=97	n=96
GG	44.7 ± 11.0 (80)	42.1 ± 11.2 (71)	160.3 ± 7.6 (78)	82.1 ± 18.3 (79)	32.0 ± 6.8 (78)
GC+CC[a]	49.3 ± 7.9 (18)	42.4 ± 8.9 (16)	158.3 ± 6.8 (18)	84.6 ± 18.5 (18)	33.7 ± 7.6 (18)
p-value	0.095	0.903	0.322	0.604	0.369
CON	n=144	-	n=144	n=143	n=142
GG	40.1 ± 10.2 (117)	-	160.3 ± 7.8 (117)	77.8 ± 17.4 (116)	30.5 ± 7.0 (115)
GC	41.3 ± 8.0 (27)	-	160.4 ± 8.0 (27)	81.4 ± 25.9 (27)	30.2 ± 6.4 (27)
p-value	0.572	-	0.951	0.384	0.857

Values are expressed as a mean ± standard deviation with the number of participants with non-missing data is indicated in parentheses. The maximum number (n) of participants in each group is also indicated.

yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, meter.

[a] Only 2 CC genotypes were present, therefore the GC and CC genotypes were combined to form GC+CC

Supplementary table 7.3 General characteristics for the *IL-6R* rs2228145 genotype groups for all participants as well as the carpal tunnel syndrome (CTS) and the control (CON) participants.

	Age of recruitment (yrs)	**Age of surgery** (yrs)	**Height** (cm)	**Weight** (kg)	**BMI** (kg/m^2)
All	n=206		n=204	n=205	n=203
AA	43.1 ± 9.5 (144)		160.2 ± 7.4 (142)	80.3 ± 15.7 (144)	31.4 ± 6.3 (142)
AC+CC[a]	43.5 ± 11.4 (62)		159.7 ± 8.1 (62)	82.0 ± 21.0 (61)	32.2 ± 8.1 (61)
p-value	0.804		0.672	0.512	0.464
CTS	n=84	n=75	n=82	n=83	n=83
AA	45.6 ± 9.6 (49)	41.6 ± 9.9 (42)	161.0 ± 8.5 (47)	84.2 ± 16.3 (48)	32.7 ± 6.4 (47)
AC+CC	45.6 ± 9.6 (35)	44.1 ± 11.7 (33)	157.8 ± 6.7 (35)	81.5 ± 19.2 (35)	32.7 ± 7.3 (35)
p-value	0.343	0.320	0.068	0.485	0.965
CON	n=121		n=121	n=121	n=120
AA	41.6 ± 9.2 (94)		159.8 ± 6.8 (94)	78.3 ± 15.1 (95)	30.7 ± 6.2 (94)
AC+CC[a]	37.9 ± 8.8 (27)		162.1 ± 9.1 (27)	82.8 ± 23.6 (26)	31.5 ± 9.1 (26)
p-value	0.059		0.154	0.241	0.594

Values are expressed as a mean ± standard deviation with the number of participants with non-missing data is indicated in parentheses. The maximum number (n) of participants in each group is also indicated.

yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, meter.

[a] Only 2 CC genotypes were present, therefore the AC and CC genotypes were combined to form AC+CC

Supplementary table 7.4 General characteristics for the *CASP8* rs3834129 (I/D) genotype groups for all participants as well as the carpal tunnel syndrome (CTS) and the control (CON) participants.

	Age of recruitment (yrs)	**Age of surgery** (yrs)	**Height** (cm)	**Weight** (kg)	**BMI** (kg/m^2)
All	n=225		n=223	n=223	n=221
I/I	42.5 ± 9.6 (117)		160.6 ± 8.3 (116)	81.6 ± 18.5 (115)	31.7 ± 6.8 (115)
I/D	41.9 ± 11.3 (86)		160.4 ± 7.63 (85)	77.4 ± 18.1 (86)	29.9 ± 6.4 (84)[a]
D/D	43.8 ± 11.1 (22)		158.5 ± 7.2 (22)	84.2 ± 22.1 (22)	33.6 ± 8.4 (22)[a]
p-value	0.754		0.498	0.172	**0.045**
CTS	n=94	n=84	n=92	n=93	n=92
I/I	45.7 ± 9.7 (47)	41.6 ± 9.7 (42)	160.2 ± 9.0 (46)	84.9 ± 18.4 (46)	33.0 ± 6.8 (46)
I/D	44.1 ± 11.5 (39)	41.5 ± 11.3 (34)	159.3 ± 6.1 (38)	79.2 ± 16.7 (39)	31.4 ± 6.9 (38)
D/D	48.9 ± 5.9 (8)	45.8 ± 5.7 (8)	159.8 ± 6.0 (8)	88.9 ± 21.0 (8)	35.4 ± 8.7 (8)
p-value	0.463	0.541	0.809	0.218	0.303
CON	n=131		n=131	n=130	n=129
I/I	40.3 ± 9.0 (70)		160.8 ± 7.8 (70)	79.4 ± 18.3 (69)	30.8 ± 6.7 (69)
I/D	40.1 ± 11.0 (47)		161.3 ± 8.1 (47)	76.0 ± 19.2 (47)	28.7 ± 5.7 (46)
D/D	40.9 ± 12.4 (14)		158.3 ± 8.1 (14)	81.6 ± 23.0 (14)	32.6 ± 8.4 (14)
p-value	0.971		0.464	0.514	0.087

