Risk Factors Associated with Blood Pressure Variation in Sickle Cell Disease in Cameroon

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Thesis Presented for the Degree of Doctor of Philosophy

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August 2023

Declaration

I, Arthemon Nguweneza, hereby declare that this dissertation/thesis is based on my original work (except where acknowledgments indicate otherwise) and that neither the whole work nor any part of it has been or is being submitted for another degree in this or any other university.

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I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publications in my PhD thesis, and where co-authorships are involved, my co-authors have agreed that I may include these publications:

1. **Nguweneza A**, Oosterwyk C, Banda K, Nembaware V, Mazandu G, Kengne AP, et al. Factors associated with Blood Pressure variation in sickle cell disease patients: a systematic review and meta-analyses. Expert Rev Hematol. (2022) 15:359–68. doi: 10.1080/17474086.2022.2043743
2. **Nguweneza A**, Ngo Bitoungui VJ, Mnika K, Mazandu G, Nembaware V, Kengne AP and Wonkam A (2022) Clinical characteristics and risk factors of Relative Systemic Hypertensionand Hypertension among sickle cell patients in Cameroon. Front. Med. 9:924722. doi: 10.3389/fmed.2022.924722

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Acknowledgments

This research was supported by the National Heart, Lung and Blood Institute of the National Institutes of Health.

First and foremost, I would like to thank my supervisor, Professor Ambroise Wonkam, for his unwavering support, mentorship, and patience throughout this study. His extensive knowledge and thorough feedback have been invaluable to me, and I will always be grateful that he took me on as a student.

I also want to express my sincere appreciation to Dr. NGO BITOUNGUI Epse ESSOMBA Valentina Josiane for providing the data and recruiting Cameroonian sickle cell patients for this study, as well as the patients themselves for participating and making this research possible.

I am also particularly grateful to Dr. Victoria Nembaware, the manager of the SaDaCC project manager, consistently provided me with support when needed. I also want to thank Mr. Kevin Esoh, Dr. Jack Morrice and Dr. Khuthala Mnika for their technical support.

I want to extend my gratitude to the entire GeneMap team for their encouragement as well as to my colleagues in the Division of Human Genetics at the University of Cape Town and my international collaborators for their contributions to this work.

I am deeply grateful to my friends and family for their support, especially my mother, Bibiane Mukamwiza, for her enduring love and support. My siblings Josiane Iradukunda and Nadine Chivieda Niwemfura have also been a constant source of motivation. To all my friends, Pierre-Axel Siffon, Jurgen Muess, Kambe Banda, Amanda Mfuyo, and Ransom Okereke Oluah thank you for their constant motivation and support. I am truly blessed to have you all in my life. May God bless you all

List of publications

Publications from this thesis

1. **Nguweneza A**, Oosterwyk C, Banda K, Nembaware V, Mazandu G, Kengne AP, et al. Factors associated with Blood Pressure variation in sickle cell disease patients: a systematic review and meta-analyses. Expert Rev Hematol. (2022) 15:359–68. doi: 10.1080/17474086.2022.2043743

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3. Genome wide association of Blood Pressure variation and Body Mass Index among Sickle Cell disease patients in Cameroon. *In print*

Publications the candidate contributed to (excluded)

The following papers are from joint effort within the group during this PhD's thesis.

1. Nembaware V, Mazandu GK, Hotchkiss J, Serufuri JS, Kent J, Kengne AP, et al. The Sickle Cell Disease Ontology: of New Planetary Health Applications. OMICS: A Journal of Integrative Biology. 2020; 24:559–67.

2. Brown BJ, Madu A, Sangeda RZ, Nkya S, Peprah E, Paintsil V, et al. Utilization of Pneumococcal Vaccine and Penicillin Prophylaxis in Sickle Cell Disease in Three African Countries: Assessment among Healthcare Providers in SickleInAfrica. Hemoglobin. 2021; 45:163–70.

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List of abbreviations

Abbreviations	Explanation
BMI	Body Mass Index
BP	Blood Pressure
DBP	Diastolic Blood Pressure
eGFR	Estimated Glomerular Filtration Rate
eQTL	Expression quantitative trait loci
FUMA	Functional mapping and annotation
GWAS	Genome wide association study
HBB	The β-globin gene
HbS	Sickle haemoglobin
LD	Linkage Disequilibrium
MAGMA	Multi-marker analysis of GenoMic annotation
MAF	Minor Allele Frequency
RSH	Relative Systemic Hypertension
SBP	Systolic Blood Pressure
SCD	Sickle Cell Disease
SITT	Silent Infarct Transfusion Trial
SNP	Single Nucleotide Polymorphism
TRV	Tricuspid Regurgitant Jet Velocity

Preface

Sickle cell disease (SCD) is an inherited autosomal recessive condition caused by a mutation in the β-globin gene (*HBB*). SCD patients show substantial heterogeneity in the presentation and clinical course of the disease. SCD patients, generally, have lower systolic, diastolic, and mean Blood Pressure compared to age and sex-matched controls. However, SCD patients are at increased risk of pulmonary Hypertension and renal dysfunctions. In fact, studies have shown that increased BP is associated with higher risk of stroke and mortality in SCD patients, even in a range of systolic and diastolic BPs (SBP, DBP) that are considered relatively normal for the general population (i.e., lower than 140 mmHg). BP seems to be a potential modulator of clinical severity in SCD patients. Understanding the aetiology of BP variation in SCD patients and ways of controlling/ managing BP variation could lead to the prevention of BP-related mortality in SCD patients. We investigated clinical, genetic, and epidemiological risk factors associated with RSH or Systemic Hypertension in SCD patients in Cameroon to gain insight into the pathophysiology of BP variation in this disease in an African setting.

Research Concept and Funding

The candidate worked with Professor Ambroise Wonkam to come up with the idea for the study and develop the methodology. The candidate then created a research proposal, analysed the data, interpreted the findings, with the guidance of the supervisor. The candidate also drafted the full manuscripts and made revisions based on feedback from co-authors and journal reviewers. This work was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health (Award Number U24HL135600) and (Award Number 1U24HL135881).

Data collection, experimentation, and data analysis

This a retrospective analysis of a data and samples obtained from a cross-sectional study of 1142 SCD patients conducted in Cameroon from May 2016 to July 2018. The data and samples were collected from nine hospitals from five cities in Cameroon, including Yaoundé, Douala, Bafoussam, Bertoua, and Maroua. 815 SCD patients with complete socio-demographic, clinical, laboratory variables, and complete systolic and diastolic Blood Pressure measurements were included in the study in our retrospective analysis. A proposal obtained ethical approval from the University of Cape Town, Faculty of Health Sciences Human Research Ethics Committee, Cape Town, South Africa by the candidate. Epidemiological data management, cleaning, and analysis was performed by the candidate. Where sample preparation or laboratory assistance was necessary, the involvement of collaborators was requested by the principal investigator. Where bioinformatics support was necessary, the involvement of collaborators was requested by

the principal investigator. The contribution and work of these collaborators on the study is recognized and acknowledged in each publication where applicable.

Publications

The candidate was responsible for synthesizing the results and drafting the manuscripts for the publications included in this thesis. These drafts were then revised by all co-authors and submitted to the journal by the principal investigator. After review, the candidate worked with the principal investigator to address all reviewer comments. The candidate's specific role in each publication is stated in each chapter.

Published (or accepted for publication) articles to be included and co-author contributions.

Contribution to authorship for the different publications was jointly agreed by all authors. Arthemon Nguweneza contributions ranged from: Conception and design of the work, methodology, analysis, data interpretation, drafting and revising the final manuscripts.

The following are the list of papers to be included in Arthemon Nguweneza PhD's thesis:

1. **Nguweneza A**, Oosterwyk C, Banda K, Nembaware V, Mazandu G, Kengne AP, et al. Factors associated with Blood Pressure variation in sickle cell disease patients: a systematic review and meta-analyses. Expert Rev Hematol. (2022) 15:359–68. doi: 10.1080/17474086.2022.2043743

   Author contribution: A.W. conceived the study. **Arthemon Nguweneza** developed the protocol. **Arthemon Nguweneza** and C.O. did the literature search, selected studies, and extracted relevant data. **Arthemon Nguweneza** performed a meta-analysis and interpreted the data. **Arthemon Nguweneza** issued the first draft of the paper, C.O., K.B., V.N., G.M., A.P.K. and A.W. critically revised successive drafts of the manuscript. A.W. supervised the project. **Arthemon Nguweneza** is the guarantor of this manuscripts. All authors have read and agreed to the published version of the manuscript.

2. **Nguweneza A**, Ngo Bitoungui VJ, Mnika K, Mazandu G, Nembaware V, Kengne AP and Wonkam A (2022) Clinical characteristics and risk factors of Relative Systemic Hypertensionand Hypertension among sickle cell patients in Cameroon. Front. Med. 9:924722. doi: 10.3389/fmed.2022.924722

   Author contribution: AW conceived the study. **Arthemon Nguweneza**, VN, GM, and AW made substantial contributions to the conception, design of the work, methodology, analysis, data interpretation, and wrote the final manuscript. **Arthemon Nguweneza** and GM analysed and interpreted the data. **Arthemon Nguweneza** issued the first draft of the paper. **Arthemon Nguweneza**, VN, KM, GM, VN, AK, and AW critically revised successive drafts of the

manuscript. VN, GM, AK, and AW supervised the project and compiled the revisions. All authors have read and agreed to the published version of the manuscript.

The findings of this thesis are presented in Chapters 3, 4 and 5: Chapter 3) A systematic review and meta-analysis of BP variation among SCD patients; Chapter 4) Clinical and epidemiological risk factors for Relative Systemic Hypertension (RSH) and Systemic Hypertension in SCD patients in Cameroon; Chapter 5) Identification of genetic variants contributing to BP variation in SCD patients in Cameroon and pathways and mechanisms underlying BP variation in SCD patients.

The candidate has met all requirements and approval of UCT's Doctoral Degrees Board, under Rules GP6.7 as follows:

a. The candidate's proposal to include publications in the current thesis was approved by the UCT Faculty of Health Sciences Doctoral Degree Board.

b. The thesis contains a summary, an introduction, a chapter on the aims and objectives, a comprehensive academic discussion of the results, forming the basis of the conclusions and perspectives drawn from this research.

c. Each results chapter with publications included is preceded by a synopsis of how the publications directly tie to the aims and objectives of the project, as well as to the thesis.

d. All included publications were written and published during the candidate's tenure as a PhD student since 2019.

Signed by candidate

Yours sincerely,
Arthemon Nguweneza

Chapter 1: Rationale, aims and objectives.

Synopsis: This chapter will give a broad overview, rationale, aims and objectives of the entire thesis.

Background

Sickle cell disease (SCD) is a genetic disorder that results from a mutation in the *HBB* gene on chromosome 11[1]. This mutation involves the replacement of glutamic acid with valine in a specific nucleotide (SNP) in the sixth codon of the gene. The normal sequence in this location is GTG, but in individuals with SCD, the sequence is GAG [1]. This mutation leads to the production of sickle haemoglobin (HbS), with altered physical properties of haemoglobin (HbA) [1]. Sickle cell disease (SCD) is inherited as an autosomal codominant trait. This means that individuals who are heterozygous for the (βs) allele carry the sickle cell trait (HbAS) but will not have SCD. However, individuals who are homozygous for the (βs) allele will have SCD [1]. Sickle cell disease (SCD) is a lifelong disease characterized by chronic haemolytic anaemia and episodic painful vaso-occlusion of variable severity, which can lead to organ damage and a shortened lifespan[2–4]. Heterozygotes, or individuals with one copy of the normal gene (HbA) and one copy of the mutated gene (HbS) in their red blood cells, are highly resistant to malaria. The distribution and prevalence of the (βs) mutation, which causes SCD, is largely influenced by the historical prevalence of malaria[5–7]. In locations where malaria was highly prevalent, the frequency of the (βs) mutation is higher because it provided some protection against malaria[5].

Approximately 3.2 million individuals are living with SCD, 43 million individuals have sickle cell trait, and 176,000 people die each year due to SCD-related complications [8]. The most common and severe form of SCD is homozygous HbSS, also known as sickle cell anemia (SCA). In individuals of African ancestry, and with regional variation in Africa, this genotype is responsible for 65-70% of SCD cases, followed by HbSC (30%), which is caused by the inheritance of the βs gene in combination with the haemoglobin C gene. Another form of SCD is HbS-βThal, which is caused by the inheritance of the βs gene in combination with a β-thalassemia mutation[5, 9].

The impact of SCD on the African continent is significant, with symptoms ranging from mild to severe. In sub-Saharan Africa, most babies with SCD rarely survive past the age of 5 if they do not receive treatment[10, 11]. Whereas, in high income countries, individuals with SCD have experienced significant increases in life expectancy due to improved access to medical treatment and preventative measures, such as new-born screening, penicillin prophylaxis, pneumococcal vaccinations, blood transfusions, and hydroxyurea therapy, which promotes the production of fetal haemoglobin. With proper medical management, individuals with sickle cell disease may have the potential to live well into

their 70s[12]. As life expectancy in SCD patients improves due to advances in medical care, the risk of developing cardiovascular complications increases due to persistent intravascular haemolysis. Cardiovascular complications associated with SCD include proliferative systemic vasculopathy, impaired left ventricular diastolic function, stroke, kidney disease, and pulmonary Hypertension[12]. These factors are thought to be indicators of disease severity and predictors of early mortality in individuals with sickle cell disease[12].

Regarding cardiovascular health, variation in Blood Pressure (BP) is a primary risk factors for stroke, heart failure, and kidney failure in individuals with SCD. BP refers to the force of blood pushing against the walls of the arteries as the heart pumps it[13]. When BP is consistently elevated, it can lead to organ damage[13]. Studies have shown that individuals with SCD typically have lower systolic, diastolic, and mean BP compared to healthy individuals of the same age and gender[14]. Increased BP has also been linked to an increased risk of stroke and mortality in individuals with SCD, even when BP is within what is considered a normal BP range for the general population (below 140 mmHg)[14]. This phenomenon has been referred to as Relative Systemic Hypertension(RSH) for SCD individuals with BP in the range of 120-139/70-89 mmHg, and Systemic Hypertension for SCD patients with BP in the range of 140/90 mmHg[14]. The management and treatment of high BP has contributed to the declining trend in morbidity and mortality over the past three decades. However, there is a lack of data on the prevalence of high BP and its risk factors among African populations, especially in SCD patients. Currently, there are no recommendations for how to define RSH and Systemic Hypertension in individuals with SCD. The lack of specific criteria for defining Relative Systemic Hypertension (RSH) and Systemic Hypertension in SCD patients is a concern, as variations in BP may impact the severity of SCD.

Rationale for this study

There is limited data available on the clinical and genetic factors that may contribute to variations in BP in SCD patients, especially in Africa. It is known that BP is a trait that can be inherited, and estimates suggest that 30-70% of the variation in BP may be due to genetic factors. A recent study in Africa (Cameroon) found that in SCD patients, long survival to be linked to be associated with recurrent deleterious mutations in *CLCN6* gene. Rare, deleterious mutations in this gene have been associated with lower BP[15]. A study that was done in African American cohort identified the variation (rs7952106 in *DRD2*) gene in modulation of SBP [16]. To investigate genetic factors that associated with certain traits such as BP, researchers have made progress in identifying some of these genetic factors using study design such as genome wide association studies (GWAS)[17, 18]. These studies

compare genetic variations in individuals with a specific disease with those of healthy individuals in the same population. Single nucleotide polymorphisms (SNP) are often examined in these studies[17, 18]. Variations in BP among individuals with SCD may be related to differences in environmental factor such as nutrition, socioeconomic status, and climate factor at different geographic locations and that might influence the natural history of disease [19].

In high-income countries, SCD research has been conducted extensively, highlighting its status as a major global public health concern. However, SCD is particularly prevalent in Africa, where it has not been as thoroughly characterized. The genetic and non-genetic factors that contribute to SCD may be different in African populations due to the high burden of malaria and other infections, limited access to medical care, and high rates of undernutrition.

It is important to study clinical, anthropometric, and genetic factors associated with BP variation in SCD patients in Africa, particularly Cameroon, for several reasons:

1. First, SCD is a major public health concern in Africa, and understanding the factors that contribute to BP variation in SCD patients can help improve the diagnosis, treatment, and management of the disease. This can ultimately improve the quality of life for SCD patients and reduce the burden of SCD on the healthcare system.

2. Second, studying these factors in Africa, particularly Cameroon, can help researchers understand how these factors may differ from those in other populations, which can be useful for developing more targeted prevention and treatment strategies. For example, the heavy burden of infectious diseases and high rates of undernutrition in Africa may contribute to BP variation in SCD patients in a way that is different from what is observed in other populations. Understanding these differences can help researchers tailor interventions to the specific needs of SCD patients in Africa.

3. Thirdly, studying clinical, anthropometric, and genetic factors associated with BP variation in SCD patients in Africa can help reduce health disparities by improving our understanding of the disease and its impact on diverse populations. This can help ensure that SCD patients in Africa have access to the same high-quality care and treatment as those in other parts of the world.

4. Finally, in addition to exploring genetic factors associated with BP variation in patients with SCD, this study also investigates potential associations between genetic variants and BMI. BMI,

a measure of body fat based on height and weight, serves as a key indicator of overall health and nutritional status, and is associated with cardiovascular complications[20]. Therefore, analysing both BMI and BP together in a GWAS allows for a more comprehensive exploration of shared genetic risk factors [20, 21] underlying cardiovascular risk in SCD patient.

Aims and objectives.

This project aims to investigate clinical, genetic, and epidemiological risk factors associated with BP variation in SCD patients in Cameroon to better understand the prevalence and pathophysiology of this disease in an African setting. The specific objectives of the project are:

1. To review existing literature on risk factors associated with BP variation in SCD patients.
2. To identify clinical and epidemiological risk factors for BP variation in SCD patients in Cameroon
3. To use genome-wide association studies to identify genetic variants associated with BP variation in SCD patients.
4. To investigate the pathways and functional mechanisms underlying BP variation in SCD patients.

Chapter 2: Methods

Synopsis: This chapter will give an overview of all the methods used in the overall project and show how each is connected to the other. Specific and detailed methods will be highlighted in each chapter.

Materials and Methods

This study was approved by the University of Cape Town, Faculty of Health Sciences Human Research Ethics Committee, Cape Town, South Africa (HREC: 142/2022). This study consisted of two parts: the first was a systematic review and meta-analysis, and the second was an analysis of an existing dataset. The systematic review focused on identifying and analysing existing research related to aetiology of BP variation in SCD patients. We then used the results of the systematic review and meta-analysis to develop a framework for analysing a dataset consisting of Cameroonian SCD patients.

2. 1 Systematic review and meta-analysis

For the systematic review and meta-analysis, we developed a PRISMA Flow diagram to map out the process and to ensure a comprehensive search. We then conducted a quality assessment of the studies included in our review, based on criteria such as study design, sample size, and participant characteristics. Finally, we conducted a meta-analysis of all the studies included to identify any common trends in the data. Chapter 3 provides in-depth description of the methods used to conduct our systematic review and meta-analysis.

2. 2 Retrospective cohort analysis of Cameroonian SCD patients

The Cameroonian SCD patient dataset was analysed using the framework developed from the systematic review and meta-analysis. Figure 2.1 shows an overview of the methodology employed in this analysis. Figure 2.3 shows the numbers of included study participants and samples throughout the analysis in chapter 4 and chapter 5. 1) To investigate the socio-demographic, anthropometric, clinical, laboratory risk factors, we included all patients with complete information. Chapter 4 provides in-depth descriptions on characteristics on study participants and methods used and the results for this analysis. 2) To investigate for genetic risk factors, we conducted a Genome wide association study (GWAS) and meta-analysis, chapter 5 provides a detailed description of the methods used and the results for this analysis.

The results of this analysis were then used to draw conclusions about this study in chapter 6.

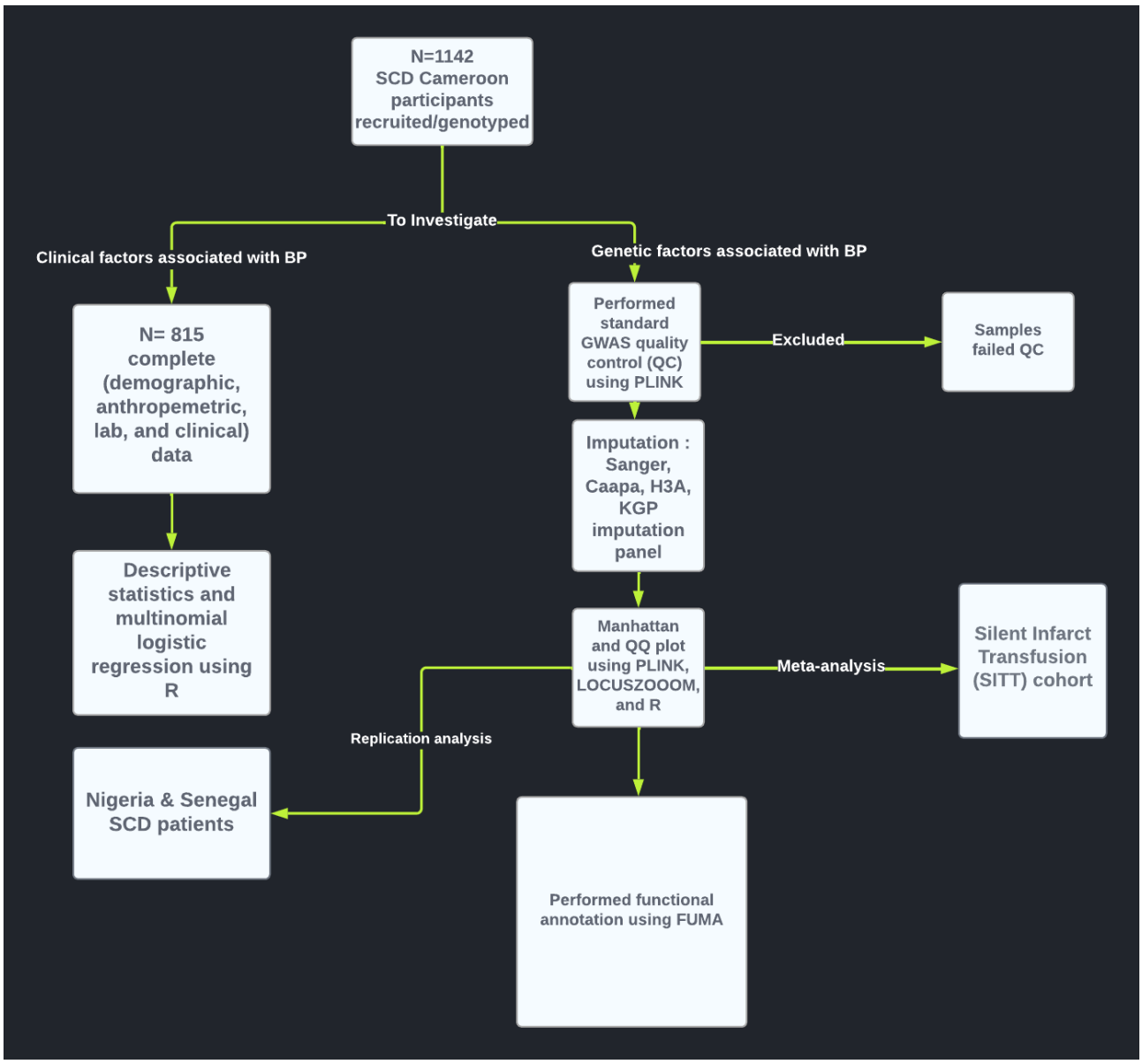

Figure 2.1: Flowchart of the methodology employed in this study.

Functional Mapping and Annotation of Genome-Wide Association Studies (FUMA) version 1.3.6 was used for the annotation of SNPs generated from GWAS summary statistics[22]. It is a comprehensive web-based tool (https://fuma.ctglab.nl/) designed to streamline the analysis of GWAS summary statistics. FUMA integrates data from 18 biological repositories to facilitate the identification of potential causal variants and associated genes from GWAS summary statistics[22].

One of FUMA's key functionalities is SNP annotation, which is done using the SNP2GENE module (Figure 2.2). This module takes GWAS summary statistics as an input and annotates all SNPs based on their biological relevance and association with specific the genes. As illustrated in Figure 2.2, SNP2GENE characterise genomic loci by identifying independent significant SNPs and candidate SNPs, defining leading SNPs, and genomic risk loci. It then annotates candidate SNPs in genomic loci,

tests such as expression quantitative trait loci (eQTL), and chromatin interactions. The output from SNP2GENE provides functional gene mapping, which serves as an input for the GENE2FUNC module of FUMA[22].

Functional annotation of mapped genes was conducted using the GENE2FUNC function, leveraging the output from SNP2GENE. Gene-based analyses were conducted with MAGMA [23], initially computing gene-based p-values. Subsequently, gene set p-values were calculated using curated gene sets and Gene Ontology terms from MsigDB v5.2. To address multiple testing, Bonferroni correction and False Discovery Rate (FDR) adjustment were applied to gene and gene-set analyses, respectively [24].