Values are expressed as a mean ± standard deviation with the number of participants with non-missing data is indicated in parentheses. The maximum number (n) of participants in each group is also indicated.

yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, meter.

[a] I/D vs D/D (p=0.024)

Supplementary table 7.5 General characteristics for the *VEGFA* rs699947 genotype groups for all participants as well as the carpal tunnel syndrome (CTS) and the control (CON) participants.

	Age of recruitment (yrs)	**Age of surgery** (yrs)	**Height** (cm)	**Weight** (kg)	**BMI** (kg/m^2)
All	n=214		n=212	n=212	n=210
C/C	41.0 ± 10.1 (124)		160.2 ± 8.4 (122)	79.5 ± 19.7 (122)	30.9 ± 7.2 (121)
C/A	43.5 ± 10.4 (80)		161.3 ± 8.7 (80)	82.6 ± 17.5 (80)	31.8 ± 6.3 (79)
A/A	45.9 ± 13.2 (10)		159.9 ± 7.3 (10)	68.2 ± 11.4 (10)	26.7 ±5.0 (10)
p-value	0.129		0.577	0.063	0.080
CTS	n=84	n=75	n=82	n=83	n=82
C/C	42.7 ± 9.7 (43)	38.5 ± 9.7 (37)	160.7 ± 8.0 (42)	83.6 ± 17.9 (42)	32.3 ± 6.9 (42)
C/A	48.0 ± 10.5 (36)	43.8 ± 11.1 (33)	159.9 ± 7.3 (35)	82.8 ± 16.8 (36)	32.6 ± 6.5 (35)
A/A	50.0 ± 13.9 (5)	48.2 ± 14.1 (5)	159.0 ± 6.5 (5)	67.2 ± 12.0 (5)	26.6 ± 4.4 (5)
p-value	0.053	0.105	0.832	0.133	0.162
CON	n=130		n=130	n=129	n=128
C/C	40.1 ± 10.2 (81)		159.9 ± 8.6 (80)	77.3 ± 20.4 (80)	30.1 ± 7.3 (79)
C/A	39.8 ± 8.9 (44)		162.4 ± 6.0 (45)	82.3 ± 18.2 (44)	31.1 ± 6.0 (44)
A/A	41.8 ± 12.4 (5)		160.8 ± 8.6 (5)	69.2 ± 12.1 (5)	26.8 ± 6.1 (5)
p-value	0.915		0.228	0.213	0.377

Values are expressed as a mean ± standard deviation with the number of participants with non-missing data is indicated in parentheses. The maximum number (n) of participants in each group is also indicated.

yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, meter.