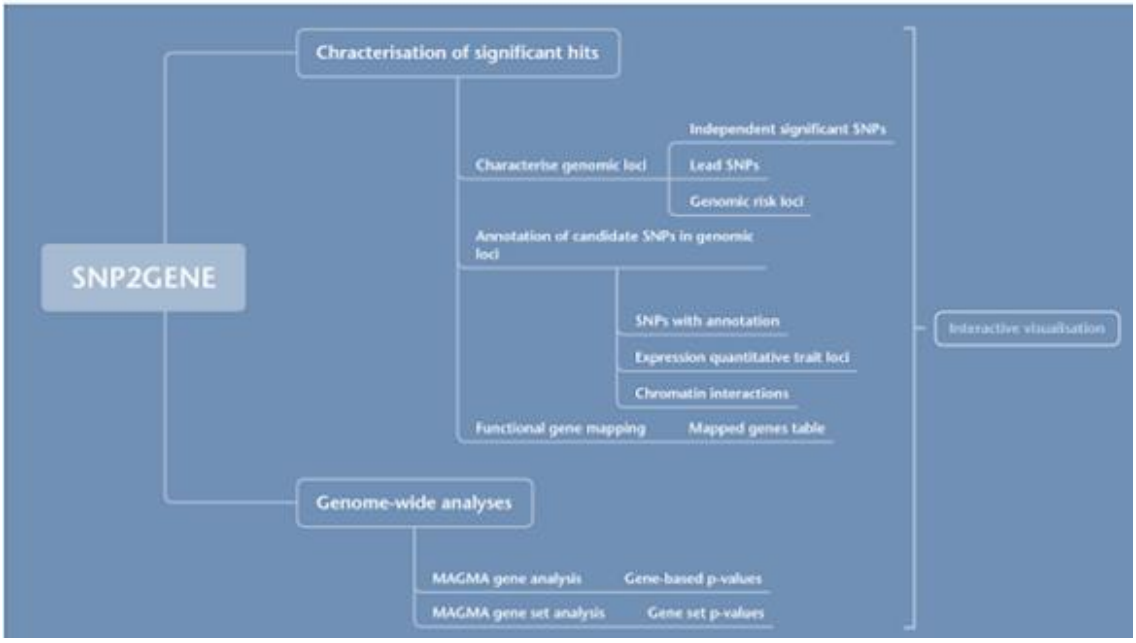

Figure 2.2: Illustrates SNP2GENE function of FUMA.

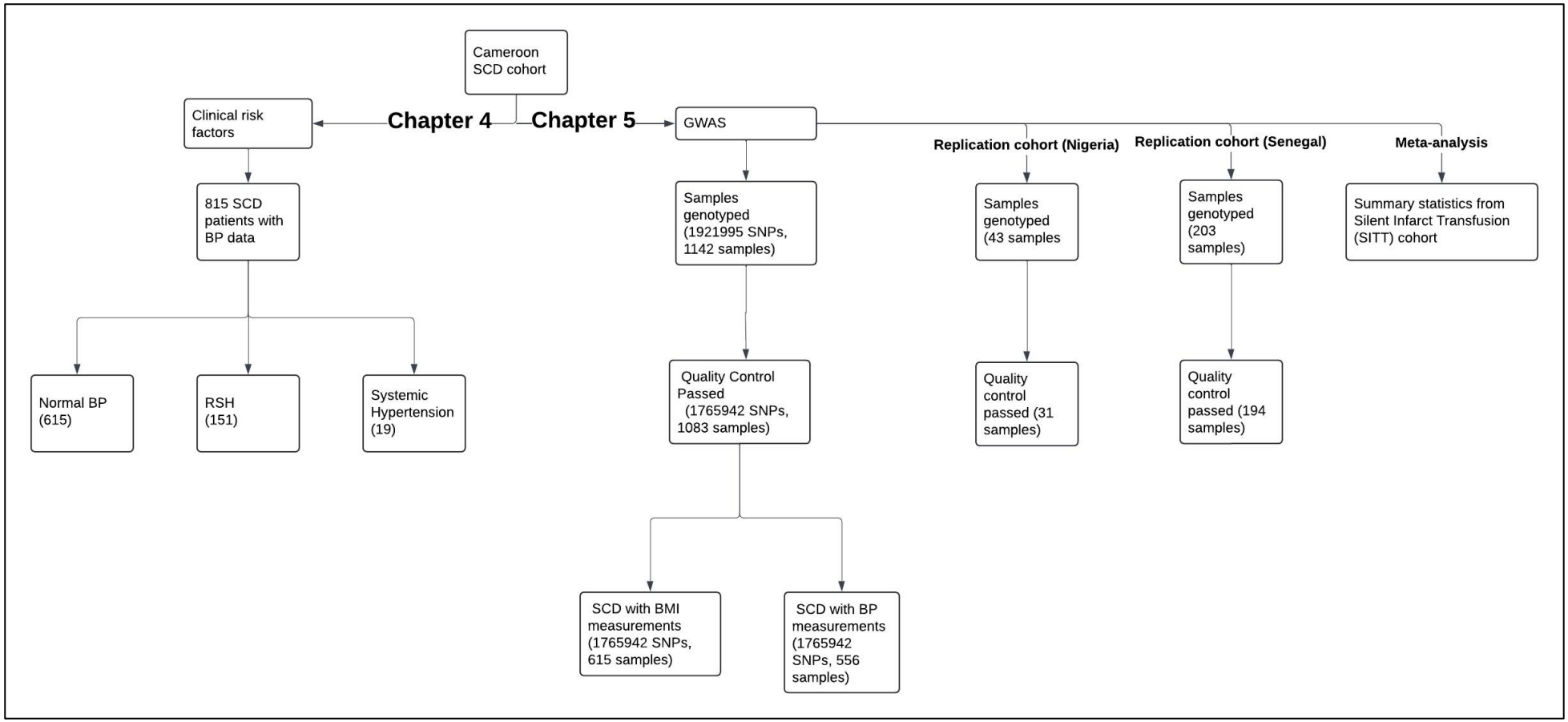

Figure 2.3: Flowchart of SCD participants included in the analysis

Chapter 3: Systematic review and meta-analysis of risk factors associated with BP variation among SCD patients.

Synopsis: This is the first reported systematic review and meta-analysis that provides information on a wide range of risk factors associated with BP variation among SCD patients

3.1 Nguweneza A, Oosterwyk C, Banda K, Nembaware V, Mazandu G, Kengne AP, et al. Factors associated with Blood Pressure variation in sickle cell disease patients: a systematic review and meta-analyses. Expert Rev Hematol. (2022) 15:359–68. doi: 10.1080/17474086.2022.2043743

- **Nature of Publication:** Original Article
- **Journal/Publisher:** Expert Review of Hematology; Informa Publishing Group; ISSN: 1747-4086; Peer-reviewed.
- **Candidate contribution**: **Arthemon Nguweneza** developed the protocol. **Arthemon Nguweneza** and C.O. did the literature search, selected studies, and extracted relevant data. **Arthemon Nguweneza** performed a meta-analysis and interpreted the data. **Arthemon Nguweneza** issued the first draft of the paper.
- **Co-Authors Contributions:** A.W. conceived the study. C.O., K.B., V.N., G.M., A.P.K. and A.W. critically revised successive drafts of the manuscript. A.W. supervised the project. **Arthemon Nguweneza** is the guarantor of this manuscripts. All authors have read and agreed to the published version of the manuscript.

Factors associated with Blood Pressure variation in sickle cell disease patients: a systematic review and meta-analysis.

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Abstract

Introduction: Blood Pressure (BP) values ≥ 120/70 mmHg considerably increase the risk for pulmonary Hypertension and renal dysfunction in Sickle Cell Disease (CSD) patients, and ultimately increased morbidity and mortality. This has led to the development of the term Relative Systemic Hypertension (RSH). RSH was defined as Systolic BP 120–139 mm Hg or diastolic BP 70–89 mm Hg, whereas Systemic Hypertension is defined as Systolic BP >= 140 mm Hg or diastolic BP >=90 mm Hg. Systematic identification and analysis of BP variations and risk factors in SCD patients could promote effective management of the patients.

Area covered: This review aimed to identify factors associated with BP variation among SCD patients. We searched PubMed, Scopus, Web of Science and Google Scholar up to December 2020 with no geographical or language restrictions. Two reviewers independently screened, assessed for quality and summarized data from eligible studies.

Expert opinion: Advancing age, gender, higher body weight, haemoglobin, eGFR, triglycerides, greater haematocrit, higher blood viscosity, history of blood transfusion, and targeted variants in DRD2 and MIR4301 genes were independently associated with the risk of relative systemic or Systemic Hypertension in SCD patients. Therefore, interventions that consider these risk factors may potentially contribute to lower BP pressure in SCD patients and prevent developing severe complications.

Keywords: Relative Hypertension, risk factors, Hypertension, sickle cell, Blood Pressure, systolic, Diastolic

Article highlights box.

- After identifying and screening 2420 records, 18 studies published between 1981-2018 met the inclusion criteria.
- 14 of the included studies had a moderate risk of bias, 2 had a high risk of bias, and 2 had a low risk of bias. 12 studies were conducted in high income countries, while the remaining 6 studies were conducted in low- and middle-income countries.
- Results from this systematic review and meta-analysis compared BP indices in SCD patients and those of non-SCD individuals from observational studies with great consistency across the studies, confirming that BP is lower in SCD patients compared to the general population.
- Demographic variables (Age, gender), anthropometric variables (Body Mass Index (BMI), waist and neck circumference), and biological variables (haemoglobin, estimated glomerular filtration rate (eGFR), absolute neutrophil count, triglycerides, greater heamatocrit, higher blood viscosity blood viscosity) and selected genetic causes (variants in DRD2 and MIR4301 genes) were identified as factors associated with BP variations in SCD patients.

1.Introduction

Sickle cell disease (SCD) is an inherited, autosomal recessive condition caused by several mutations in the β-globin gene (HBB). SCD encompasses any one of the syndromes which arise from the mutation that leads to substitution of valine for glutamic acid at a single nucleotide polymorphism (SNP) in the sixth codon of the HBB gene on chromosome 11. This mutation leading to the production of sickle haemoglobin (HbS), with altered physical properties of haemoglobin (Hb)[25–27]. According to a systematic review by the Global Burden of Disease Study, 3.2 million people live with SCD, 43 million people have sickle cell trait (i.e., are carriers of the mutation), and 176,000 people die due to SCD related complications per year [28] . The most common and the most severe form of SCD is homozygous HbSS (sickle cell anaemia), resulting from the inheritance of βS gene from both parents. The HbSS genotype accounts for 70% of cases of SCD in patients of African ancestry. Other forms of SCD include the inheritance of the βs gene in combination with hemoglobin C or β- Thalassemia mutations, resulting in HbSC or HbSβThal genotypes, respectively [25, 26].

Sickle cell disease (SCD) patients show substantial heterogeneity in the presentation and clinical course of the disease [25]. Typically, the pathobiology of SCD is a vicious cycle of four major processes. Firstly, under deoxygenation conditions, HbS molecules polymerize to form bundles. The polymer bundles form long fibres that impair the erythrocyte membrane, leading to erythrocyte sickling. Secondly, the normally freely flowing cytosol of the erythrocyte becomes viscous making the cell much less deformable. This leads to the blockage of blood vessels, disrupting the blood supply, referred to as vaso-occlusion. Thirdly, the polymer bundles also promote hemolysis causing chronic anemia with Hb levels ranging from 6–11 g/dl. The severity of this anaemia is dependent on primary genotype, and genetic modifiers of fetal haemoglobin levels, the strongest known modifier of the SCD clinical expression. Patients with higher rates of hemolysis are more likely to develop vascular injury and multi-organs dysfunction. Lastly, hemolysis by-product such as erythrocyte damage-associated molecular patterns (eDAMPS) along with ischemia-reperfusion injury caused by vaso-oclussion, promote sterile inflammation and oxidative stress. These events further amplify vaso-occlusion through a feedback loop by promoting adhesion of neutrophils, platelets, and endothelial cells. These molecular, cellular, and biophysical processes work in synergy to promote acute and chronic pain, ischemic reperfusion injury and infarctions of vital organs such as spleen, heart, kidneys, liver, muscle, brain, lung, and bone.

While in Sub-Saharan Africa, without treatment, most babies born with SCD rarely live beyond five years of age [10, 11] the life expectancy has improved drastically in high income countries; This is mainly due to the widely implemented new-born screening, followed by a comprehensive care,

including penicillin prophylaxis, pneumococcal immunization, availability of red cell transfusions, and use of the fetal haemoglobin-inducing therapy Hydroxyurea. Some patients have potential to live well into their 7th decade[12]. However, with improved life expectancy, cardiovascular complications become more common, due to persistent intravascular haemolysis [29]. Cardiovascular complications such as proliferative systemic vasculopathy, left ventricular diastolic dysfunction and pulmonary Hypertension have been reported. These factors are known predictors of mortality in SCD patients [12, 30].

Individuals with SCD commonly have lower diastolic, systolic, and mean Blood Pressure in comparison to the general population. Notably, the incidence of pulmonary Hypertension and renal insufficiency at relatively normal BP (≥ 120/70 mmHg) have been reported in some SCD patients [14]. Previous studies in SCD patients having labelled this phenomenon "relative Systemic Hypertension" (RSH) [14]. RSH was defined as Systolic BP (SBP) 120–139 mm Hg or diastolic BP (DBP) 70–89 mm Hg and systematic Hypertension as SBP 140 mm Hg or DBP 90 mm Hg or higher. However, it's important to note that the current cut-off or definition of RSH is applied uniformly across both paediatric and adult SCD patients. Given the potential differences in BP norms across age groups, there is a need for age-specific values for RSH and Systemic Hypertension. Such definition would not only enhance the understanding of variation of BP in SCD patients, but also allows more accurate comparisons across studies.

Blood Pressure (BP) is a potential modulator of clinical severity in SCD patients, and recurrent deleterious and loss of functions mutation with genes associated with lowering BP has been recently associate with long survival in SCD in Africa[15]. Identifying factors associated with BP variation is key to controlling BP, as well as preventing associated causes of mortality in SCD patients. Herein, we present a systematic review on risk factors associated with BP variation in SCD patients.

2.Methods

2.1 Protocol registration

This review was registered with the International Prospective Register of Systematic Reviews (PROSPERO), registration number CRD42020168798.

2.2 Search Strategy and Eligibility Criteria

The Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) served as a

template for reporting the present review [31].

Two reviewers (AN and CO) with the help of an expert librarian, developed a comprehensive literature search strategy in the following databases:

- MEDLINE/PubMed.
- Scopus.
- Web of Science.

The PubMed database search syntaxes are presented in Appendix 1. This syntax is a combination of MeSH terms, keywords and tags and was adapted for other databases. AN and CO searched for studies using the following three concepts: SCD patients, risk factors, and Blood Pressure. The Boolean operators 'OR' were used with synonyms within each concept and then the search results for different concepts were combined with the 'AND' operator. In addition, AN and CO searched other sources using Google Scholar search engine and checked the reference lists of relevant studies. AN and CO completed the search process by manually searching Google. All the identified articles were imported into Mendeley (reference manager) software. Duplicate studies from the different electronic databases were removed through the Mendeley reference manager. Independently, AN and CO screened titles and abstracts of the search results for potentially eligible studies. Full texts of the remaining articles were retrieved and further assessed independently by AN and CO using eligibility criteria. Any discrepancies were resolved through consensus.

The following exclusion and inclusion eligibility criteria were used to screen full text:

- Only observational studies addressing the risk factors of Blood Pressure variation among SCD patients were included.
- Risk factors included but not limited to age, sex, demographic and genetic variants, epigenetic, socio-economic, psychological, anthropometric, biological, and disease-related factors.
- We excluded review articles without original data, case reports and expert opinion commentaries, and studies whose main outcome was not BP in SCD patients.
- Eligible studies were included regardless of language, year of publication or geographical location.

2.3 Assessment of the methodological quality

The Newcastle-Ottawa Scale (NOS) and Q-Genie were used to evaluate the methodological quality of observational and genetic studies included in this review, respectively. Since there was no validation

study to provide a cut-off score for rating low-quality studies, we considered 0–4, 5–7, and 8–10 stars as indicative of high, moderate, and low risk of bias, respectively. Two investigators (AN and CO) independently assessed study quality and disagreements were resolved by consensus.

2.4 Data extraction

The study inclusion criteria were the study population, the study design, the country, the outcomes. Relevant data were independently extracted by the two reviewers (AN and CO) using a standardised extraction form in Microsoft Excel. The data extracted included the citation, language of the paper, study period, study location, study objectives, study design, study period, characteristics of participants, sample size and sampling technique, explanatory and outcome variables, and the major findings. Specific data such as the demographic and socio-economic characteristics, any anthropometric measurements, Blood Pressure measurement procedures were also extracted. The risk factors that are associated with BP variations were categorised into demographic, anthropometrical, biological, and genetic.

2.5 Data synthesis

We conducted as a meta-analysis and a narrative synthesis where a meta-analysis was not possible and descriptive synthesis. The descriptive synthesis included: (a) PRISMA Flow; (b) a quality assessment; (c) a description of the study characteristics and findings (Table 2); and (d) a narrative synthesis. Furthermore, the results of meta-analysis are presented as a forest plot (Figure 2). 95 % confidence intervals (CI), and the standardized mean difference (std Mean difference) were calculated for each study and used for the meta-analysis. We used inverse-variance weighted random effects meta-analysis to pool the study-specific estimates. The choice of a random effects model was made a priori to allow for inter-study heterogeneity of results. inter-study heterogeneity was evaluated by the X2 test on Cochrane's Q statistic and its magnitude estimated using the I2 values. These values represent the percentage of variation in effect size between studies that can be attributed to genuine variability rather than chance. The I2 values of 25%, 50% and 75% represent low, medium, and high heterogeneity, respectively. Quantitative data was pooled in a statistical meta-analysis using Review Manager (RevMan) software.

3. Results

3.1 The review process

The database search yielded 2477 citations and a further 30 articles were identified by manual screening of citations. After the elimination of duplicates, 2420 records remained. After screening titles and abstracts, we found that 2325 records were irrelevant and excluded them. After assessing the full texts of the remaining 95 papers for eligibility, 77 of them were excluded. 16 studies were part of the qualitative synthesis, and 7 studies were included in the meta-analysis.

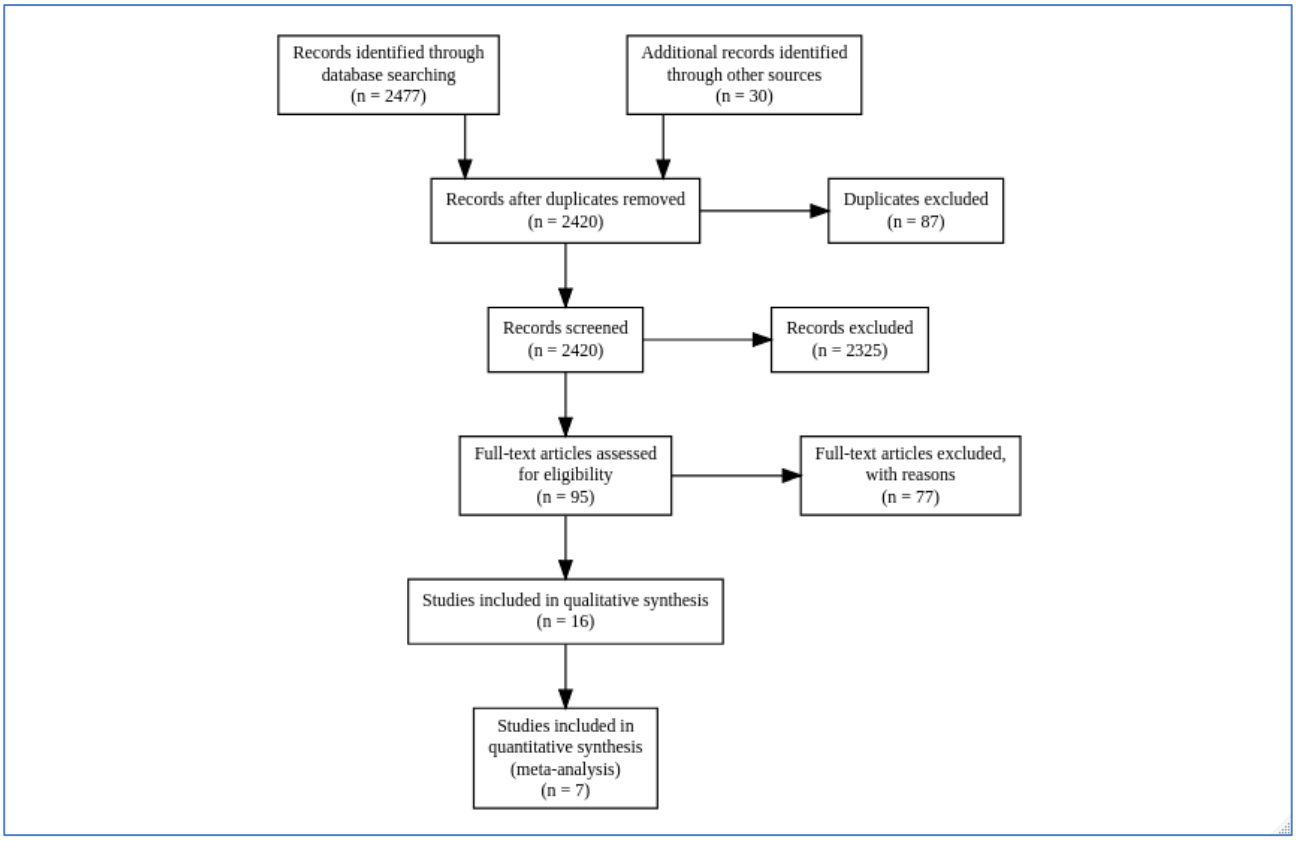


Figure 1. Flow chart of study selection.

3.2 Assessment of risk of bias

All 16 studies included in the review were considered for critical appraisal. The results of the critical appraisal were used to generate a description of the risk of bias for each included study (Table 1). Studies were classified as having low (n=2), moderate (n=12) and high (n= 2) risk of bias. In most cases, the selection of non-representative samples was the source bias.

Table 1. Risk of bias in the included studies

Reference	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Risk of bias score	Risk of bias
Genetic data											
Bhatnagar	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	7	Moderate
Descriptive / Cross-sectional studies											
Campbell	No	Yes	Yes	Yes	Yes	NA	No	Yes	Yes	6	Moderate
Desai	No	No	No	Yes	Yes	NA	No	Yes	Yes	4	High
Johnson	No	Yes	Yes	Yes	Yes	NA	No	Yes	Yes	6	Moderate
Karayayla	No	No	Yes	Yes	Yes	NA	No	Yes	Yes	6	Moderate
Lamarre	No	Yes	Yes	Yes	Yes	NA	No	Yes	Yes	6	Moderate
Lemonne	No	Yes	Yes	Yes	Yes	NA	No	Yes	Yes	6	Moderate
Oguanobi	No	No	Yes	Yes	Yes	NA	No	Yes	Yes	5	High
Pegelow	No	Yes	Yes	Yes	Yes	NA	No	Yes	Yes	6	Moderate
Pikilidou	No	Yes	Yes	Yes	Yes	NA	No	Yes	Yes	6	Moderate
Cohort / Case control studies											
Gordeuk	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes	7	Moderate
Grell	No	Yes	Yes	Unclear	Yes	Yes	Unclear	Yes	Yes	6	Moderate
Homi	No	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	7	Moderate
Kuma	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	8	Low
Makubi	No	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	7	Moderate
Novelli	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	8	Low

Description of study characteristics

The studies' sample sizes ranged from 38 to 3317 participants. Study participants' mean age ranged from 6 to 81 years. Included studies were published between 1981 to 2018. Studies had a cross-sectional (n=9), retrospective cohort (n=6), and genome-wide meta-analysis (n=1) design. The study settings included outpatient or in-hospital SCD participants. None of the studies included community-based recruitment of SCD participants. Studies were carried out in North America (n=11), in Africa (=3) and Europe (n=2). Details on the characteristics of the 16 included studies are reported in Table 2. Studies described BP and risk factors associated in either SCD patients versus healthy controls or SCD patients only.

Table 2: Characteristics of included studies.

Studies	Year	Countries	Study settings	Sample size	Methodology	Characteristics of study participants	Outcomes	Identified factors
Bhatnagar	2013	United States (USA)	SCD cohorts of African American ancestry	1,617 patients	Genome-wide meta-analysis	This study includes two African American ancestry SCD cohorts. Gender: 843 males, 774 females. Average age was 8.96 from one cohort and 9.57 years from the second cohort	Systolic Blood Pressure (SBP)	Genetic factors

Campbell	2007	Jamaica	SCD clinic	51 subjects with HbSC and 88 subjects with HbSS	Cross-sectional	51 subjects with HbSC (29 males, 22 females) and 88 subjects with HbSS (43 males, 45 females). Age: Age ranged from 23.1 to 31.6 years.	Blood Pressure (BP) and BP by genotype	Anthropometric, haematological, and renal functional factors
Desai	2012	USA	SCD patient's cohort at University of North Carolina (UNC) clinic	156 SCD patients in the UNC cohort	Cross-sectional	Age: Mean age was 36 years (range 27-47) Gender: 97 (62%) females Ethnicity: 154 (99%) African American	SBP and DBP	Clinical and laboratory factors
Gordeuk	2008	USA	Patients from Sickle Cell Pulmonary	163 SCD patients	Retrospective cohort	Adults with haemoglobin SS or Sβ0	Relative Systemic Hypertension (RSH) (i.e., SBP 120–139 mm	Complications associated

			Hypertension Screening Study			thalassemia patients	Hg or DBP 70–89 mm Hg)	

Grell	1981	Jamaica	SCD clinic of the University Hospital in Jamaica.	70 SCD patients	Retrospective cohort	Participants were aged over 30 years. Gender: 29 males and 41 females. Age: age ranged from 30 to 69 years.	SBP and DBP	Age and gender
Homi	1993	Jamaica	SCD clinic of the University Hospital of the West Indies, Kingston, Jamaica.	342 SCD patients	Retrospective cohort	SCD patients aged 9.5 to 18.5 years.220 with homozygous SS and 122 with hemoglobin C	BP	Weight
Johnson	1981	USA	SCD clinics at the Los Angeles County-University of Southern	187 SCD patients	Cross-sectional	Gender: 81 males, 106 females Age: mean age was 29 years (range	Prevalence and severity of Hypertension in SCD	Haemoglobinopathy, age and advancing age

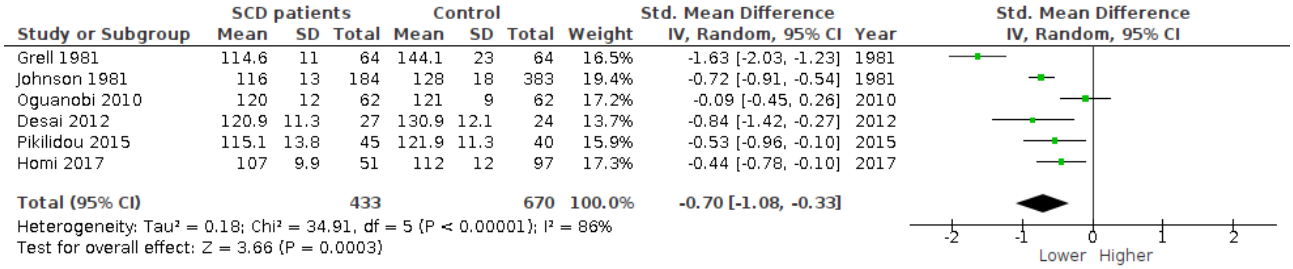
			California Medical Center and The Martin Luther King, Jnr General Hospital.			18-67). 130 sickle cell anaemia, 43 sickle cell-haemoglobin C, 15 had sickle cell thalassemia		
Strumph	2021	USA	Children's hospital	101 patients with SCD	Cross-sectional	100 SCD patients with age ranging from 5 to 21	Nocturnal Hypertension	Complication associated
Kuma	2018	Ghana	The Ghana Institute of Clinical Genetics	875 with homozygous or compound heterozygous SCD	Retrospective cohort	Participants were adults aged >18 years. Gender: 63 % were females. Age: Median age was 31 (range 23–44)	Prevalence of RSH	Complications associated

Lamarre	2013	(Guadeloupe, French West Indies)	SCD Center at the Academic Hospital of Pointe-a`-Pitre.	97 SCD patients	Cross-sectional	Adults aged ≥18 years. Gender: 43 male and 54 females	RSH and systematic Hypertension	Biological factors
Lemonne	2014	(Guadeloupe, French West Indies).	SCD Center at the Academic Hospital of Pointe-a`-Pitre.	89 SCD patients (sickle cell-hemoglobin C)	Cross-sectional	Gender: 40 males, 49 females Age: mean age was 38 ± 13 years	RSH	Blood Viscosity
Makubi	2017	Tanzania	Patient were identified at paediatric or haematology clinics or during hospitalization	1013 SCD patients	Retrospective cohort	Gender: 56% were females Age: Median age was 17 (range15–22)	RSH and systematic Hypertension	Any risk factors
Novelli	2014	USA	Multicentre international sickle cell trial (USA and	661 SCD patients	Retrospective cohort	Participants were adults: 500 HbSS and 161 HbSC, with mean age	Pulse pressure	Markers of haemolysis

			United Kingdom)			34 (range 25–45) and 41 (range 28 –51)		
Oguanobi	2010	Nigeria	Outpatient sickle cell clinics and the cardiac center of the University of Nigeria Teaching Hospital, Enugu, Nigeria	62 SCD patients	Cross-sectional	Participants were adults. Age: Mean age was 28.27(range 18-44)	Arterial BP	Age, gender, and anthropometric data
Pegelow	1997	USA	Cooperative Study of Sickle Cell Disease (CSSCD) at 23 clinical centres, USA	3,317 SCD patients	Cross-sectional	Participants included were children and adults (2 years old or older)	BP	Age, gender, anthropometric, clinical and laboratory data
Pikilidou	2015	Greece	SCD center	45 SCD patients	Cross-sectional	Participants were adults. Age: Mean age	BP	Arterial stiffness

						was 43 years (range 26-65) Body Mass Index (BMI):(24.1 ± 3.6 kg/m2 Gender: 30 males and 15 females		

(a)



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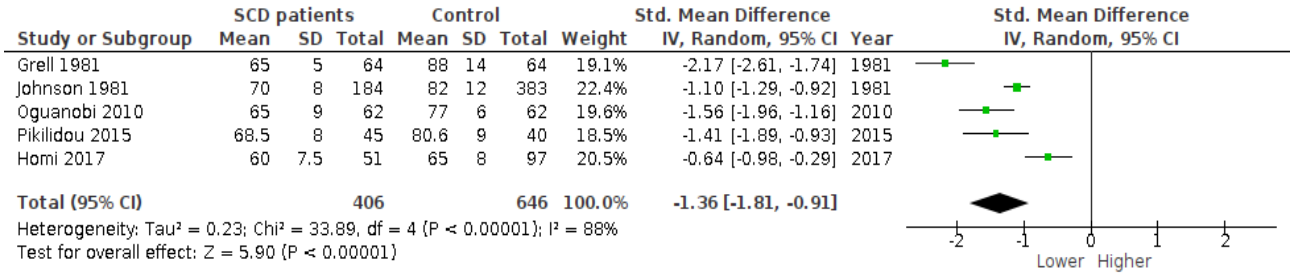

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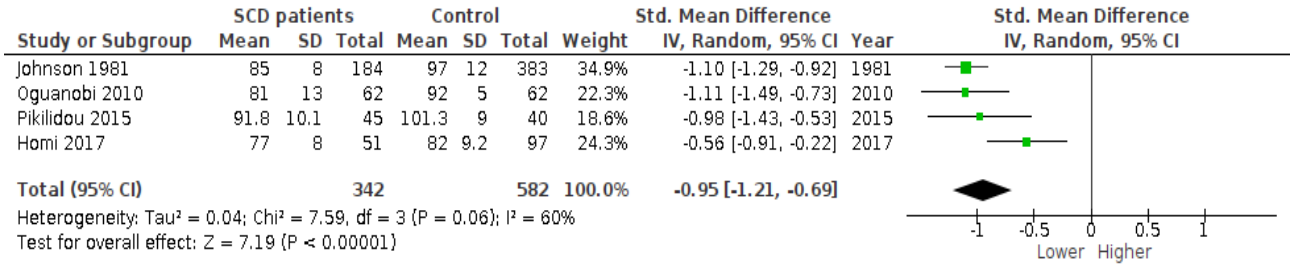
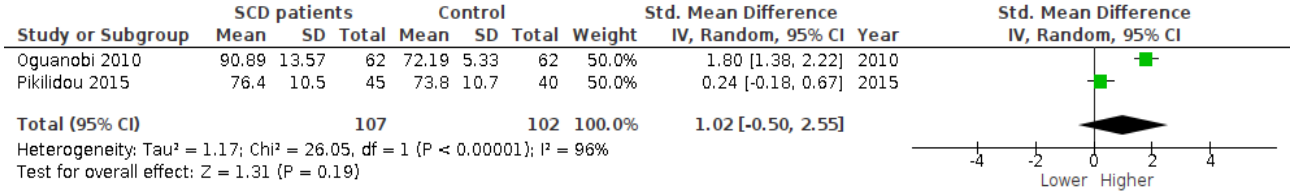


(d)



(e)

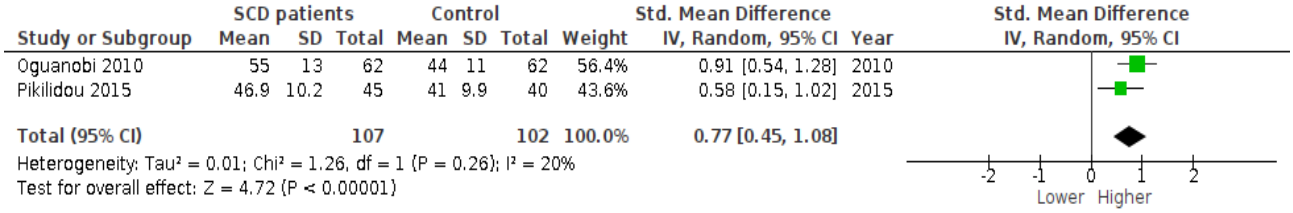


Figure 2: BP indices in SCD patients versus controls. (a) = systolic BP, (b) =diastolic BP, (c) =mean arterial pressure; (d) = Pulse rate, (e) = Pulse pressure.

3.3 Meta-analysis

Due to limited data, we could only conduct a meta-analysis on 7 studies that looked at BP in SCD patients versus controls. The meta-analysis results are presented in Figure 2. From the pooled results systolic, diastolic, and mean arterial pressure were lower in SCD patients compared to control groups, whereas and pulse rate and pulse pressure were higher in SCD patients compared to control groups. Heterogeneity (I2) ranged from 20 % to 96 %, indicating substantial heterogeneity between studies (Figure 2c). In the sensitivity analysis, we identified outliers[32]. The two studies substantially affected the heterogeneity of the pooled results. After excluding these two studies, the heterogeneity decreased from 96 to 60%. Furthermore, mean arterial pressure remained lower in SCD patients than in controls. We were unable to conduct a sensitivity/ subgroup analysis for pulse rate and pulse pressure, due to having fewer studies. The results that were not included in the meta-analysis are summarized in the narrative synthesis below.

3.4 Narrative synthesis

Although BP is a multifactorial condition, we present our results in categories of risk factors, namely demographic, anthropometric, biological, and genetic.

3.4.1 Demographic risk factors:

Age was assessed in four studies. Studies reported a lower BP in SCD patients than age- and sex-matched controls [33–36]. However, a common finding was that age is significantly associated with BP in SCD and that the BP increases rapidly with advancing age in SCD patients starting in the early twenties. Gender was assessed in five studies [34, 35, 37–39]. One study reported a significantly lower SBP in male compared to females SCD patients, but after adjusting for weight this difference disappeared [39]. The expected DBP rise with advancing age was not present in male participants [34]. Pulse pressure was higher in males of all age[10, 35, 40].

3.4.2 Anthropometric risk factors:

BMI was assessed in five studies [33, 36–38, 41]. Studies found that, after controlling for other covariates, SBP was significantly associated with BMI [36]. BMI is independently associated with Hypertension [33, 37, 41]. In addition, SCD patients with elevated BP had significantly higher body weight, waist, and neck circumference [38].

3.4.3 Biological risk factors:

After controlling for other covariates, four studies found that DBP was independently associated with the level of haemoglobin [33, 36, 41, 42]. DBP showed a positive correlation with age, Body Mass Index in those over 17, haemoglobin concentration in women, blood urea nitrogen in males under 18, and a negative correlation with estimated glomerular filtration rate (eGFR) in patients under age 17[41]. Triglycerides, increased haematocrit, and higher blood viscosity were independent risk factors for RSH in SCD patients [42, 43]. Furthermore, pulse pressure and a history of blood transfusion were independently associated with Systemic Hypertension in SCD patients [33]. Markers of haemolysis are associated with higher pulse pressure in SCD patients[43]. Augmentation index, a measure of arterial stiffness, was significantly higher in SCD patients compared with healthy controls [40]. On multivariate analysis, there was a significant correlation between SBP and age, BMI, history of Hypertension, and absolute neutrophil count (estimate: 21.18, P 5 0.023) [36].

3.4.4 Genetic risk factors:

We found limited data investigating the underlying genetic factors contributing to Blood Pressure variation particularly in SCD patients. We found one study which was a single genome-wide meta-analysis of SBP. It included 1617 African- American SCD children and identified a suggestive candidate locus at rs7952106 in DRD2 gene, and in MIR4301 gene[16].

3.4.5 Clinical complications and Blood Pressure

Gordeuk et al found that patients with SBP > 120 or DBP > 70mm Hg may represent relative Hypertension in SCD patient and identify patient at increased risk for pulmonary arterial Hypertension and renal dysfunction. When they stratified patients by tricuspid regurgitant jet

velocity (TRV) and serum creatinine concentration according to three Blood Pressure categories (Normal, RSH, Systemic Hypertension), TRV was 2.5 m/sec in 27% of the patients with normal BP, in 37% of the patients with RSH, and in 93% of the patients with Systemic Hypertension. Serum creatinine concentration was 1.0 mg/dL or higher in 7% of patients with normal BP, in 17% with the patients with RSH and in 50% of the patients with Systemic Hypertension. At the two-year follow up evaluation, Systemic Hypertension was observed in (16%) of those with RSH at initial evaluation. Over two years of follow-up, there were trends for more frequent progression to elevated TRV or creatinine values in RSH and Systemic Hypertension groups[14]. DeBaun et al, reported that higher baseline systolic Blood Pressure (P=0.018), and male sex (P=0.030) were statistically significantly associated with an increased risk of a silent cerebral infarcts[44]. Contrary to similar studies, Kuma et al, found a high prevalence of RSH and Hypertension with a relatively low frequency of renal insufficiency, and no association between RSH and stroke[35]. Novelli et al, found that higher pulse pressure was associated with markers of haemolysis, elevated serum creatinine, and with proteinuria in SCD patients[43]. Additionally, Kuma et al, found a higher pulse pressure in males of all ages compared to females [35]. A recent cross-sectional study looking at the association between twenty-four-hour ambulatory BP monitoring and cerebrovascular outcomes found an association between nocturnal Hypertension and a higher prevalence of silent cerebral infarcts (SCI), and stroke in children with SCD[45].

4. Discussion

To our knowledge, this is the first reported systematic review and meta-analysis that provides information on a wide range of risk factors associated with BP variation among SCD patients. Advancing age, gender, BMI, higher body weight, waist and neck circumference, haemoglobin, BUN, eGFR, triglycerides, greater haematocrit, higher blood viscosity, absolute neutrophil count, history of blood transfusion, and potentially variant in MIR4301 gene were independently associated with the risk of RSH or Systemic Hypertension in SCD patients. Furthermore, our pooled estimates illustrate that SBP, DBP and mean arterial pressure were lower in SCD patients. Pulse pressure and pulse rate were higher in SCD patients compared to the general population, with a heterogeneity ranging from low to high.

Previous studies explained that lower BP in SCD patients may potentially be due to renal tubular defects or hyposthenuria, increased Sodium and water loss [34, 41], lower BMI, and alteration in peripheral vascular resistance and vasodilation [39] which affects cardiac parameters including cardiac output, cardiac index, ejection fraction, and stroke volume. It is well documented that SCD patients with RSH or Systemic Hypertension are at increased risk of renal insufficiency, pulmonary Hypertension and silent cerebral infarction or stroke [14, 35, 42, 44]. It has also been reported that the risk of occlusive stroke increases with SBP. No significant relationship between DBP and occlusive stroke was observed. No association was found between BP and a history of painful vaso-occlusive crisis[37]. However, an association between increased triglycerides (a known risk factor for RSH and Systemic Hypertension) and occurrence of vaso-occlusive crises was observed. In addition, pulse rate and pulse pressure being higher in SCD patients is a concern because pulse pressure is a well-documented predictor of sudden death [12, 46]. Akingbola et al, reported that history of stroke was associated with higher SBP and BMI on univariate analyse ($p<0.006$)[47], however, Kuma et al, found a high prevalence of RSH and Hypertension with a relatively low frequency of renal insufficiency, and no association between RSH and stroke. The study attributed these differences mostly to selection bias and under documentation of complications in their study design [35].

In SCD patients, elevated pulse pressure has been reported to be associated with haemolysis, proteinuria and chronic kidney disease[35, 43]. Elevated TRV and serum creatinine concentrations in RSH and Systemic Hypertension in SCD [14] reinforces the importance of systemic Blood Pressure as a predictor of kidney deterioration in SCD and pulmonary arterial Hypertension.

This review also highlights a wide range of risk factors associated with BP variation in SCD patients and subsequently lead to complications described above. Previous studies show that risk of RSH and Systemic Hypertension in SCD patients increases with advancing age, with an onset in the early twenties [41, 48]. In addition, gender dependent factors such as nitric oxide production, which is higher in females compared to males, may explain the higher pulse pressure observed among SCD male patients[12]. Nitric oxide is thought to be important in maintaining vasomotor tone, limiting platelet aggregation, inhibiting ischemia-reperfusion injury, and modulating endothelial adhesion molecule expression [49]. Therefore, being an adult and/or male SCD patient could potentially increase the risk of developing RSH and Systemic Hypertension. Furthermore, SCD patients with elevated BP have significantly higher waist and neck circumference and elevated BMI and BP have

been reported to be associated with history of stroke [35, 38, 50]. Elevated BP has been reported to be associated with a history of stroke[47], higher prevalence of silent cerebral infarcts (SCI), and stroke in children with SCD[45]. Therefore, the ability to identify individuals prior to the development of cardiovascular complications is of paramount importance, particularly in SCD patients[50].

Further evidence of the role of biological factors in BP variation in SCD patients is increased haemoglobin, increased haematocrit or blood viscosity, triglycerides, history of blood transfusion and prior diagnosis of Hypertension[37]. These factors are also risk factors for RSH and Systemic Hypertension in SCD patients. The findings on genetic factors relating to BP in SCD patients are consistent with a previous review [51]. Since genetic factors were only investigated in one study, no solid inference can be drawn on whether the same genetic variants influence BP across different human populations. This highlights the urgent need for studies aimed at identifying specific SNPs associated with BP and cardiovascular complications in SCD patient populations, particularly in Africa.

Even though we conducted a comprehensive search and most of our included observation studies were low to moderate risk of bias, this review is not without limitations. When we pooled point estimates in a meta-analysis, we observed heterogeneity ranging from low to high, thus limiting our interpretation of pooled estimates. Contributors to this unexplained heterogeneity may be due to a wide range of study methodologies, study settings, populations, and periods studied in our included studies. Most of the included studies were from North America, which highlights a gap in comparable studies from lower income countries which carry the greatest burden of SCD. In addition, all the included studies recruited their study participants from SCD clinics or referral hospitals. Thus, these findings may not be generalisable to the whole SCD population. Nevertheless, the study still provides a comprehensive evaluation of risk factors associated with BP in a SCD clinic or hospital setting.

5. Conclusion

Blood Pressure is lower in SCD patients compared to general population, however, SCD patient with RSH or Systemic Hypertension are at increased risk of morbidity and mortality. In addition, risk factors such as age, gender, triglycerides, blood transfusion and haemoglobin level are significant determinants of BP variation in SCD patients. Interventions that consider these risk factors may potentially lower BP pressure in SCD patients and prevent developing severe complications.

6. Expert Opinion

The study provides a comprehensive evaluation of risk factors associated with BP in a SCD clinic or hospital setting. Despite study limitations, our study updates current research on a range of factors that may contribute to the risk of RSH and systematic Hypertension in SCD patients. Consequently, personalised early interventions aimed to lower elevated BP may improve life expectancy among SCD patients by inhibiting progression to pulmonary arterial Hypertension, advanced renal disease, and other vascular complications. However, high quality evidence from clinical trials is needed to guide initiation of therapy and treatment goals in SCD patients. Similarly, public health interventions such as population-based health education and lifestyle modification, within the cultural context, may play a role in preventing elevated BP in SCD patients. However, clinical trials and longitudinal studies should be conducted to confirm these hypotheses. Our systematic review identified methodological limitations and gaps in knowledge that should be addressed in future studies. Limited genetic studies have been done to identify SNPs associated with BP and cardiovascular complications in SCD patients, particularly in Africa. There is a gap in comparable studies from lower income countries which carry the greatest burden of SCD. Most importantly, there is a need for clinical trials that aim to determine whether BP interventions can be reduced associated complications in SCD patients as they are in the general population. Lastly, the current proposed cutoff values for RSH is defined as Systolic BP 120–139 mm Hg or diastolic BP 70–89 mm Hg, whereas Systemic Hypertension is defined as Systolic BP >= 140 mm Hg or diastolic BP >=90 mm Hg in all SCD patients. However, providing age specific values for RSH and Systemic Hypertension would make it easier for readers and allow comparison across studies.

7. Five-year view

A combination of genetic and large longitudinal cohort studies may be able to identify SCD patients who are at highest risk of cardiovascular complications. Over the next 5 years, we will hopefully have more genetic studies looking SNPs associated with BP and larger longitudinal cohort studies with scientifically rigorous methods in understanding risk factors associated with BP in SCD patients. The establishment of the SickleInAfrica consortium which aims to facilitate research in SCD, may provide more prospective observational studies on BP and SCD, particularly in Africa. Additionally, there is a need to establish the optimal BP threshold above which to initiate therapy for patients with SCD and to make comparison with earlier studies easy.

Authors' contributions

A.W. conceived the study. AN developed the protocol. AN and CO did the literature search, selected studies, and extracted relevant data. AN analysed and interpreted the data. AN issued the first draft of the paper. AN, CO, KB, VN, GM, AK and A.W. critically revised successive drafts of the manuscript. VN, GM, AK and A.W. supervised the project and compiled the revisions. AN is the guarantor of this review. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health (Award Number U24HL135600) and (Award Number 1U24HL135881)

Declaration of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or conflict with the subject matter or materials discussed in this manuscript apart from those disclosed.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Acknowledgements

Gill Morgan, Health Science Library, University of Cape Town, who assisted with developing the search strategy.

Chapter 4: Clinical characteristics and risk factors of Relative Systemic Hypertensionand Hypertension among sickle cell patients in Cameroon.

Synopsis: This chapter will present the findings on demographic, anthropometric, clinical and laboratory factors associated with RSH and Hypertension among SCD patients in Cameroon, one of the rare attempts from Africa.

4.1 Nguweneza A, Ngo Bitoungui VJ, Mnika K, Mazandu G, Nembaware V, Kengne AP and Wonkam A (2022) Clinical characteristics and risk factors of Relative Systemic Hypertension and Hypertension among sickle cell patients in Cameroon. Front. Med. 9:924722. doi: 10.3389/fmed.2022.924722

- **Nature of Publication:** Original Article
- **Journal/Publisher:** Frontiers in Medicine; Frontiers Media Publishing Group; ISSN: 2296-858X; Peer-reviewed.
- **Candidate contribution**: AW conceived the study. **Arthemon Nguweneza**, VN, GM, and AW made substantial contributions to the conception, design of the work, methodology, analysis, data interpretation, and wrote the final manuscript. **Arthemon Nguweneza** and GM analysed and interpreted the data. **Arthemon Nguweneza** issued the first draft of the paper.
- **Co-Authors Contributions:** AW conceived the study. AN, VN, KM, GM, VN, AK, and AW critically revised successive drafts of the manuscript. VN, GM, AK, and AW supervised the project and compiled the revisions. All authors have read and agreed to the published version of the manuscript.