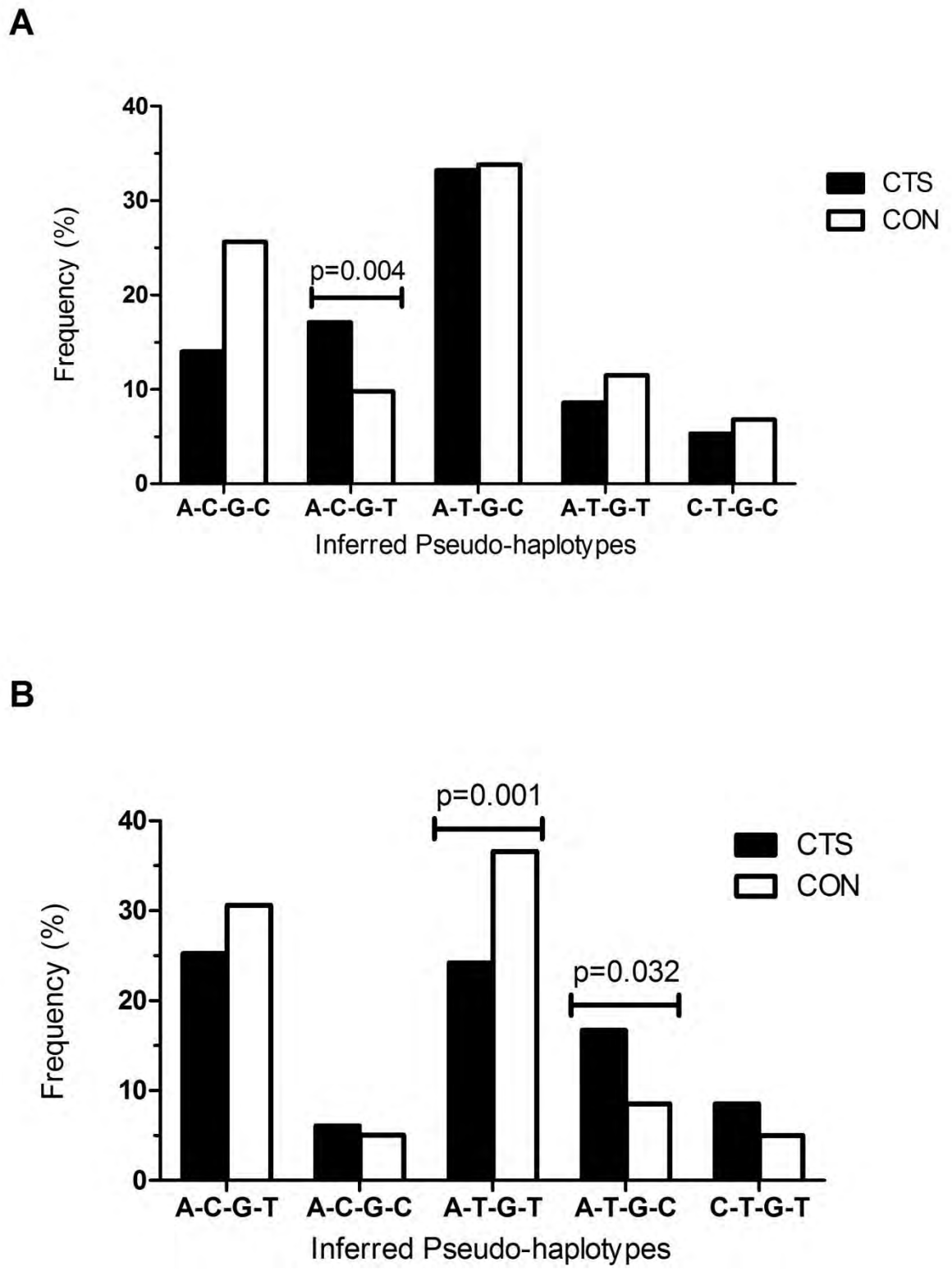
Supplementary table 7.6 Genotype frequency distributions of the *IL-1β* rs16944 (C/T), *IL-6* rs1800795 (C/G), *IL-6R* rs2228145 (A/C), *CASP8* rs3834129 (I/D) and *VEGFA* rs699947 (C/A) variants in carpal tunnel syndrome (CTS) and control (CON) groups for all participants (All) as well as the female participants (Female) excluding medical conditions that are suggested to be associated with CTS.

	All		**Female**	
	CTS	**CON**	**CTS**	**CON**
***IL-1β* rs16944 (C/T)**	n=74	n=108	n=67	n=94
TT	28.4 (21)	34.3 (37)	26.9 (18)	29.8 (28)
TC	55.4 (41)	43.5 (47)	55.2 (37)	45.7 (43)
CC	16.2 (12)	22.2 (24)	17.9 (12)	24.5 (23)
Genotype p-value	0.278		0.450	
C Minor allele	43.9 (65)	44.0 (95)	45.5 (61)	47.3 (89)
Allele p-value	0.991		0.747	
***IL-6* rs1800795 (C/G)**	n=72	n=123	n=66	n=106
GG	87.5 (63)	78.7 (96)	89.4 (59)	78.3 (83)
GC	11.1 (8)	21.3 (26)	9.1 (6)	21.7 (23)
CC	1.4 (1)	0.0 (1)	1.5 (1)	0.0 (0)
Genotype p-value [a]	0.127		0.067	
C Minor allele	6.9 (10)	11.4 (28)	6.1 (8)	10.8 (23)
Allele p-value	0.154		0.132	
***IL-6R* rs2228145 (A/C)**	n=61	n=100	n=55	n=86
AA	62.3 (38)	77.0 (77)	58.2 (32)	80.2 (69)
AC	36.1 (22)	22.0 (22)	40.0 (22)	18.6 (16)
CC	1.6 (1)	1.0 (1)	1.8 (1)	1.2 (1)
Genotype p-value [b]	**0.050**		**0.007**	
C Minor allele	19.7 (24)	12.0 (24)	21.8 (24)	10.5 (18)
Allele p-value	0.061		**0.009**	
***CASP8* rs3834129 (I/D)**	n=69	n=114	n=64	n=98
I/I	47.8 (33)	50.9 (58)	46.9 (30)	51.0 (50)