Clinical characteristics and risk factors of Relative Systemic Hypertension and Hypertension among Sickle Cell Patients in Cameroon

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Abstract

Increased Blood Pressure (BP) has been associated with higher risk of stroke and mortality in Sickle Cell Disease (SCD). We investigated risk factors associated with Relative Systemic Hypertension (RSH) or Systemic Hypertension in SCD patients in Cameroon. Using R, Multivariate multinomial logistic regression modelling was used to examine the effects of the demographic, anthropometric, clinical, and laboratory factors to determine risk factors. A total of 815 individuals with SCD, including 380 (46.6%) males were analysed. At baseline, the median age [interquartile range] was 18.0 [12.0–25.0] years, ranging from 3 to 66 years. Approximately three-quarters of the patients (n = 645; 79.1%) had normal BP, 151 (18.5%) had RSH and 19 (2.3%) had Hypertension. Age (P <.001) and gender (P = 0.022) were significantly different across the BP categories. Weight (P <.001), height (P <.001), BMI (P <.001), pulse pressure (P= 0.020), history of stroke (P= 0.012), haemoglobin level (P = 0.002), red blood cell count (P = 0.031), creatinine (P <.001), and (estimated glomerular filtration rate) eGFR (P = 0.002) was also significantly different across the three BP categories. After adjustment, the significantly associated factors of RSH in the SCD patients were age [OR = 1.03, 95% CI = 1.01-1.06), p <.010], male gender [OR = 1.54, 95% CI = 1.04- 2.27), P=0.029], BMI [OR = 1.10, 95% CI = 1.04-1.17), P=0.001]. After adjustment, the independent variables significantly associated factors of Hypertension in the SCD patients were age [OR = 1.05, 95% CI = 1.01–1.10), p = 0.034], male gender [OR = 3.31, 95% CI = 1.04–10.52), p = 0.042], BMI [OR = 1.14, 95% CI = 1.01-1.29), p = 0.027]. Creatinine was significantly associated with RSH [OR =1.31(1.05-1.63, p = 0.016]. SCD patients with RSH or Hypertension maybe at increased risk of renal dysfunction. We found the relatively high prevalence of RSH and Hypertension (20.8%) in SCD patients in Cameroon. Tailored interventions that consider major risk factors (age, gender, and BMI) may lower BP pressure and prevent severe complications.

Keywords: Relative Hypertension; Hypertension; risk factors; sickle cell disease, Cameroon; Africa

4.2 Introduction

Sickle cell disease (SCD) patients, generally, have lower systolic, diastolic, and mean Blood Pressure (BP) compared to age and sex-matched controls [14, 41]. There are no specific recommendations proposed regarding the defining criteria (and management) of Hypertension in SCD patients. The lack of recommendations is a major concern since increased BP has been associated with higher risk of stroke and mortality in SCD patients, even in a range of systolic and diastolic BPs (SBP, DBP) that are considered relatively normal for the general population (i.e lower than 140 mmHg)[14].

Blood Pressure (BP) is a potential modulator of clinical severity in SCD patients, recent studies showed that Relative Systemic Hypertension(RSH), defined as BP120-139/70–89 mmHg, and Systemic Hypertension (BP >140/>90), considerably increased the risk of pulmonary Hypertension and renal dysfunction[14]. Previous studies have reported demographic, biological, anthropometric, and genetic factors to be associate with BP in SCD patients[16, 36, 37, 42, 43, 52]. BP is a heritable trait with estimates of heritability indicating that 30–70% of the trait variance is attributable to genetic variation and a recurrent deleterious and loss of functions mutation with genes associated with lowering BP has been recently associate with long survival in SCD in Africa[15].

Identification of risk factors associated with BP variation in different populations is key to controlling BP, as well as preventing associated causes of mortality in SCD patients. We investigated risk factors associated with RSH or Systemic Hypertension in SCD patients in Cameroon to gain insight into the pathophysiology of BP variation in this disease in an African setting.

4.3 Patients and Methods

4.3.1 Ethical approval

A proposal was submitted to the University of Cape Town, Faculty of Health Sciences Human Research Ethics Committee, Cape Town, South Africa (HREC/REF: 142/2022). All patients older than 18 years signed consent forms, while informed consent was given by the parents or guardians for participants younger than 18 years old, in accordance with the Declaration of Helsinki. This

study was approved by the National Ethical Committee of the Ministry of Public Health of Cameroon (No 193/CNE/SE/15).
Written and signed informed consent forms were obtained from adult participants and parents/guardians of minor patients. An assent was also obtained from the participants of more than seven years old.

4.3.2 Participants' recruitment

All SCD patients with complete socio-demographic, clinical, laboratory variables, and complete systolic and diastolic BP measurements were included in the study. The data were obtained from a cross-sectional study conducted in Cameroon from May 2016 to July 2018. The data were collected from nine hospitals from five cities in Cameroon, including Yaoundé, Douala, Bafoussam, Bertoua, and Maroua. Patients who have not experienced a painful crisis a month before, and who had not received a blood transfusion in the past 6 months, were recruited irrespective of age and gender.

4.3.3 Use of variables

Dependent variables: SCD patients with a SBP within the range of 120–139 mmHg and/or DBP within the range of 80–89 mmHg is defined as having RSH. Systemic Hypertension is further defined as SBPs greater than 140 mmHg or DBPs greater than 90 mmHg. Participants who had incomplete/out of range BP readings were excluded from the analysis.

Independent variables: Information on demographics, including age, residential location, sex, ethnicity, educational level, marital status, and household income status, was collected using a standard questionnaire involving the household and individual levels. Clinical information and laboratory information were also collected. Those who had incomplete/out of range relevant information such as age, gender, BMI, demographic, clinical, laboratory information were also excluded from the dataset.

4.3.4 Statistical analysis

All our analysis was analysed using R (version 4.0.2). Continuous variables were presented as median and interquartile range (IQR) and categorical variables as percentages (%).

Categorical variables were compared using X^2-test or Fisher exact test if the expected count in a cell was less than five while continuous variables were compared according to BP category with the Kruskal–Wallis test.

Multivariate multinomial logistic regression modelling was used to examine the effects of the demographic, anthropometric, clinical, and laboratory factors to determine the potential independent risk factors for RSH and Systemic Hypertension.

A final model was created that included all the predictors and interactions that were significantly associated at the level of $p < 0.05$. The findings presented as crude and adjusted odds ratios with their 95% confidence intervals (CI).

4.4 Results

4.4.1 Baseline characteristics

Table I: Shows the demographic, anthropometric, clinical and laboratory characteristics of the BP categories. Our analysis included 815 individuals with SCD, of whom 645 (79%) had normal BP, 151 (19%) had RSH, 19 (2%) had Systemic Hypertension. 380 (46.6%) were males. At baseline, the median age [interquartile range] was 18.0 [12.0–25.0] years, ranging from 3 to 66 years. Approximately three-quarters of the patients (645 or 79.1%) were normal BP, 151 (18.5%) had relative Hypertension and 19 (2.3%) had Hypertension. Age (P <.001) and gender (P = 0.022) were significantly different across the BP categories, with age increasing with BP. Weight (P <.001), height (P <.001), BMI (P <.001), pulse pressure (P= 0.020), history of stroke (P= 0.012), haemoglobin (P = 0.002), red blood cell count (P = 0.031), creatinine (P <.001), and eGFR (P = 0.002) were also significantly different across the three BP categories.

4.4.2 Univariate and multivariate analysis

The normal BP group versus RSH group: Among SCD patients, univariate analyses indicated that these variables were significantly more common risk factors for higher BP values among patients with RSH than those with normal BP: Age (P<.001), patients > 18 years (P<.001), weight (P<.001), height (P<.001), BMI(P<.001), pulse pressure (P=0.046), creatinine (P<.001), eGFR (P<.001) and haemoglobin (P=0.020) (Table II). Multivariate analyses found that age [OR = 1.02, 95% CI = 1.01-1.05), p =0.021], creatinine [OR = 1.310, 95% CI = 1.05- 1.63, P=0.016], BMI [OR = 1.09, 95% CI = 1.03-1.16), P=0.002] were independent risk factors for high BP values in SCD patients with RSH compared with SCD patients with normal BP values (Table III).

The normal BP group versus Systemic Hypertension group: Among SCD patients, univariate analyses indicated that these variables were significantly more common risk factors for higher BP values among SCD patients with Hypertension than those SCD patients with normal BP: Age (P<.001), patients > 18 years (P = 0.013), male gender (P =0.019), weight (P<.001), BMI(P<.001), pulse pressure (P=0.019) , haemoglobin (P<.001), creatinine (P<.001) and Red blood cell count (P=0.03) (Table 2). Multivariate analyses found that age [OR = 1.05, 95% CI = 1.01-1.10), p = 0.034], male gender [OR = 3.31, 95% CI = 1.04–10.52), p = 0.042], BMI [OR = 1.14, 95% CI = 1.01-1.29), p = 0.027] were independent risk factors for higher BP values in SCD patients with Hypertension compared with SCD patients with normal BP values (Table III).

Additionally, figure 1 Illustrates the relationship between BP and age, gender, BMI. As age increases the probability of SCD patients having RSH or Hypertension increases (Figure 1a). Secondly, Males have a higher probability of having RSH or Hypertension than females among SCD patients (Figure 1b). For every increase in BMI units, the probability of having RSH or Hypertension increases among SCD patients (Figure 1c). Lastly, for every increase in creatine units, the probability of having RSH or Hypertension increases among SCD patients (Figure 1d).

4.5 Discussion

This study determined the role of demographic, anthropometric, clinical and laboratory factors associated with RSH and Hypertension among SCD patients in Cameroon, one of the rare attempts from Africa. The main findings, from this relatively large dataset are as follows. Approximately one quarter of our 815 SCD patients were classified in either RSH or Systemic Hypertension group. At baseline, we observed statistically significant differences in age, gender, weight, height, BMI, pulse pressure, a history of stroke, haemoglobin, and red blood cell count across our three BP groups (normal BP, RSH, Hypertension). We found that age, BMI, creatinine, and male gender were independently associated with an increased risk of RSH and Systemic Hypertension after adjusting for other variables.

The nearly 19% prevalence of RSH reported in this study was similar to that of 17% reported in studies from North America by Becker et al.[53], and Bodas et al.[54]. In similar setting as our study, RSH was lower to that of 45% reported by Benneh Akwasi Kuma et al.[35] and 44% found by Makubi et al.[33], the participants in these studies were adult patients, whereas our study included both paediatric and adult patients. The 2% prevalence of Systemic Hypertension reported in this study from Cameroon also agrees with previous reports from both high and low incomes settings, which have reported the prevalence of Systemic Hypertension in SCD patients to be lower than that of the general population (2%-8% versus 28%, respectively)[33, 55, 56]. Potential explanations of low prevalence of RSH and Systemic Hypertension in SCD patients include Sodium and water wasting due to the medullary defect[57], systemic vasodilatation compensating for microcirculatory flow disturbances[56], increased production of prostaglandins and nitric oxide[58], reduced vascular reactivity[57], and premature deaths that remove those individuals whose BP might reach hypertensive levels in middle adulthood[33].

Unsurprisingly, this study also found that age was significantly associated with BP in SCD patients, SCD patients with RSH and Systemic Hypertension were older than SCD patients with normal BP values suggesting that advancing age contributed to their higher BP values. This finding corroborates with previous reports in developed countries[36, 41]; and in Africa[47], that reported that BP rapidly increases age in SCD patients. With the improved survival of patients with SCD patients, the incidence of RSH or Systemic Hypertension is expected to rise, thus screening and

awareness are necessary to prevent the expected complications, in all part of the world. Indeed, mortality in adult with SCD in the USA and other high-income countries have not changed over the past four decades, mostly dues to debilitating and severe cardiovascular complications[59]. Most of the previous data is from 18-year-olds. However, younger patients may already show elevated BP and risk for complications. In addition, previous studies have highlighted that BP increases rapidly with age, starting in early twenties in people with SCD [41]. However, previous studies have also indicated that elevated BP is not uncommon in children with SCD [54, 60]. In the general population, the clinical definition of high BP varies between paediatric patients and adults [61]. Whereas, in literature, the definition of RSH and Systemic Hypertension has not been established for paediatric or adolescent SCD patients. There is a need to establish age-specific values for RSH and Systemic Hypertension for SCD patients. Such an approach would not only improve our understanding of BP in SCD patients but also enable more accurate comparisons across studies. Pegelow et al[41] demonstrated that BP values were higher in males than in females, which is consistent with our results showing that male gender is independently associated with RSH and Systemic Hypertension in SCD patients. This gender disparity in BP is likely due to gender-related differences in SCD biology or health-seeking behaviour between genders[40, 62]. For instance, older males with elevated BP relative to the SCD population are at increased risk of stroke than age-matched females[35]. SCD males have higher pulse pressure, a predictor of all-cause mortality, than age-matched SCD female patients[35, 40] which further highlights the risk of adverse outcomes associated with RSH and Systemic Hypertension in males. Another study suggests that regular medical visits are critical for improving Hypertension awareness among young adults and reducing gender disparities in cardiovascular health[62].

Consistent with previous studies, Oguanobi et al.[38] in Nigeria, and Pegelow et al.[41] who reported that BMI correlates positively with SBP and DBP and Homi et al. who reported that low weight is a risk factor for low BP. In this study, we found that BMI correlates positively with BP, and BMI was independently associated with RSH and Systemic Hypertension among SCD patients. Suggesting that a higher BMI in SCD patients with RSH or Systemic Hypertension may contribute to their higher BP values compared to the SCD patients with normal BP. SCD patients have lower BMI compared to general population but increased BMI in SCD patients has potential to modulate BP[36]. In addition, the prevalence of obesity in patients with SCD seems to be on the increase.

Obesity is a risk factor for other diseases, including, but not limited to, type 2 diabetes, Hypertension, sleep apnea, cardiovascular disease[63]. These diseases, in turn, worsen the clinical picture of SCD and increase the frequency of vaso-occlusive crises (VOCs)[63]. Because of the clinical importance as well as public health importance of RSH or Systemic Hypertension, the ability to identify otherwise normal BMI is of paramount importance, particularly in SCD patients.

Furthermore, measuring BMI alone, in SCD, is sufficient to screen for adiposity and obesity. Previous reports show the body composition of SCD patients with normal mean BMI (22.6 kg/m2), showed a 32.6% fat composition, indicating high levels of adiposity. Since fat accumulation and adipocyte secretion are responsible for many hormonal changes playing a role in the development of vascular dysfunction and Hypertension in the general population, this could be the case in SCD patients too, even if BMI values are normal. Therefore, further studies are needed to better understand the relationship between BMI; hormonal status and BP in SCD.

Previous studies reported that SCD patients with SBP 120–139 mm Hg or DBP 70–89 mm Hg had elevated levels of creatinine compared to SCD patients with SBP <120 mm Hg and DBP <70 mm Hg.[2] In this study we found that creatinine was independently associated with RSH. Additionally, SCD patients in RSH and Systemic Hypertension group had a higher creatinine compared to SCD patients in the normal group. Suggesting that SCD patients with RSH or Hypertension are at increased risk of renal dysfunction. Longitudinal studies are needed to better understand temporal relationship between renal dysfunction and RSH.

Previous studies found increasing haemoglobin, blood viscosity and blood transfusion to be independent risk factors for RSH or Hypertension in SCD patients. However, in this study we did not find these factors to be significantly associated with BP among SCD patients[64].These observed differences may be explained by differences in study design, patient's clinical characteristics and thresholds used to define RSH or Systemic Hypertension.

Our participants were recruited form referral hospitals. Thus, the findings may not be representative of RSH or Systemic Hypertension seen in a community. Nevertheless, our analysis is based on the large and well-characterized homozygous study population in a resource-limited country. Therefore, these findings expand the understanding of risk factors for RSH and Systemic Hypertension in SCD

beyond what has been reported from resource-limited settings.

The exclusion of incomplete records with missing BP might have introduced some bias. Additionally, BP was measured at single time point which might have increased some patients' likelihood of developing white coat Hypertension. Previous studies have highlighted the importance of 24-hour ambulatory BP monitoring in diagnosing masked Hypertension[65].

The inability to follow up the cohort as a longitudinal study is a limitation.

In conclusion, this study found evidence of the prevalence of RSH and Hypertension in the SCD patients in Cameroon. Age, male gender, BMI was found to be independently associated factors of RSH and Hypertension in the SCD patients in Cameroon. Tailored Interventions that consider these risk factors have potential to lower BP pressure in SCD patients and prevent developing severe complications.

Acknowledgments

Sickle Africa Data Coordinating Centre (SADaCC) team, Human genetics Division, Department of Pathology, Health Science Faculty, University of Cape Town.

This work was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health (Award Number U24HL135600) and (Award Number 1U24HL135881).

Authors' contributions

AW conceived the study. AN, VJNB, GM, AW made substantial contributions to the conception, design of the work, methodology, analysis, data interpretation and wrote the final manuscript. AN, GM analysed and interpreted the data. AN issued the first draft of the paper. AN, VJNB, KN, GM, VN, AK and A.W critically revised successive drafts of the manuscript. VN, GM, AK and A.W supervised the project and compiled the revisions. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

Table 1. Baseline demographic, anthropometric, clinical and laboratory characteristics of Cameroonian SCD patients by BP levels.

Characteristics	All (n/N, %)	Normal n (n=, %) or Median (IQR)	RSH (n=, %) or Median (IQR)	Hypertension (n=, %) or Median (IQR)	P-value
Demographics					
Age, years	815/815 (100.0)	17.0 (11.0–24.0)	22.0(18.0–28.0)	24.0 (18.0–40.5)	<.001
Aged less than 18	373/815 (45.8)	336/645 (52.0)	33 /151 (22.0)	4/19 (21.1)	<.001
Aged older or equal 18	442/815 (54.2)	309/645 (48.0)	118/151 (78.0)	15/19 (78.9)	
Gender, male	380/815 (46.6)	289/645 (44.7)	77/151 (51.3)	14/19 (73.7)	0.022
Anthropometric and Clinical					
Weight	815	46.0 (30.0–55.0)	56.0 (50.0-62.0)	60.0 (50.0–68.0)	<.001
Height	815	1.58(1.37-1.67)	1.67 (1.60–1.73)	1.64 (1.58–1.77)	<.001

Body Mass Index (BMI)	815	18.0 (16.0 -20.0)	20.0 (18.0-22.0)	21.0 (19.5- 23.0)	<.001
Pulse pressure[a]	789	91.0(81.3–101)	88.0 (80.3–96.8)	84.0 (76.0–92.5)	0.020
History of stroke[a]	28/803 (3.5)	20/637(3.1)	5/147 (3.4)	3/19 (15.8)	0.012
History of kidney disease[a]	82/807 (10.2)	69/640 (10.8)	10/148 (6.8)	3/19 (15.8)	0.246
History of Pulmonary Hypertension[a]	68/807 (8.4)	52/640(8.1)	13/148 (8.8)	3/19 (15.8)	0.4888
History of transfusion[a]	634/813 (78.0)	504/644(78.3)	118/150(78.7)	12/19 (63.2)	0.286
HydroxuriAfrican-american	72 /807 (8.9)	58/640 (9.1)	14/148 (9.5)	0/19 (0.0)	0.381
Biological data					
Haemoglobin (g/dl)	797/815	7.60(6.80-8.50)	8.00(7.10–8.90)	8.10(7.80–10.8)	0.0020
Haemoglobin F (%)	794 /815	6.40(3.80–11.6)	6.30(3.40–11.5)	8.35(4.73–12.3)	0.459
White blood cell count (10^9/l)	797/815	10.3(7.80–13.0)	9.80(7.77–12.6)	9.45(8.30–11.3)	0.637
Mean corpuscular volume (fl)	798/815	88.0(82.0–95.0)	89.0(83.0–95.0)	85.0(77.5–92.0)	0.360
Red blood cell	794 /815	2.70(2.30-3.13)	2.83(2.40–3.19)	2.87(2.63–3.38)	0.031

count					
Creatinine (mg/dl)	770/815	0.45(0.37-0.60)	0.60(0.40-0.78)	0.65(0.50-0.87)	<.001
(Estimated glomerular filtration rate) eGFR	760/815	175 (151-204)	158 (136-178)	150 9119-182)	0.002

[a]Total number of children may differ because of missing data; IQR, interquartile range.

Table 2. Univariate multinomial logistic regression analyses of factors associated with RSV and Hypertension among SCD patients in Cameroon (reference: Normal BP)

Factors	RSH vs Normal		Hypertension vs Normal BP	
	cOR (95% CI)	P-value	cOR (95% CI)	P-value
Demographics				
Age*	1.05 (1.03–1.06)	<.001	1.08(1.04–1.12)	<.001
Aged less than 18 (ref.)				
Aged older or equal 18*	3.89(2.56–5.89)	<.001	4.07 (1.34–12.41)	0.013
Gender, female (ref.)				
Gender, male*	1.28 (0.89–1.83)	0.170	3.45(1.22-9.69)	0.019

Anthropometric and Clinical				
Weight* (Kilograms)	1.05 (1.04-1.07)	<.001	1.07(1.03-1.11)	<.001
Height (meters)*	76.1 (21.77-265.89)	<.001	15.9(0.96–264.38)	0.054
Body Mass Index*	1.16 (1.10–1.23)	<.001	1.22(1.11–1.35)	<.001
Pulse pressure*	0.98 (0.97–1.00)	0.046	0.95(0.92–1.00)	0.019
History of stroke* vs No (ref)	1.03 (0.38–2.77)	0.957	5.59(1.51–20.66)	0.010
History of kidney disease vs No (ref)	0.60(030–1.19)	0.145	1.57(0.44–5.54)	0.480
History of pulmonary Hypertension vs No (ref)	1.11(058–2.10)	0.749	2.20(0.26-7.78)	0.223
History of transfusion vs No (ref)	1.00 (0.65–1.54)	0.985	0.47(0.18–1.23)	0128
Hydroxuria	1.00 (0.53–1.89)	0.992	0.00(-7.07-1.7)	0.762

Biological data				
Haemoglobin (g/dl) *	1.12(1.01–1.23)	0.020	1.36 (1.14–1.61)	<.001
Haemoglobin F (%)	0.99 (0.97-1.03)	0.746	1.04 (0.97-1.11)	0.226
White blood cell count (10^9/l)	0.99(0.95–1.09)	0.782	0.95(0.85.1.08)	0.485
Mean corpuscular volume (fl)	1.00 (1.00-1.02)	0.528	1.00(0.94–1.01)	0.062
Red blood cell count*	1.23(1.00–1.53)	0.061	1.9(1.24–2.94)	0.03
Creatinine (mg/dL)	1.24(1.15-1.34)	<.001	1.38(1.17-1.62)	<.001
eGFR	1.00(0.98-1.00)	<.001	0.99(0.98-1.00)	0.007

cOR: Crude odds ratio; CI: Confidence interval; *denotes significant at the 5% level

Table III: Multivariate multinomial logistic regression analyses of factors associated with RSV and Hypertension among SCD patients in Cameroon (reference: Normal BP)

Factors	RSH vs Normal BP		Hypertension vs Normal BP	
	aOR (95% CI)	P-value	aOR (95% CI)	P-value
Age, years*	1.02(1.01-1.05)	0.021	1.05(1.01-1.10)	0.034
Gender, male vs female (ref.) *	1.20(1.10-1.80)	0.372	3.31(1.04-10.52)	0.042

Body Mass Index (BMI)*	1.09(1.03-1.10)	0.002	1.14(1.01-1.29)	0.027
Pulse pressure	1.00(0.98-1.01)	0.679	0.98(0.93-1.01)	0.289
History of stroke vs No (ref)	0.89(0.30-2.56	0.830	2.24(0.42-11.79)	0.339
Creatinine (mg/dL	1.31(1.05-1.63)	0.016	1.26(0.75-2.09)	0.373
Haemoglobin (g/dl)	1.00 (0.70 – 1.40)	0.234	0.88(0.35 – 2.47)	0.572
Red blood cell count	1.02(0.87 – 1.13)	0.684	1.15 (0.81 – 2.13)	0.145

aOR: Adjusted odds ratio; CI: Confidence interval; *denotes significant at the 5% level. All the variables that were significant (P< 0.005) in the univariate analysis were entered in our model.

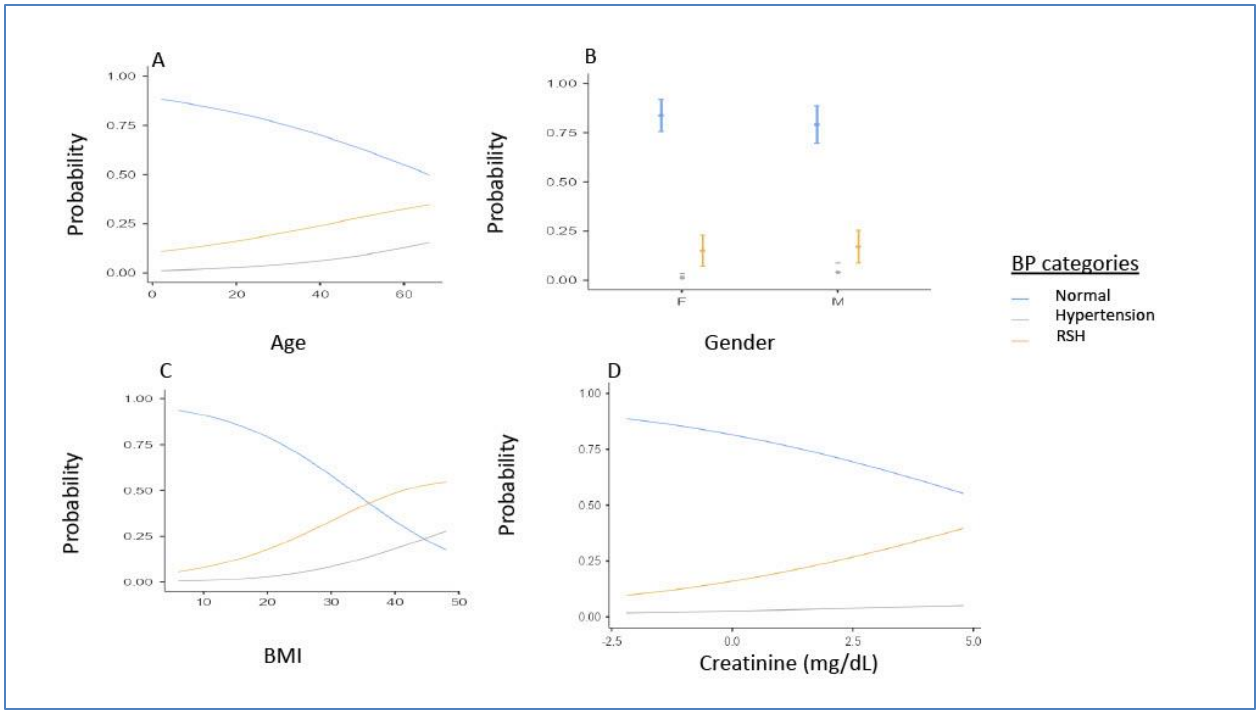

Figure 1. Estimated Marginal Means of a) Age, b) Gender, c) BMI by BP categories, d) creatine (mg/dL). Abbreviations: BP, Blood Pressure; BMI, Body Mass Index; RSH, Relative Systemic Hypertension; F, Females; M, Males.

Chapter 5: Genome-wide Association of Blood Pressure variation and Body Mass Index among Sickle Cell Disease Patients in Cameroon

Synopsis: This chapter will present the findings on genetic variants associated with RSH and Hypertension among SCD patients in Cameroon, one of the rare attempts from Africa.

Genome wide association of Blood Pressure variation and Body Mass Index among Sickle Cell disease patients in Cameroon

Abstract:

Introduction: Blood Pressure (BP) is a heritable, known risk factor for stroke and cardiovascular disease, which are major causes of global mortality and morbidity. We aimed to identify genetic variants associated with BP using genome-wide association study (GWAS) in Cameroon SCD patients.

Methods: Using the H3Africa Array enriched with variants from Genomes of Sub-Saharan individuals, GWAS was conducted using linear mixed model regression to account for population structure. In Cameroonian cohort (discovery cohort), linear regression analysis was used to assess variants associated with BP. For replication, we examined these variants in cohorts of SCD patients from Nigeria and Senegal. A meta-analysis was performed using summary statistics from the Cameroonian cohort and Silent Infarct Transfusion (SIT) cohort. The GWAS summary statistics were then annotated by the online annotation software called Functional Mapping and Annotation (FUMA).

Results: Based on a GWAS analysis of 556 patients (aged 18.0 (16.0-21.0) with data on SBP and DBP, we detected two SNPs that were statistically associated with SBP: (rs146072506, p=4.88915e-08) located in *RP11-727A23.5* on chromosome 11 and (rs35715722, p= 3.40829e-08) located in *DLGAP1* gene on chromosome 18. Two other SNPs located in the intergenic regions on chromosome 11 were also statistically associated with SBP. For DBP, the only significant SNP was in an intergenic region. For 615 patients (aged 22.0 (18.0–28.0) with data for BMI, we found one SNP that was statistically

associated with BMI, (rs186536474, p=8.90x10-09) located on gene *GPR39* on chromosome 2. Identified variants in intergenic regions may play a role in modulating the regulation of genes involved in BP variations. Interestingly, the statistically significantly associated variants for SBP, DBP and BMI are African specific. For SBP, we validated SNPs from the Cameroon SCD cohort by the combining summary statistics from the Cameroon SCD cohort, with an African America SCD cohort, i.e., Silent Infarct Transfusion Trial (SITT) cohorts. Based on the summary statistics, the P-values for 3 variants associated with SBP were all suggestive around e-07 after adjusting for false discovery rate (FDR), indicating the potential transferability of our results to other cohorts. With targeted variants analysis, rs78291268 and rs62640055 are replicated at the nominal P values ($P<0.02$) in The Nigerian Cohort for BMI. Similarly, rs1385729 and rs74952753 ($P<0.002$) are replicated in the Senegalese cohort for BMI. However, these results are not confirmed after adjusting for false discovery rate (FDR).

For *In silico* functional investigations, after performing genetic analysis and Gene-set analysis with Functional mapping and annotation (FUMA) none of the identified genes or gene-set reached a significant threshold. Additionally, GTEx's tissue expression analysis of 53 specific tissue types found that the expression in the adrenal gland had the lowest p-value of 0.004 for SBP, the brain hypothalamus had the lowest p-value of 0.082 for DBP, and the breast mammary tissue had the lowest p-value of 0.027 for BMI. The results suggest a need to increase sample size and possibly additional African cohorts for further exploration to elucidate functional implications of our findings.

Conclusion: This study, with an African Specific GWAS array, has identified suggestive genetic loci and genes, with African specific variants, that may influence BP and BMI in individuals with SCD from Africa (Cameroon). This study also highlights the importance of studying disease relevant phenotypes in a large homogenous population.

5. 1 Background

Blood Pressure (BP) is a known risk factor for stroke and cardiovascular disease, which are major causes of global mortality and morbidity [66]. Studies have shown that a significant portion of the variance in BP is due to environmental and genetic contributions, with heritability estimates ranging from 30-70%[67]. However, the genetic variation identified to date, in the general population,

explains only ~2%[67, 68].

Despite the identification of several genetic markers associated with BP or through large-scale analyses of candidate genes, linkage studies, and genome-wide association studies (GWAS)[67, 75–84], the genetic variation identified to date explains only a small fraction of the variance in BP. Most GWAS have been conducted on individuals of European ancestry, and studies based on African ancestry participants comprised mainly of African Americans[85]. There are multiple causes of BP variability, including genetics and certain behavioural factors like diet, lifestyle, and obesity [86–90], which can differ among different racial and ethnic groups. A large proportion of BP genetic variation in African population remains unaccounted for, which justifies the need for genetic studies of BP in Africa, and no GWAS study has been reported for a relatively large-scale study for SCD in Africa.

Similarly, Body Mass Index (BMI), often used as a measure of obesity, is influenced by genetic and environmental factors. Obesity is a well-established risk factor for cardiovascular diseases. Around 40–70% of inter-individual variability in BMI has been attributed to genetic factors[69–71] understanding its genetic underpinnings maybe crucial for effective prevention and management cardiovascular complications.

While studies have identified genetic markers associated with BP and BMI in diverse populations, there remains limited research focusing on African populations, particularly in SCD patients. In addition, the interplay between genetic factors, BMI, and BP in SCD patients remains poorly understood. Elucidating the genetic architecture of BMI may provide insights into the mechanisms underlying BP variation in SCD.

In the clinical context of SCD, BP may be a potential modulator of clinical severity in patients. Recurrent deleterious and loss of function mutations with genes associated with lowering BP, e.g. variants in *CLCN6,* have been recently associated with long survival in SCD in Cameroon (Africa) [15]. Rare mutations in renal salt regulation genes have been linked to these differences in BP [72–74], but these mutations are too rare to explain a substantial proportion of inter-individual BP variation in the general population. Additionally, demographic, biological, and anthropometric factors have been reported to be associate with BP in SCD patients[64, 91]. Nevertheless, BP is

generally lower in SCD patients compared to the general population[41]. Thus, description of Relative Systemic Hypertension (RSH) for SCD patients is a BP ranging from 120–139/70–89 mmHg, and Systemic Hypertension for SCD patients with BP above 140/90 mmHg. Understanding the aetiology of BP variation in SCD patients, assessing up to date evidence is key to controlling BP variation. Additionally, early prediction of the severity could lead to better prevention and treatment of SCD and its associated causes of mortality. Herein, we investigated (clinical, genetic, and epidemiological) risk factors associated with BP variation in SCD patients in Cameroon to gain insight into the pathophysiology of BP variation in this disease in an African setting. In addition to exploring genetic factors associated with BP variation in patients with SCD, this study also investigates potential associations between genetic variants and BMI.

5.2 Materials and Methods

Ethical approval:

This study was approved by the University of Cape Town, Faculty of Health Sciences (HREC/REF: 142/2022). Details on Ethical considerations have been discussed in section 4.3.1.

Study participant and recruitment:

The study participants were SCD patients who had complete socio-demographic, clinical, and laboratory data, as well as complete systolic, diastolic BP, and BMI measurements. Our discovery cohort was SCD patients from Cameroon. The characteristics of participants in our discovery cohort have been discussed previously [91]. We investigated a total of 1083 patients' samples that passed quality control (QC) in our discovery cohort. We used a SCD cohorts from Nigeria and Senegal, as the replication cohorts, for targeted variants of interest, with a total of 246 samples were included in the combined dataset for the replication analysis.

Phenotypic assessment

Single measurements of SBP, DBP and BMI was obtained at participants' visit at the clinic. Our dependent variables were BMI, SBP and DBP (analysed as quantitative traits). The effect of each SNP on BP level was assessed by adjusting for age, sex, BMI, and top 10 principal components.

Genotyping, Quality control, imputation, and principal component analysis

The DNA samples of 1142 study participants were genotyped using the Human Heredity and Health in Africa (H3Africa) Consortium Illumina chip, developed from whole genome enriched for common variants in sub-Saharan Africans with 3280 individuals from 17 African countries (https://www.h3abionet.org/h3africa-chip) [92]. QC procedures[93] were implemented using PLINK to identify and remove any potential technical and genetic factors that could skew the results of the GWAS. QC measures included removing individuals who were not from Cameroon or who did not report their ethnicity. Additionally, any individuals with inconsistent sex information, such as discordance between self-reported and genotyped sex or discrepancies in sex information obtained by computing X chromosome inbreeding coefficient, were identified, and removed using the *--check-sex* function in PLINK 1.9. Before checking for sex information, the X chromosome pseudo-autosomal regions (PARs) were separated. To identify and remove any outliers in terms of ancestry or population, the *smartpca* function of the EIGENSOFT package was run with 10 iterations for outlier removal. QC for SNPs included removing any with a minor allele frequency (MAF) less than 1%, a genotype call rate less than 95%, or that failed the Hardy-Weinberg equilibrium (HWE) test at a p-value of less than 1e-4. Additional checks for missing data on the X chromosome were done after combining the X chromosome PARs.

For the replication cohort, the genotyping procedure involved the analysis of SNPs associated with systolic Blood Pressure (SBP), diastolic Blood Pressure (DBP), and Body Mass Index (BMI). Specifically, all 73 SNPs with a p-value less than $5x10^{-5}$ were selected for genotyping. SNPs were genotyped using iPLEX GoldSequenom Mass Genotyping Array (Inqaba Biotec, Pretoria, South Africa). The genotyped data was transformed into a PED file format, while the SNP information, including the reference and alternate alleles, was obtained from the dbSNP database (https://www.ncbi.nlm.nih.gov/snp/). The SNP information was then transformed into a MAP file format, resulting in the creation of PLINK major file sets. Furthermore, it should be noted that three 3 SNPs with deletions were excluded from further analysis due to difficulties in encoding them in the MAP file. Consequently, a total of 69 SNPs were included for subsequent analyses. QC procedures were applied to the genotyped data in the PLINK major file sets separately for each cohort, i.e., Nigeria and Senegal.

In the replication cohorts, QC was performed by removing SNPs with a missing genotype rate of

up to 20%. Additionally, SNPs with a minor allele frequency (MAF) less than 0.01 and SNPs that failed the HWE test at a p-value of 0.01 were excluded. Individuals with a missing genotype rate (between 25%-38%) were removed.

Phasing and Imputation

Phasing and Imputation were performed using four strategies: an in-house imputation pipeline using EAGLE v2.4 [94] for phasing and IMPUTE2 [95] for imputation against the Consortium on Asthma among African-ancestry Populations in the Americas (CAFRICAN-AMERICANPA)[96], H3AFrica[97], The Trans-Omics for Precision Medicine (TOPMed)[98], Sanger[99], and the 1000 Genomes Project (KGP)[100]. After imputation, we were left with 15 515 482, 13 831 238, 17 837 499, 16 111 957, and 19 669 885 SNPs, respectively.

Statistical analysis and meta-analysis

For the discovery cohort, association analysis and QC measures were performed using the PLINK software package, version 1.09 (http://pngu.mgh. harvard.edu/purcell/plink/). The association of each SNP on BP levels was evaluated by adjusting for age, sex, BMI, and top 10 principal components. Similarly, the association of each SNP on BMI was evaluated by adjusting for age, sex, and top 10 principal components in a multivariate linear regression, assuming an additive genetic model of inheritance. The genome-wide significant threshold was determined through permutation (P-value $<1.27X10^{-7}$). R statistical computing environment (http://www.r-project.org/) (version 2.9.0) was employed to generate quantile–quantile (Q–Q), and Manhattan plots, providing a comprehensive graphical representation of our results.

For the replication cohort, association testing was conducted separately for each cohort using the PLINK 1.9 software. The PLINK1.9 "--assoc" command was employed, with a maximum of 100,000 permutations, to perform association analysis on the separated datasets. Additionally, the PLINK1.9 "--linear" command was utilized to adjust for sex and age as covariates. In the Nigeria cohort, only 13 out of the 31 samples had complete BMI, sex, and age information, while none of the samples had complete SBP and DBP information passed the QC filters. Consequently, no samples remained for analysis regarding these traits in the Nigeria cohort. For the Senegal cohort, SBP and DBP data were not available. Among the 194 samples that successfully passed QC filters, only 89 had complete BMI, sex, and age information.

Additionally, we performed a Meta-analysis using the summary statistics obtained from the Silent Infarct Transfusion (SITT) cohort, all the participants included in cohort were of African American ancestry and had SCD. SITT cohort is a group of children with SCD and silent cerebral infarcts (SCIs) who participated in a randomized controlled trial. The STT study cohort has been previously described[101]. We combined GWAS summary statistics from both Cameroon SCD cohort and SITT cohort using inverse-variance weighted fixed-effect models as implemented in METAL[102, 103]. Only SBP data was available in the SITT cohort. Therefore, we did not perform a meta-analysis on BMI and DBP.

Functional annotation

Functional annotation was performed with Functional Mapping and Annotation of genetic associations (FUMA) software [92], an online platform for functional mapping of genetic variants from GWAS summary statistics. Independent significant SNPs for which $P<5\times10-8$ and ($r2<0.6$) were identified from GWAS summary statistics. Lead SNPs were defined further from these independent significant SNPs if pairwise SNPs had ($r2<0.1$). Genomic risk loci in which SNPs were in LD ($r2 > 0.6$) with independent significant SNPs were identified. The maximum distance between LD blocks to merge into a genomic locus was 250 kb. The genetic data of African populations in 1000G phase3 were viewed as reference data to conduct LD analyses.

Functional Mapping and Annotation of genetic associations (FUMA) incorporates 18 biological data repositories and to test whether the top scoring variants measured in each GWAS loci cluster in specific biological pathways, a gene-set analysis was produced in Multi-marker Analysis of Genomic Annotation (MAGMA). MAGMA examines sets of biologically related genes that are more strongly associated with BP and BMI than other genes. This tool uses multiple linear regression models to assess whether genes in each gene set are more strongly associated with a polygenic trait, correcting for confounding factors such as linkage disequilibrium between variants and gene size. A gene-property analysis was conducted using MAGMA to indicate the role of tissue expression to gene associations, using the GWAS summary statistics.

5.3 Results

5.3.1 GWAS results

In our discovery cohort, a total of 1142 samples were collected and genotyped from individuals who had SCD, which consisted of 1921995 SNPs. 1083 samples passed quality control requirements, which consisted of 1765942 SNPs. Characteristics of these participants are summaries in Table 1. 556 individuals (aged 18.0 (16.0-21.0) had BP values collected (52 % males), and these consisted of 1765942 SNPs. 651 individuals (aged 22.0 (18.0–28.0) had BMI values collected (53 % males), and these consisted of 1765942 SNPs (Figure 1).

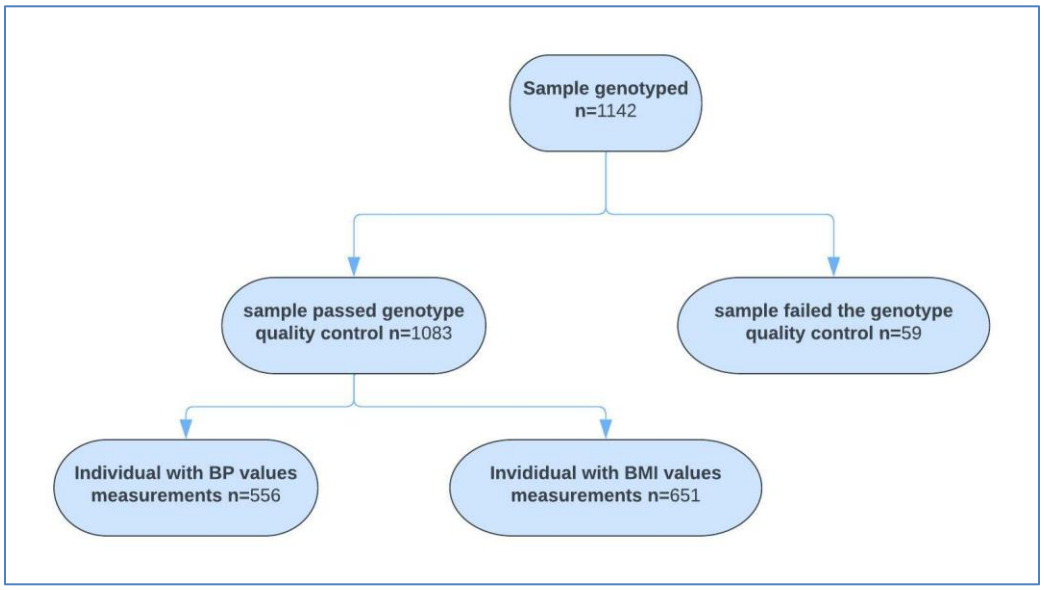

Figure 1. A flow chart diagram showing the inclusion/exclusion of samples at different stages of the study.

This study included two replication cohorts (i.e., Nigeria and Senegal); In the Nigerian cohort, 43 samples underwent QC after genotyping. Out of these samples, 31 individuals passed the QC filters. Among the 31 individuals, data on BMI, age, and sex were available for 13 individuals (aged 21(11-29), and 38% were males. None of 13 individuals had data on SBP or DBP. Whereas in the Senegal cohort, we genotyped 203 individuals. After performing QC, 194 individuals remained in the cohort. Out of these 194 individuals, data on BMI, age, and sex were available for 81 individuals (aged 20(12-26) and 44 % were males. However, data on SBP and DBP were not available for any individuals in this cohort (Table 1).

Table 1. Characteristics of SCD populations in Cameroon

	Discovery cohort (Cameroon) (N=1142)	Replication cohort (Nigeria) (N=43)	Replication cohort (Senegal) (N=203)
Number passed genotype quality control	**1083** • 556 had data on SBP/DBP • 651 had data on BMI.	**31** • 13 had data on BMI, age, sex. • None passed QC had data on SBP/DBP	**194** • 81 had data on BMI, age, sex. • SBP and DBP data was not available
Gender (% Males)	SBP/DBP 556 (52%) BMI 651 (53%)	5 (38%)	39 (44%)
Age, Y	SBP/DBP group 18.0 (16.0-21.0) BMI group 22.0 (18.0–28.0)	21(11-29)	20(12-26)

To assess the quality of the GWAS and potential biases due to population stratification or other technical issues, we constructed a Quantile-quantile (Q-Q) plot of the p-values obtained from the association tests. Figure 2 depicts a Q-Q plot of observed versus expected P-values. The observed P values for each SNP were sorted from largest to smallest and plotted against expected values from a theoretical $\chi 2$-distribution. All points (in orange) are on or near the middle line between the x-axis and the y-axis (null hypothesis), meaning that the observed values correspond to the expected values. The lack of an early departure of the observed P-values indicates that our data are not affected by issues with genotyping, imputation, uncontrolled sample relatedness, or population stratification. For three phenotypes, the genomic control (GC) was close to 1 (0.998 for SBP, 0.981 for DBP, and 1.000 for BMI), indicating that the test statistics were not significantly inflated (Figure 2). Therefore, the observed associations between the tested variants and three phenotypes are likely true associations rather than results of biases or technical problems. PCA indicates genetic variation within the population, revealing patterns of ancestry or substructure. Figure 3 illustrates the population stratification detected through PCA analysis, providing insights into the genetic diversity and clustering within the study population.

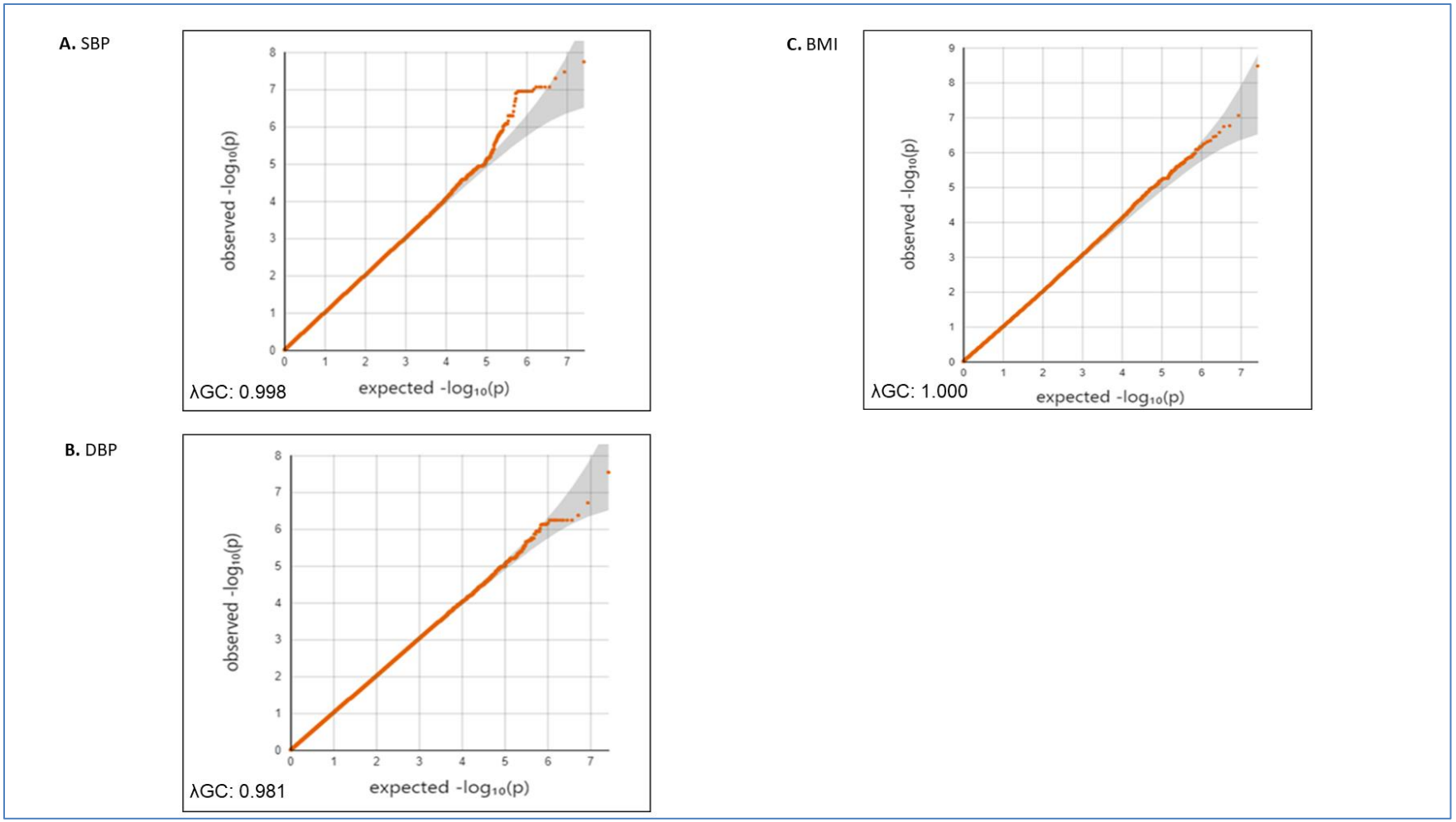

Figure 2. Quantile-quantile (Q-Q) plot for three phenotypes. A) systolic Blood Pressure (SBP), B) diastolic Blood Pressure (DBP), C) Body Mass Index (BMI). Genomic control l was 0.998 for SBP, 0.981 for DBP, and 1.000 for BMI. The observed P values for each SNP are sorted from largest to smallest and plotted against expected values from a theoretical χ2-distribution.