Supplementary table 7.6 Continued

I/D	43.5 (30)	36.8 (42)	43.8 (28)	35.7 (35)
D/D	8.7 (6)	12.3 (14)	9.4 (6)	13.3 (13)
Genotype p-value	0.587		0.528	
D Minor Allele	30.4 (42)	30.7 (70)	31.3 (40)	31.1 (61)
Allele p-value	0.957		0.981	
***VEGFA* rs699947 (C/A)**	n=61	n=112	n=56	n=96
CC	55.7 (34)	59.8 (67)	55.4 (31)	58.3 (56)
CA	39.3 (24)	36.6 (41)	39.3 (22)	38.5 (37)
AA	4.9 (3)	3.6 (4)	5.4 (3)	3.1 (3)
Genotype p-value [c]	0.631		0.737	
A Minor Allele	24.6 (30)	21.9 (49)	25.0 (28)	22.4 (43)
Allele p-value	0.565		0.605	

Values are expressed as a frequency (%) with the number of participants (n) in parentheses. The maximum number (n) of participants in each group is also indicated. Significant p-values are indicated in bold.



Supplementary figure 7.1 Inferred pseudo-haplotypes constructed from the **(A)** *IL-6R* rs2228145, *IL-1β* rs16944, *IL-6* rs1800795 and *COL5A1* rs12722 variants as well as the **(B)** *IL-6R* rs2228145, *IL-1β* rs16944, *IL-6* rs1800795 and *COL5A1* rs3949 variants. Significant differences are indicated with the p-value.

Supplementary table 8.1 Suspected and actual minor allele frequencies of the investigated variants

Gene & variant	Minor allele	Suspected range	Actual frequency
MMP10 rs486055 (C/T)	T	0.6 - 10.5	4.6
MMP1 rs1799750 (G/GG)	G/GG	G: 47.0 - 47.9 GG: 37.5 - 43.3	G: 42.4
MMP3 rs679620 (A/G)	A	23.0 - 48.3	30.6
MMP12 rs2276109 (A/G)	G	0.0 - 15.9	5.5
COL5A1 rs13946 (T/C)	C	10.5 - 38.4	22.0
COL5A1 rs12722 (C/T)	T/C	T: 13.6 - 22.1 C:43.4	T: 30.4
COL5A1 rs71746744 (-/AGGG)	AGGG	14.2	38.9
BGN rs1126499 (C/T)	C/T	C: 47.2 T: 0.06 - 45.0	T: 20.2
ACAN rs1516797 (G/T)	G/T	G: 24.0 - 33.5 T: 31.6 - 46.2	T: 45.9
IL-1β rs16944 (T/C)	C	33.3 – 50.0	44.5
IL-6 rs1800795 (G/C)	C	0.0 – 35.2	9.6
IL-6R rs2228145 (A/C)	C	6.2 – 38.6	15.5
CASP8 rs3834129 (I/D)	I/D	I: 45.2 – 50.0 D: 21.7 – 50.0	D: 29.0
VEGFA rs699947 (C/A)	C/A	C: 46.0 A:11.9 – 41.0	A: 23.5

Values are presented as a range of frequencies with the actual frequency of the minor allele in the Coloured population, indicated.

Supplementary table 8.2 Percentage of successfully genotyped variants

		% successfully genotyped	
Gene	Variant	CTS (n=103)	CON (n=150)
MMP10	rs486055	94.2 (97)	87.3 (131)
MMP1	rs1179750	85.4 (88)	86.0 (129)
MMP3	rs679620	92.2 (95)	84.7 (127)
MMP12	rs2276109	93.2 (96)	87.3 (131)
COL5A1	rs13946	88.3 (91)	84.7 (127)
	rs12722	96.1 (99)	94.0 (141)
	rs71746744	80.6 (83)	82.7 (124)
ACAN	rs1516797	92.2 (95)	84.0 (126)
BGN	rs1126499	92.2 (95)	83.3 (125)
*IL-1*β	rs16944	97.1 (100)	85.3 (128)
IL-6	rs1800795	96.1 (99)	96.7 (145)
IL-6R	rs2228145	82.5 (85)	81.3 (122)
CASP8	rs3834129	91.3 (94)	88.0 (132)
VEGFA	rs699947	81.6 (84)	87.3 (131)

Values are expressed as a frequency (%) with the number of participants (n) with non-missing data indicated in parentheses. The maximum number (n) of participants in each group is also indicated.