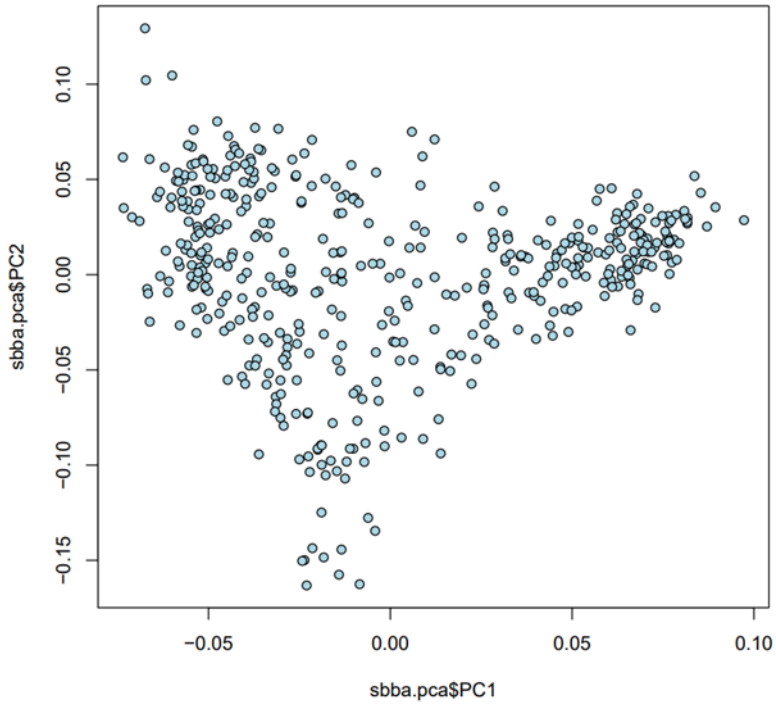

Figure 3. Principal component analysis (PCA) plot of Cameroonian SCD cohort

Figure 4.A-C shows the distribution of association P-values (Manhattan plot) for SBP (figure 4-A), DBP (Figure 4-B), and BMI level (Figure 4-C). Whereas figure 4 shows the top leading candidate SNPs that reached a threshold of $p < 5\times10^{-7}$. Figure 5 shows common genes detected using multiple imputations panels. And Figure 6 shows genes that were common in between SBP, DBP, and BMI

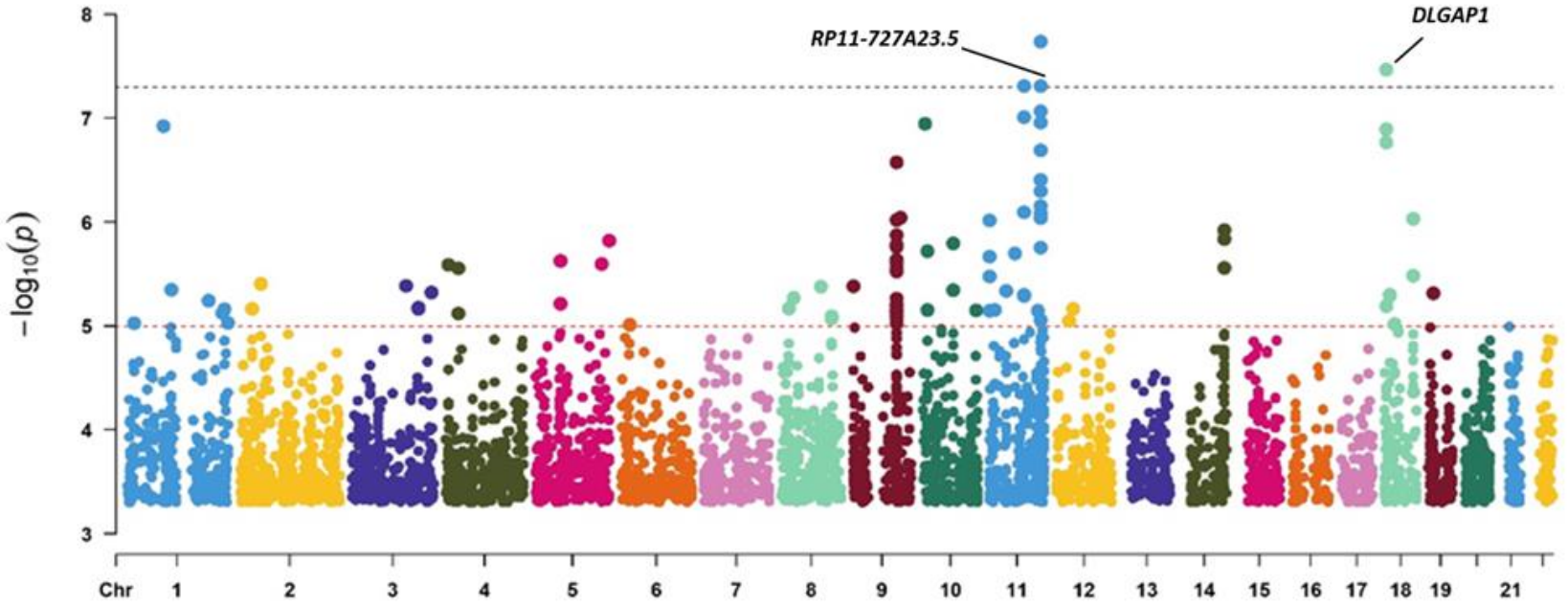

Figure 4-A. Manhattan plot showing the association of SNPs with systolic Blood Pressure (SBP) in Cameroon SCD cohort. The genome-wide distribution of -log10 P-values are plotted against the physical position of each SNP on each chromosome. The threshold for genome-wide significance ($p < 5.0x10\text{-}8$). Red line=suggestive line ($p < 5\times10\text{-}7$).

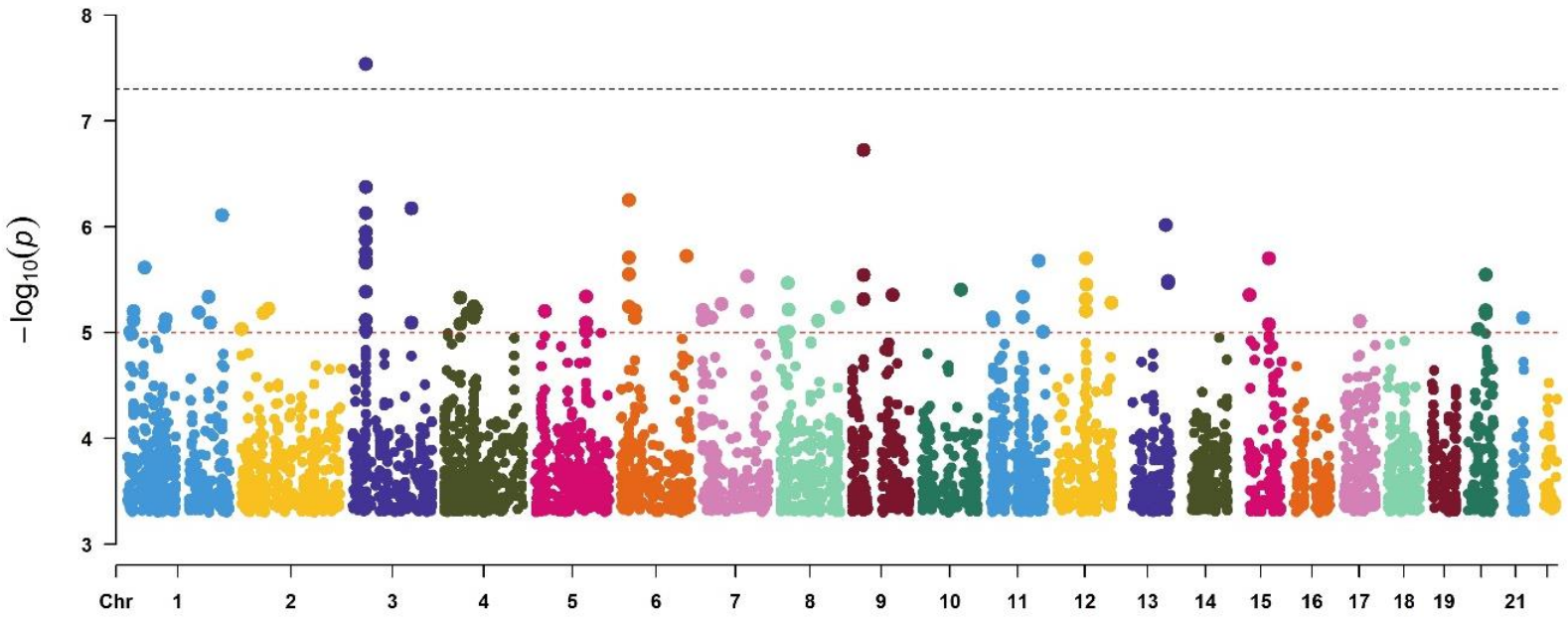

Figure 4-B. Manhattan plot showing the association of SNPs with diastolic Blood Pressure (DBP) in Cameroon SCD cohort.

The genome-wide distribution of -log10 P-values are plotted against the physical position of each SNP on each chromosome. The threshold for genome-wide significance ($p < 5.0x10\text{-}8$). Red line=suggestive line ($p < 5\times10\text{-}7$).

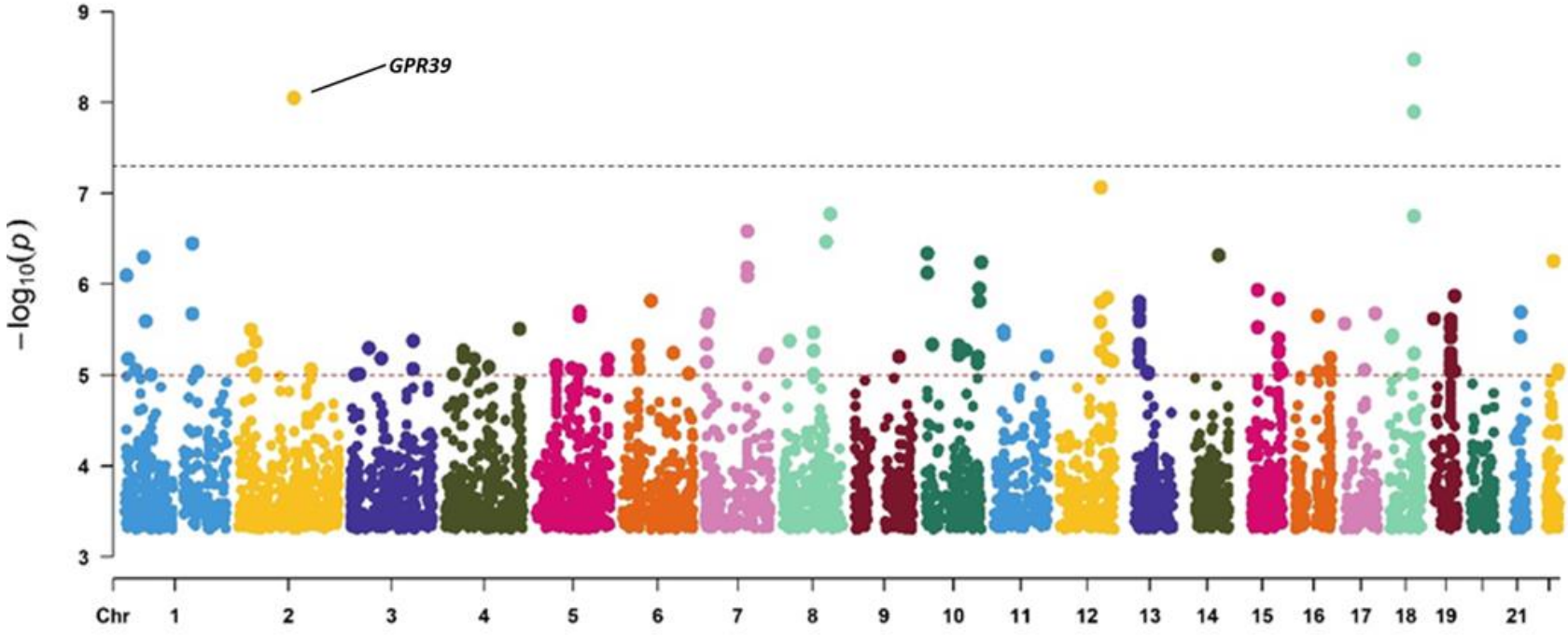

Figure 4-C. Manhattan plot showing the association of SNPs with Body Mass Index (BMI) in Cameroon SCD cohort.

The genome-wide distribution of -log10 P-values are plotted against the physical position of each SNP on each chromosome. The threshold for genome-wide significance ($p < 5.0x10\text{-}8$). Red line=suggestive line ($p < 5\times10\text{-}7$).

Figure 5 depicts a Venn diagram illustrating the integration of genome-wide association study (GWAS) findings derived from four distinct imputation panels. Specifically, the analysis encompasses single nucleotide polymorphisms (SNPs) exhibiting a significance threshold of $P < 5 \times 10^{-5}$ or lower.

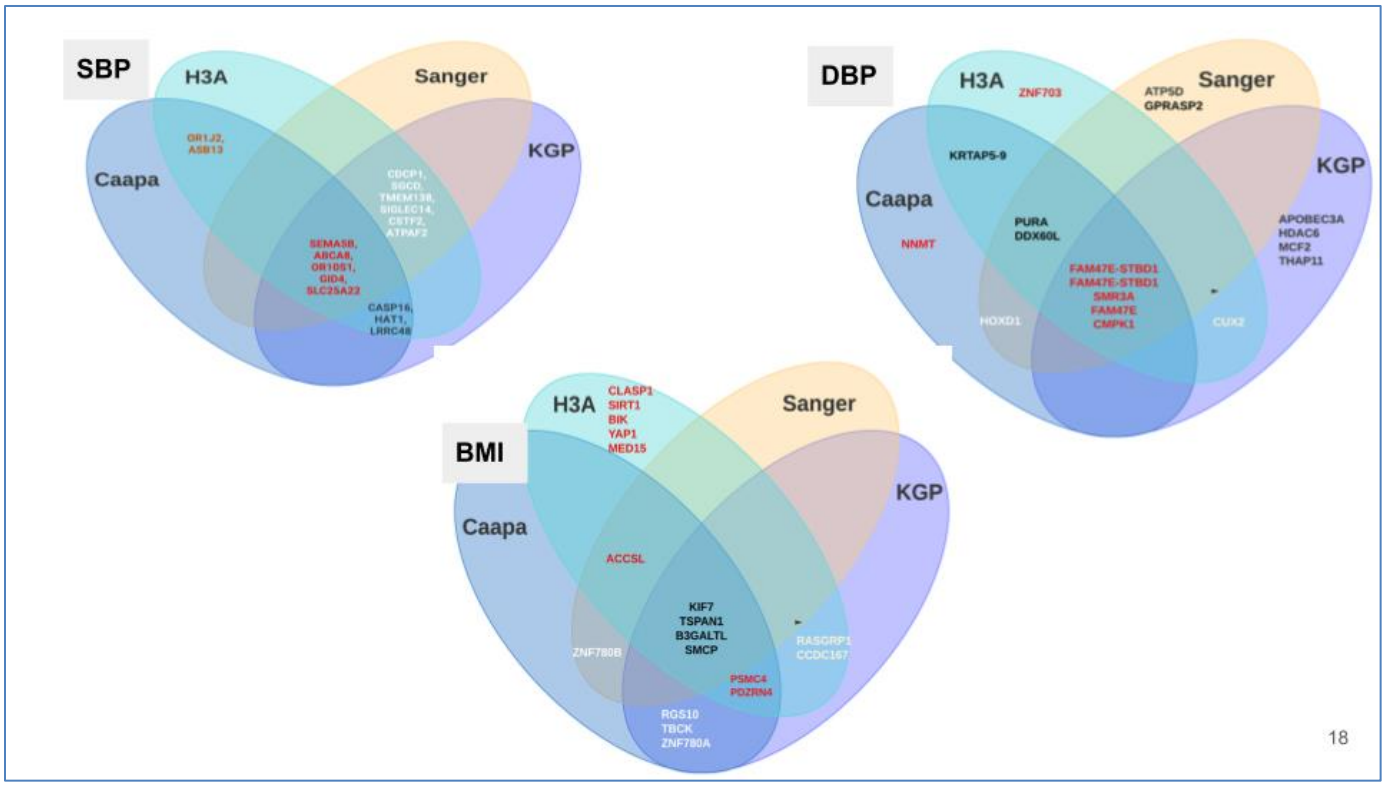

Figure 5. Genes detected using four different imputation panels. The four impation panels are: 1000 Genomes Project hosted at the Michigan Imputation Server (KGP), The Sanger Imputation serve, the consortium on Asthma among African-ancestry Populations in the Americas (CAAPA) imputation panel, and H3Africa imputation panel (H3A).

In Figure 6, For SBP, there were 8 independent significant SNPs and 6 leading SNPs, 161 candidate SNPs in LD with leading SNPs, lead SNPs could be classified into 6 genomic risk loci, 14 genes were mapped to these loci. For DBP, there were 2 independent significant SNPs and 2 leading SNPs, 17 candidate SNPs in LD with leading SNPs, lead SNPs could be classified into 2 genomic risk loci, 1 mapped gene was in these loci. For BMI, there were 10 independent significant SNPs and 10 leading SNPs, 58 candidate SNPs in LD with leading SNPs, lead SNPs could be classified into 10 genomic risk loci, 5 mapped genes located in loci (Supplementary 1, 2)

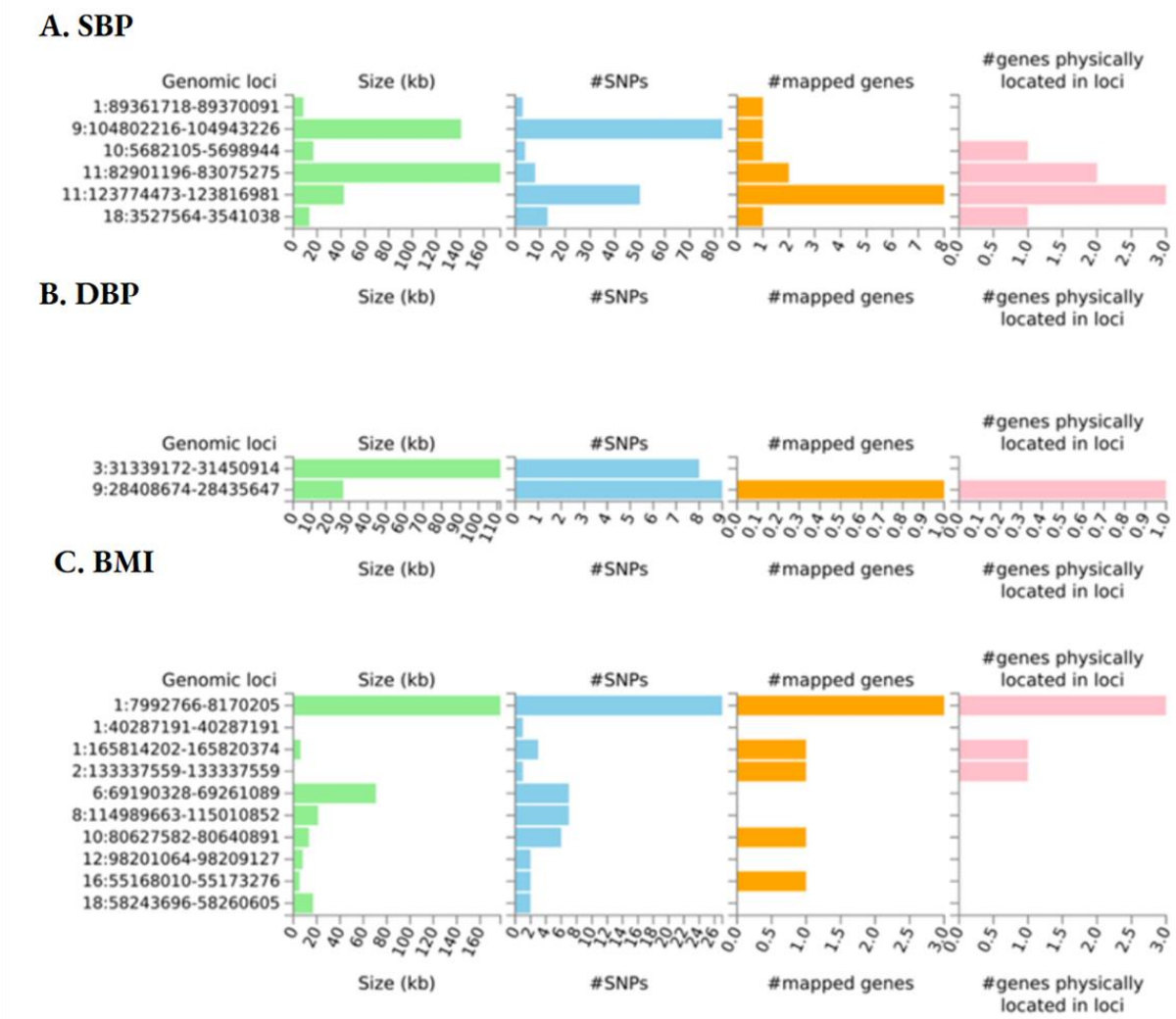
A. SBP
Genomic loci
Size (kb)
#SNPs
#mapped genes
#genes physically located in loci
1:89361718-89370091
9:104802216-104943226
10:5682105-5698944
11:82901196-83075275
11:123774473-123816981
18:3527564-3541038
B. DBP
3:31339172-31450914
9:28408674-28435647
C. BMI
1:7992766-8170205
1:40287191-40287191
1:165814202-165820374
2:133337559-133337559
6:69190328-69261089
8:114989663-115010852
10:80627582-80640891
12:98201064-98209127
16:55168010-55173276
18:58243696-58260605

Figure 6. Leading SNPs that that reached threshold of p < 5×10-7. Summary showing candidate SNPs, mapped genes per genomic risk locus.

Summary of genomic risk locus based on GWAS of A) SBP, B) DBP, C) BMI. Genomic risk loci are displayed by the 'chromosome: start position-end position' on the Y axis. Histograms from left to right show the size of the genomic locus (in green), number of candidate SNPs in the genomic locus (blue), number of mapped genes by positional mapping and eQTL mapping in the genomic locus (in orange), and the number of genes known to be located within the genomic loci (in pink), respectively.

Figure 7 displays 14 mapped genes, 1 mapped gene, and 7 mapped genes found in the genomic risk loci by FUMA for SBP, DBP, and BMI respectively. For SBP, the following genes were mapped *GTF2B, BAAT, ASB13, PCF11, ANKRD42, SCN3B, ZNF202, TMEM225, OR8D4, OR4D5, OR6T1, OR10G7, VWA5A, DLGAP1 in the genomic risk loci.* For DBP, *LINGO2* gene was mapped in genomic risk loci. And For BMI, *TNFRSF, PARK7, ERRFI1, UCK2, GPR39, ZMIZ1, IRX5* were mapped to genomic risk by FUMA (Supplementary 3).

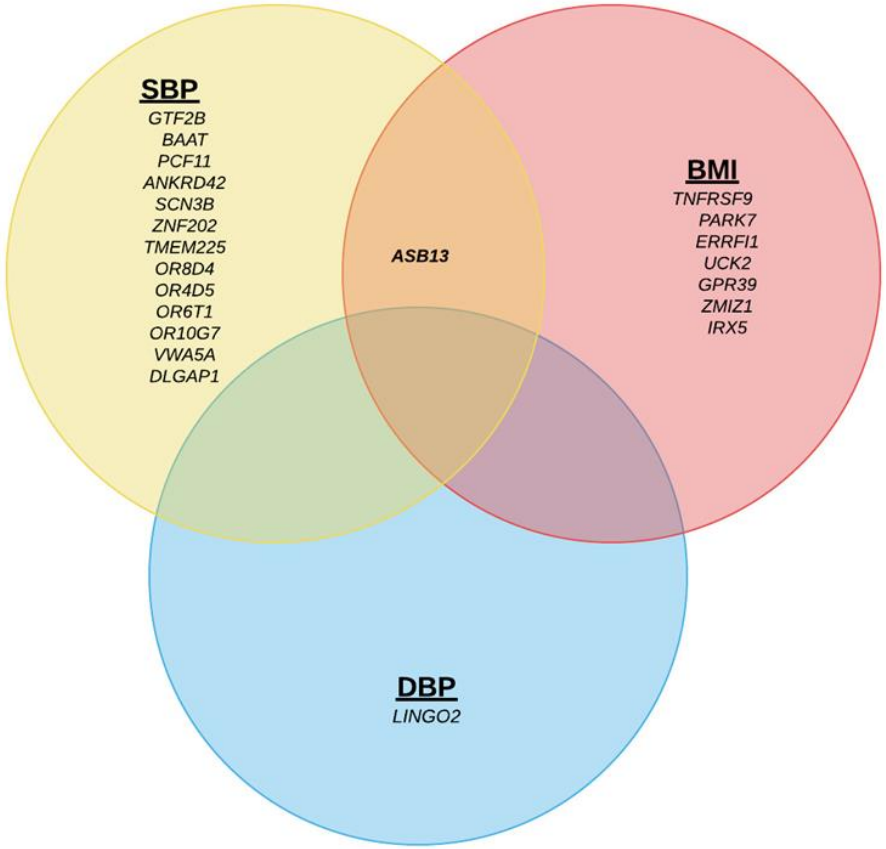

Figure 7. Number of mapped genes by positional mapping and eQTL mapping in the genomic locus in the genomic risk loci for SBP, DBP, and BMI (supplementary 3)

Table 2 includes only significant SNPs (that reached threshold of $p < 5\times10\text{-}8$) associated with SBP, DBP, and BMI in SCD patients. For SBP, with a threshold of $p < 5\times10\text{-}8$, two genetic loci including four significant and independent SNPs were found in the GWAS (Figure 4, Table 2). We found two genetic loci including independent and suggestive SNPs. The fist locus was found in *RP11-727A23.5* gene on chromosome 11 (rs146072506, $p=4.89x10^{-08}$)(Figure 8-A), the second locus was found on *DLGAP1* gene on chromosome 18 (rs35715722, $p=3.41x10^{-08}$)(Figure 8-B). Two other SNPs were found in intergenic region on chromosome 11 (rs58269424, $p=1.82x10^{-08}$) and (rs12282739, $p=4.92X10^{-08}$). Previous report identified suggestive candidate loci (rs7952106 in DRD2) gene on chromosome 11 to be associated with SBP in SCD children [16], however, (rs7952106, p= 0.0886197) was not significant in this study. For DBP, the only significant SNP was (rs75820288, $p=2.90X10^{-08}$) located in the intergenic region on chromosome 3.

For BMI, with a threshold of $p < 5\times10\text{-}8$, One genetic locus including four significant and independent SNPs were found in the GWAS (Figure 4, Table 2). Three significant SNPs were located on intergenic region on chromosome 18 (rs9955087, $p=3.37x10^{-09}$), (rs73445228, p=1.27x10-08), and chromosome 12 (rs79063750, $p=8.62x10^{-08}$). The identified locus was located on *GPR39* gene (rs186536474, p=8.90x10-09) on chromosome 2 (Figure 8-C). Figure 8 A-C shows regional plot of suggestive SNPs that are worthy of following up in larger studies.

Table 2. Summary statistics of SNPs associated with SBP, DBP, BMI in SCD patients in Cameroon.

Chr	SNP	Position	Ref/Alt allele	Gene	MAF	P-value	Beta	FDR
					SBP			
11	rs146072506	82901196	C/T	*RP11-727A23.5*	0.032	4.88915e-08	0.80	0.07
11	rs58269424	123787275	C/T	Intergenic	0.053	1.82886e-08	0.60	0.07
18	rs35715722	3534322	G/A	*DLGAP1*	0.042	3.40829e-08	0.78	0.07
11	rs12282739	123798774	G/A	Intergenic	0.078	4.92465e-08	0.52	0.07
					DBP			
3	rs75820288	31357861	G/A	Intergenic	0.013	2.9029e-08	1.00	0.32
					BMI			

18	rs9955087	58243950	G/A	Intergenic	0.002	3.37879e-09	1.28	0.04
2	rs186536474	133337559	T/C	*GPR39*	0.020	8.90144e-09	0.86	0.10
12	rs79063750	98209127	C/T	Intergenic	0.012	8.61816e-08	1.12	0.58
18	rs73445228	58260605	C/T	Intergenic	0.005	1.2727e-08	1.19	0.10

Chr: chromosome, SNP: single nucleotide polymorphisms, MAF: minor allele frequency, FDR: false discovery rate,

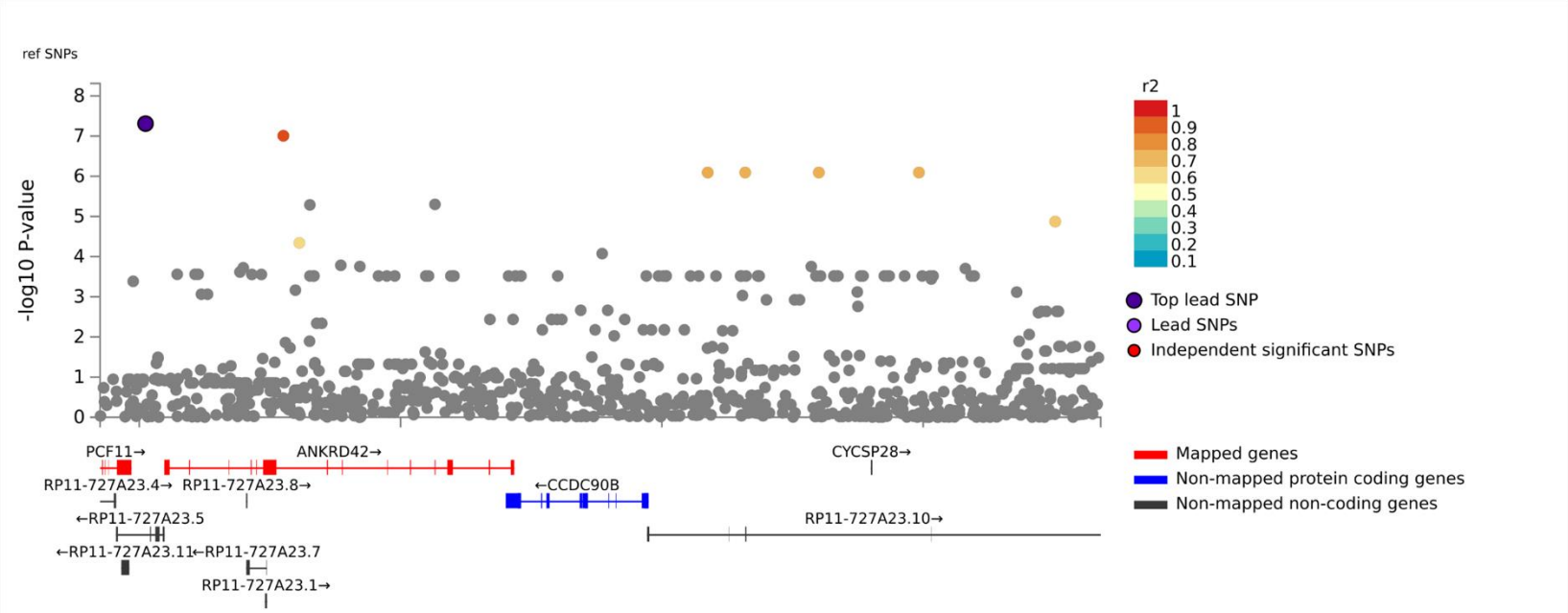

Figure 8-A. Regional plot of rs146072506 on chromosome 11. Mapped genes in red. Non mapped protein coding genes in blue. Non-mapped non-coding genes

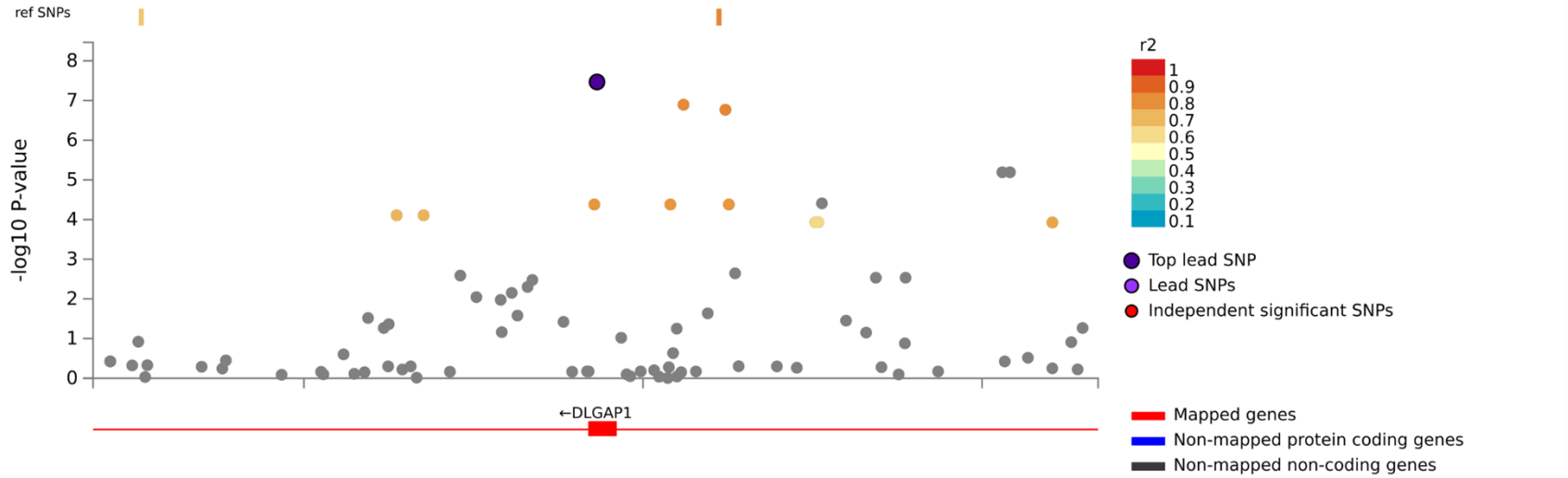

Figure 8-B. Regional plot of rs35715722 on chromosome 18. Mapped genes in red. Non mapped protein coding genes in blue. Non-mapped non-coding genes

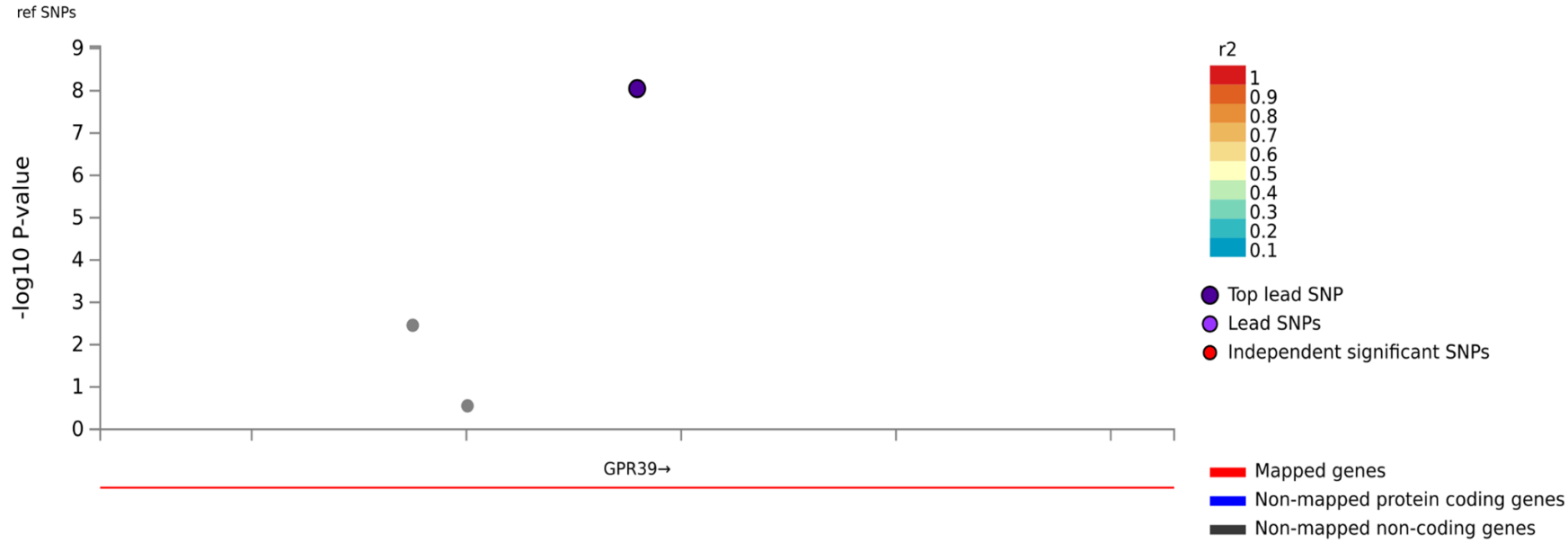

Figure 8-C. Regional plot of rs186536474 on chromosome 2. Mapped genes in red. Non mapped protein coding genes in blue. Non-mapped non-coding genes.

Global comparison of MAF of the suggestive variants

After comparing the minor allele frequency (MAF) of suggestive SNPs associated with SBP (rs146072506, rs58269424, rs35715722, and rs12282739), DBP (rs75820288), and BMI (rs9955087, rs186536474, rs79063750, and rs73445228) in different populations as shown below SBP results in Figure 9A (SBP), Figure 9B (DBP), and Figure 9C (BMI).

For SBP (Figure 9A) The MAF of rs146072506 seem to be African specific. Whereas rs35715722 varied among populations, with the highest frequency observed in Cameroon SCD population, followed by other populations of African ancestry (Africans and African American). Whereas rs58269424 and rs12282739 had the lowest frequency in Cameroon SCD patients compared to other populations.

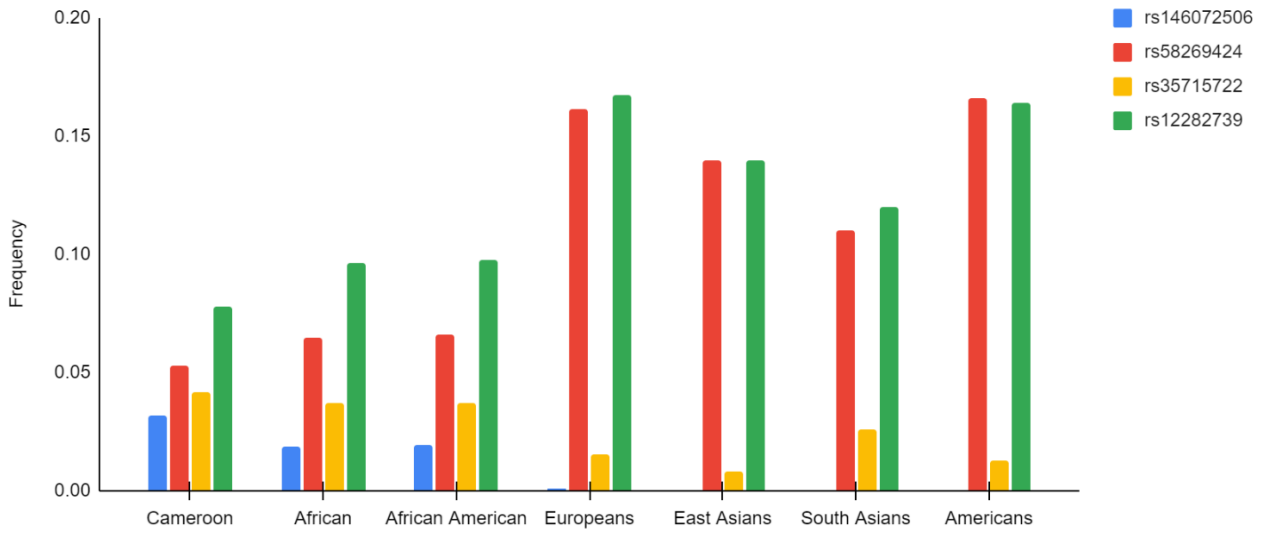

Figure 9-A: Comparison of minor allele frequency (MAF) of suggestive SNPs associated with systolic Blood Pressure (SBP) in different populations. Different colours represent each different SNP (rs146072506, rs58269424, rs35715722, and rs12282739).

For DBP (Figure 9B), nearly all the variants found are African specific. Among them, the MAF frequency of rs75820288 was more

common in African and African Americans populations, but lower in Cameroon SCD population.

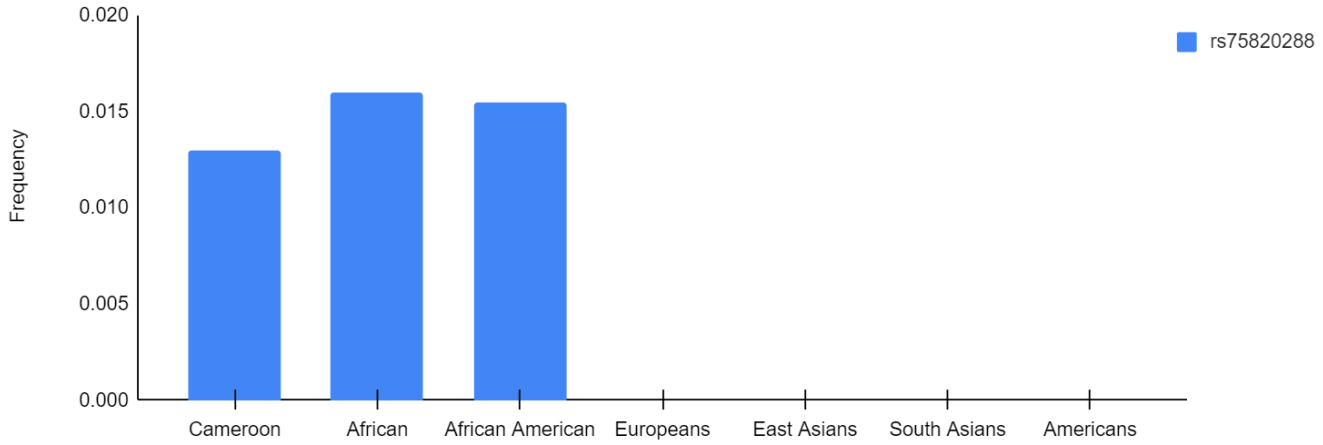

Figure 9-B: Comparison of minor allele frequency (MAF) of suggestive SNPs associated with diastolic Blood Pressure (DBP) in different populations. Different colours represent each different SNP (rs75820288).

For BMI (Figure 9C) rs9955087 appears to be African specific, with variable frequencies. That variant is higher among African American, followed by other African populations, and then lowest in Cameroon SCD population. Whereas rs186536474, 73445228 and rs79063750 had the highest frequencies in the Cameroon SCD population compared to other populations.

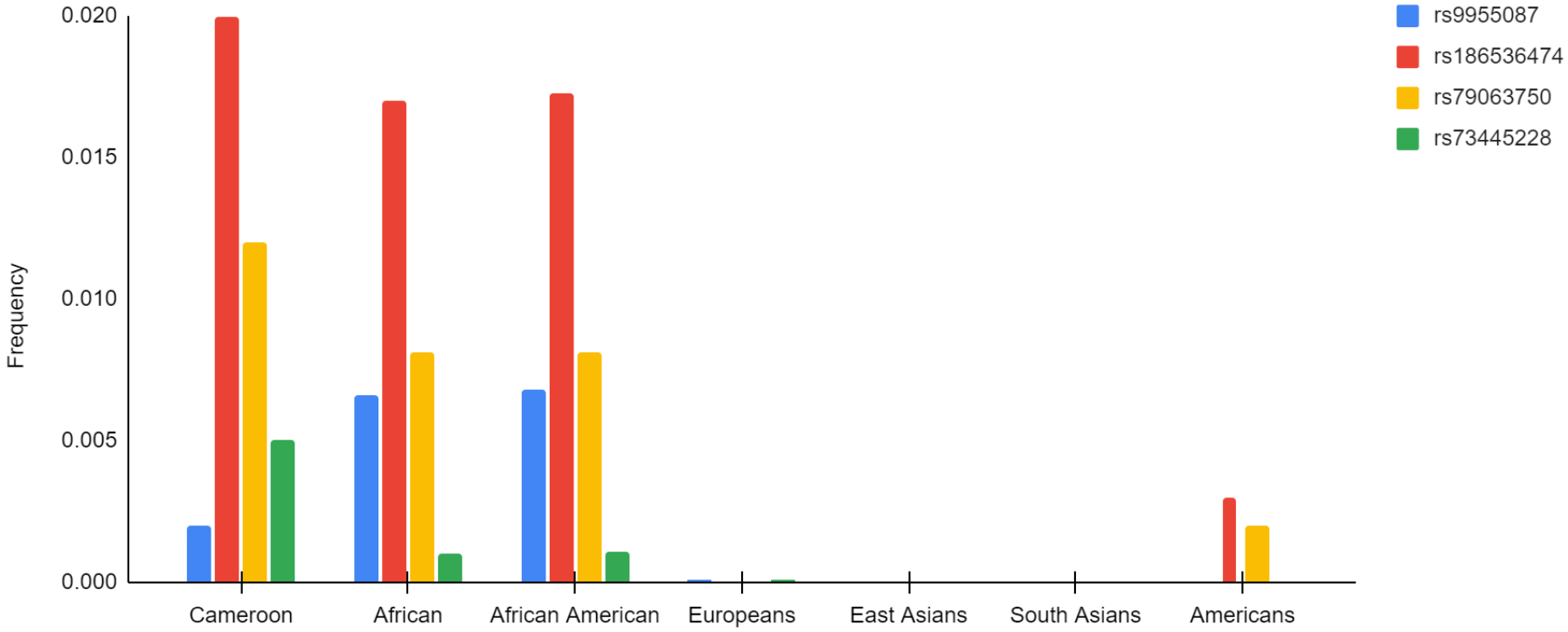

Figure 9-C. Comparison of minor allele frequency (MAF) of suggestive SNPs associated with Body Mass Index (BMI) in different populations. Different colours represent each different SNP (rs9955087, rs186536474, rs79063750, and rs73445228).

5.3.3 Meta-analysis results

Table 3: Association summary of genome-wide significant SNPs from the meta-analysis

RSID	Coordinate	Alleles	MAF	BETA	SE	P-value	FDR	Gene	Panel	Direction	HetlSeq	HetPval
rs13435015	4:6920726	T/C	0.2802	-0.2841	0.056	3.981e-07	0.15801650925	*TBC1D14*	H3A	--	19.7	0.2644
rs970584	11:123802399	A/G	0.0980	-0.4436	0.0881	4.805e-07	0.15801650925	Intergenic	H3A	-+	6.3	0.3017
rs12737457	1:198045249:	A/G	0.2189	0.3162	0.0644	9.052e-07	0.1984551428	Intergenic	H3A	++	10.7	0.2899

Abbreviations: RSID: Reference SNP IDentifier, MAF: Minor Allele Frequency, SE: Standard Error, FDR: False Discovery Rate, HetlSeq: I-squared heterogeneity, HetPval: Heterozygosity P-value.

We attempted to validate candidate loci from the Cameroon SCD cohort by the combining summary statistics from the Cameroon SCD cohort and SITT cohorts. The P-values for 3 variants associated with reference SNP identifier (RSID) were all suggestive around e-07. However, I-squared heterogeneity (HetlSeq) showed low heterogeneity and heterogeneity p-value (Het Pval) is not significant, which suggests that the studies are relatively homogeneous. After adjusting for false discovery rate (FDR) none of the identified SNPs were significant.

5.3.3 Gene Analysis and Gene-Set Analysis

In a gene-based association analysis using MAGMA (which is integrated into FUMA), all SNPs were mapped to protein-coding genes if they were located within genes. Gene sets were then tested using a default competitive test model. The analysis tested a total of 18,800 gene sets for SBP, DBP, and BMI. The most significant genes identified for each trait were *ABCA8* (chromosome 17) for SBP with a P-value of $8.3*10^{-6}$, *FAM47E-STBD1* (chromosome 4) for DBP with a P-value of $1.95*10^{-5}$, and PSMC4 (chromosome 19) for BMI with a P-value of $2.67*10^{-5}$. The threshold for genome-wide significance was set at $P = 0.05/18799 = 2.66*10^{-6}$. However, none of the identified genes reached this threshold.

In the gene set analysis by MAGMA integrated in FUMA (which is also integrated into FUMA), a total of 18,799 gene sets were tested and a default competitive test model was applied. For SBP, the gene set were the most significant (Serum uric acid levels in response to allopurinol in gout) (p=value 3.11e-09) and (Response to citalopram or escitalopram in depression) (p=value 2.22e-05) (supplementary 4). There were no significant gene-set for DBP. For BMI, the most significant gene set was (response to platinum-based chemotherapy in non-small-cell lung cancer) (p-value 2.45e-06). The top gene sets from the analysis are included in supplementary table 4.

Tissue Expression Analysis by FUMA

The expression of genes in specific tissues was studied using the GTEx (Genotype-Tissue Expression) resource. The relationship between tissue-specific gene expression and genetic associations was tested by using the average gene expression in each tissue type as a covariate. Two analyses were conducted: one that examined 30 general tissue types and the other that focused on 53 specific tissue types. The results are presented in figure 10 A-F. GTEx's tissue expression analysis of 53 specific tissue types found that the expression in the adrenal gland had the lowest p-value of 0.004 for SBP, the brain hypothalamus had the lowest p-value of 0.082 for DBP, and the breast mammary tissue had the lowest p-value of 0.027 for BMI. Additionally, GTEx's tissue expression analysis of 30 specific tissue types found that the expression in the adrenal gland had the lowest p-value of 0.006 for SBP, the prostate had the lowest p-value of 0.160 for DBP, and the breast had the lowest p-value of 0.016 for BMI. Overall, these findings a concurrent to the known relevance of adrenal gland, brain hypothalamus function in BP regulation, and the mammary tissue mostly made of adipocytes, potentially the prostate tissue that is sensitive to sympathetic and para-sympathetic nervous system regulation, in the pathophysiology of BP regulation.

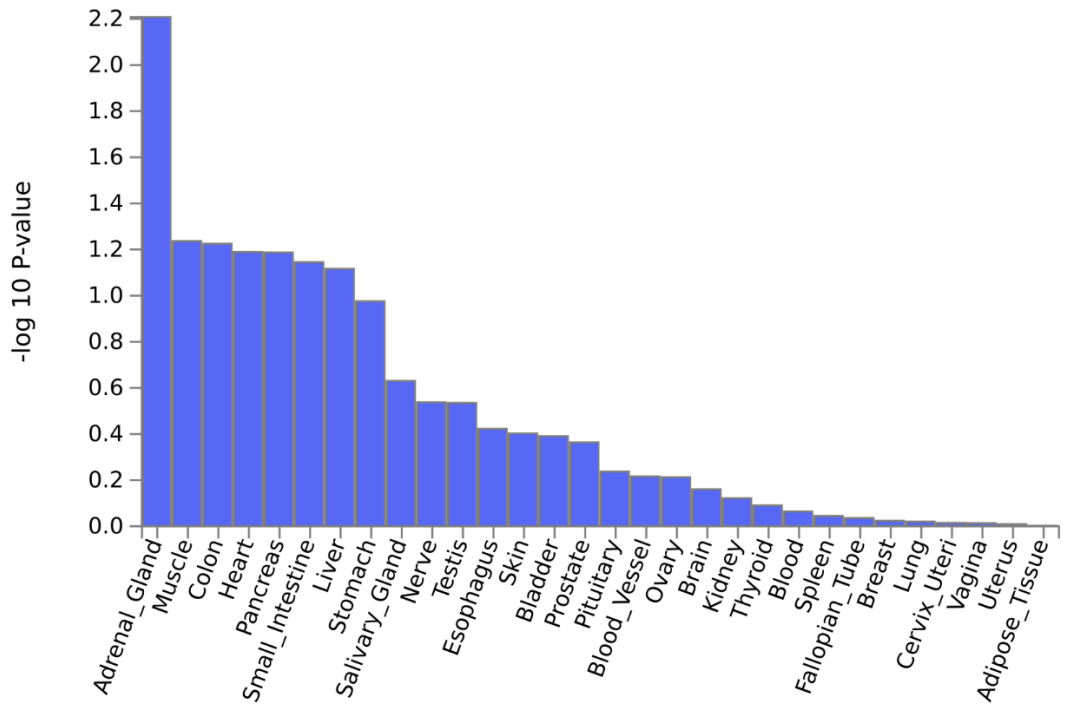

Figure 10-A: The tissue expression results on 30 specific tissue types by Genotype-Tissue Expression (GTEx) for SBP.

The top tissues, the adrenal glands which produces adrenalin, and muscle involved in vascular motility, are critical organ and tissue for BP regulation.

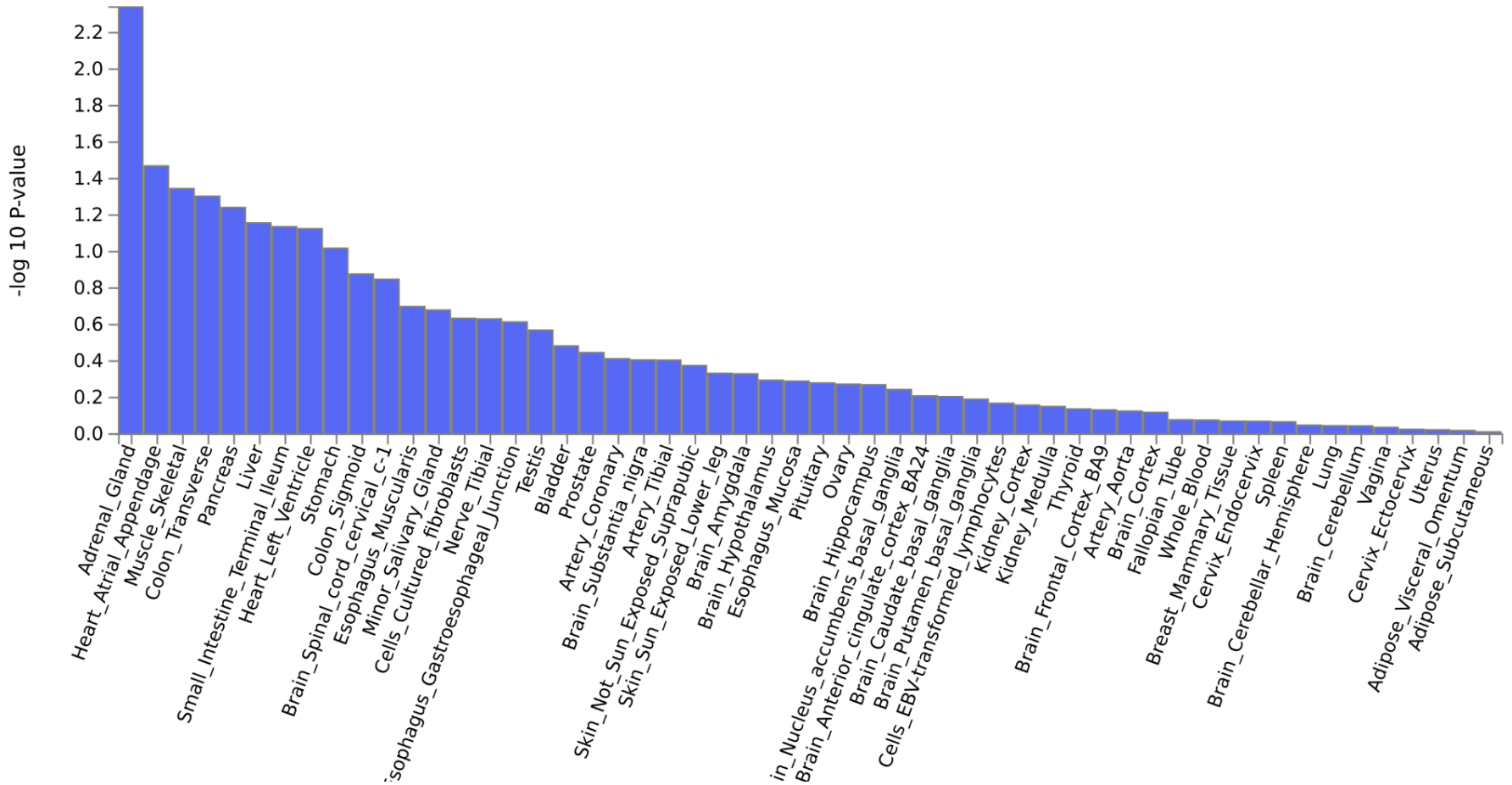

Figure 10-B: The tissue expression results on 53 specific tissue types by Genotype-Tissue Expression (GTEx) for SBP

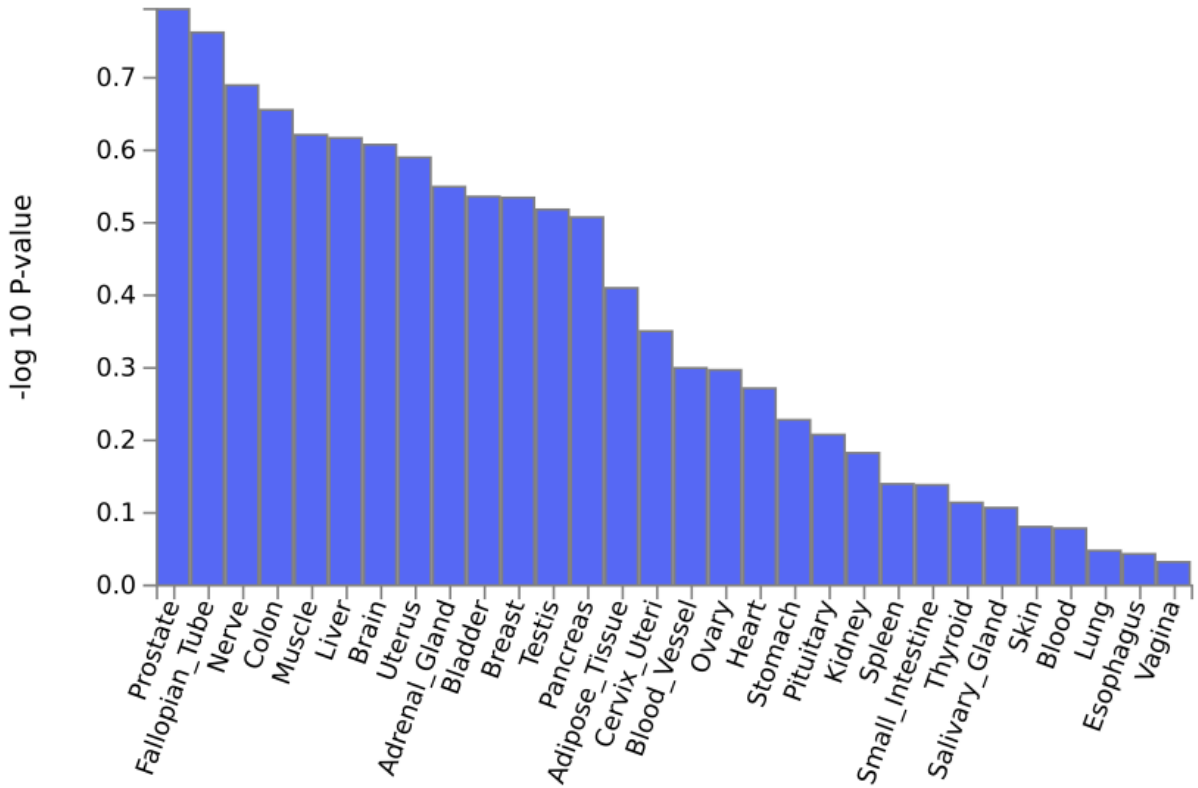

Figure 10-C: The tissue expression results on 30 specific tissue types by Genotype-Tissue Expression (GTEx) for DBP

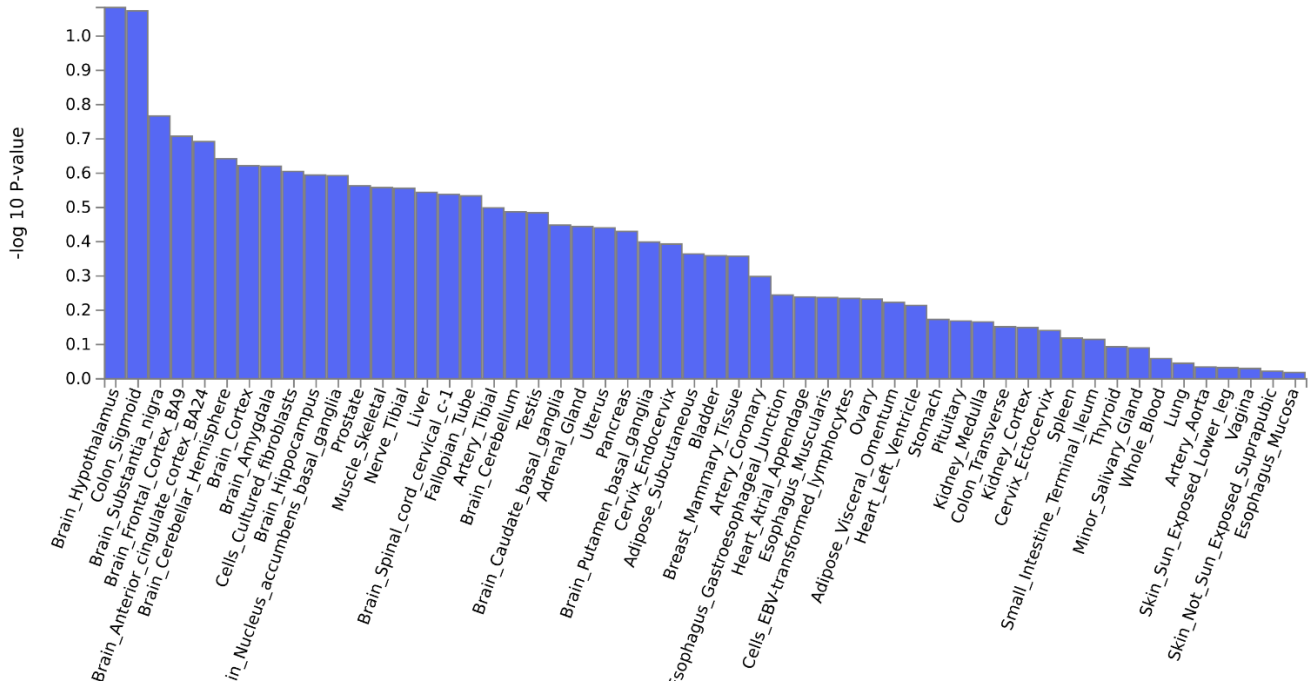

Figure 10-D: The tissue expression results on 53 specific tissue types by Genotype-Tissue Expression (GTEx) for DBP.

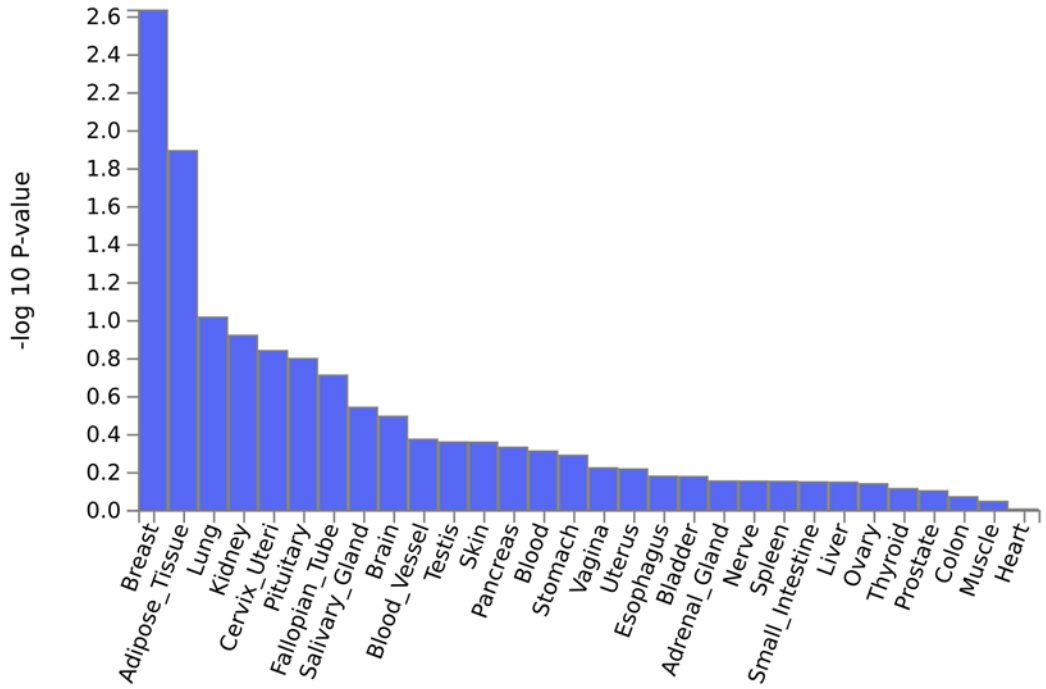

Figure 10-E: The tissue expression results on 30 specific tissue types by Genotype-Tissue Expression (GTEx) for BMI.

Breast is mainly made of adipose tissue, and not surprising that its tissue homeostasis could be link to BMI.

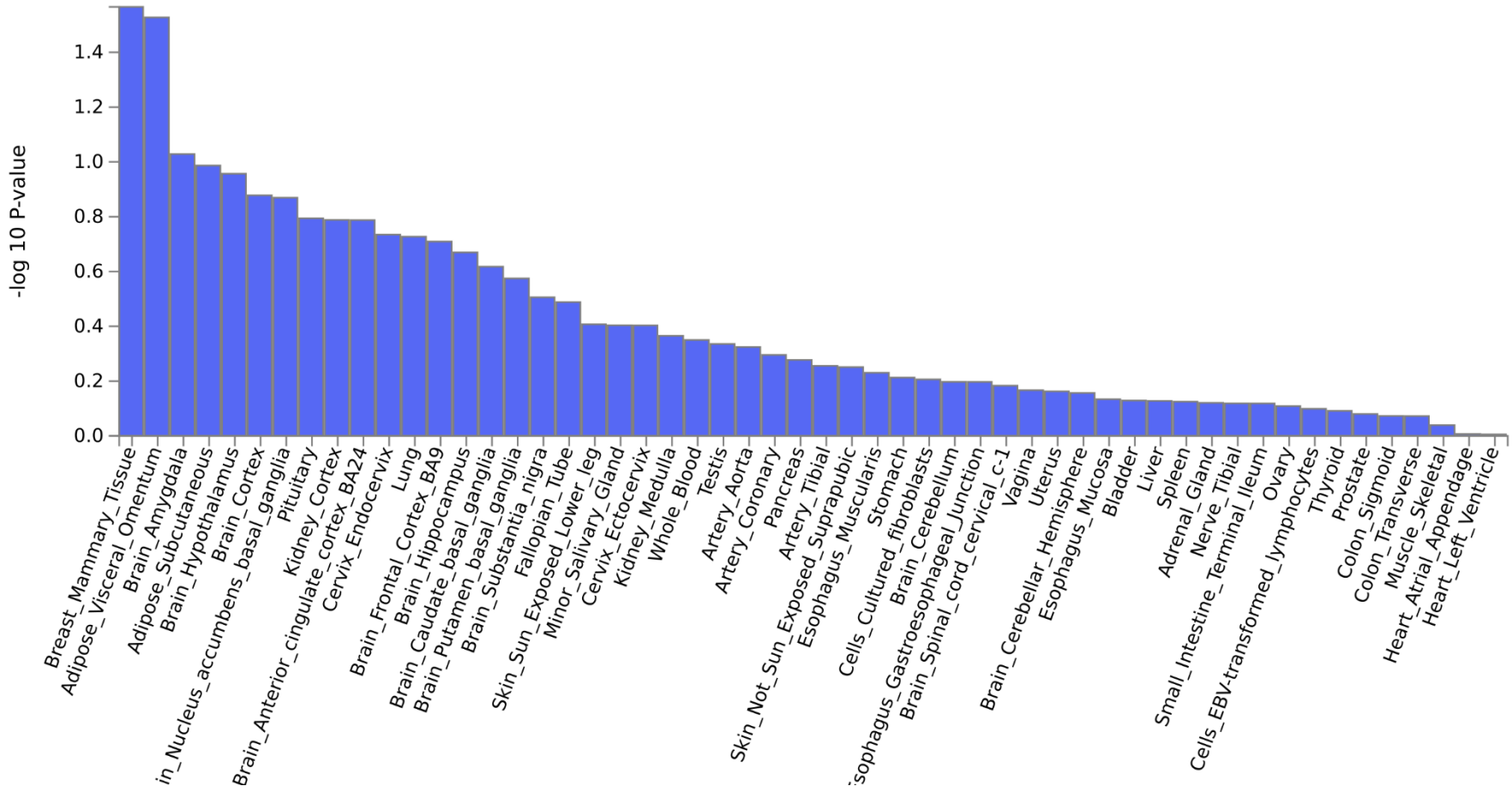

Figure 10-F: The tissue expression results on 53 specific tissue types by Genotype-Tissue Expression (GTEx) for BMI

Replication cohorts' results

For the Nigeria cohort, 26 SNPs with a missing genotype rate of up to 20% were removed. Additionally, 9 SNPs with a minor MAF less than 0.01 and SNPs that failed the HWE test at a p-value of 0.01 were excluded. 12 Individuals with a missing genotype rate of up to 38% were also removed, taking into consideration that the 25% threshold would result in the exclusion of all samples. Following these QC steps, 34 SNPs and 31 individuals passed the filters, resulting in a genotyping rate of 62.32% (Table 4).

Table 4. Association of selected SNPs and BMI among Nigerian SCD cohort.

CHR	SNP	BP	ALT	REF	BETA	SE (CI)	P	FDR(BH)	EMP1	EMP2
9	rs78291268	116798255	A	G	4.433	1.646(1.206-7.659)	0.02468	0.8024	0.173	0.9666
9	rs62640055	116812114	T	C	4.433	1.646(1.206-7.659)	0.02468	0.8024	0.173	0.9666
8	rs4872200	23692503	T	C	-3.154	1.499(-6.093-(-0.2149))	0.06477	0.7893	0.1371	0.9532
2	rs77470226	59906495	G	A	2.19	1.21(-0181-4.561)	0.1037	0.2468	0.9121	1
18	rs149054459	24195397	A	G	3.894	2.776(-1.547-9.335)	0.1943	0.9533	0.6531	1
3	rs114680749	6785582	C	T	-3.562	2.969(-9.381-2.257)	0.2608	0.2468	0.5781	1
18	rs292267	5205280	G	T	-2.887	2.75(-8.277-2.502)	0.3211	0.9382	0.3461	0.9998
23	rs148220168	13501432	G	A	-2.887	2.75(-8.277-2.502)	0.3211	0.9533	0.3461	0.998
11	rs7129598	25134788	G	A	-1.292	1.4(-4.035-1.452)	0.3802	0.8963	0.1914	0.9933
9	rs10757831	29282192	T	C	1.044	1.168(-1.246-3.335)	0.3947	0.7893	0.985	1
18	rs75733641	55139066	A	C	2.785	3.458(-3.993-9.564)	0.4414	0.9533	0.03802	0.6976
11	rs12269875	120170477	G	A	-1.206	1.643(-4.426-2.013)	0.4814	0.938	0.873	1
6	rs549202542	21255081	T	A	-1.509	3.2(-7.78-4.762)	0.6484	0.777	0.9236	1
10	rs116245687	26474131	T	C	-1.509	3.2(-7.78-4.762)	0.6484	0.8963	0.9236	1
10	rs11816159	73001055	A	G	0.9843	2.251(-3.428-5.397)	0.6723	0.8963	0.7618	1
8	rs1385729	25936301	T	G	0.4214	1.285(-2.097-2.94)	0.7504	0.7893	0.5677	1
6	rs2753912	24347624	A	T	0.6109	2.311(-3.919-5.14)	0.7975	0.7893	0.5449	1
8	rs1481608	13515457	C	T	0.1324	1.38(-2.573-2.837)	0.9257	0.7893	0.3512	1
3	rs4434100	122192272	G	C	0.1754	2.913(-5.534-5.885)	0.9533	0.4318	0.732	1

3	rs75711887	122251750	A	G	0.1754	2.913(-5.534-5.885)	0.9533	0.5184	0.732	1
1	rs142534297	80568405	G	A	NA	NA	NA	NA	0.5	1
1	rs34704551	210107045	C	A	NA	NA	NA	NA	0.5	1
2	rs144176733	66570586	T	G	NA	NA	NA	NA	0.5	1
3	rs142221779	124439970	G	A	NA	NA	NA	NA	0.5	1
7	rs114598697	152199493	A	C	NA	NA	NA	NA	0.5	1
11	rs181246540	80059714	T	C	NA	NA	NA	NA	0.5	1
11	rs146072506	82901196	T	C	NA	NA	NA	NA	0.5	1
11	rs76652740	83008777	A	G	NA	NA	NA	NA	0.5	1
11	rs114622323	115918429	T	C	NA	NA	NA	NA	0.5	1
15	rs190083650	37061325	C	T	NA	NA	NA	NA	0.5	1
15	rs74008788	37086134	G	C	NA	NA	NA	NA	0.5	1
18	rs115089532	65814470	A	C	NA	NA	NA	NA	0.5	1
22	rs11703407	38200124	G	C	NA	NA	NA	NA	0.5	1
23	rs73245703	104007736	C	G	NA	NA	NA	NA	0.5	1

Abbreviations: Chr: chromosome, SNP: single nucleotide polymorphisms, ALT: alternative allele, REF: reference allele, SE standard error, CI: confidence interval, FDR: false discovery rate, EMP: permutation p-value

For the Senegal cohort, 5 SNPs with a missing genotype rate of up to 20% were removed. Similarly, 19 SNPs with a MAF less than 0.01 (n = 19) and 3 SNPs that failed the HWE test at a p-value of 0.01 were excluded. 9 Individuals with a missing genotype rate of up to 25% were removed. After applying these QC measures, 42 SNPs and 194 individuals remained in the dataset, resulting in a genotyping rate of 93% (Table 5).

Table 5. Association of selected SNPs and BMI among Senegalese SCD cohort.

CHR	SNP	BETA	SE (CI)	P	FDR(BH)	EMP1	EMP2
8	rs1385729	-2.872	1.238(-5.298-(-0.4452))	0.02325	0.9298		
8	rs74952753	0.01441	4.034(-7.892-7.92)	0.02325	0.9972	0.1282	0.9346
1	rs75912514	3.438	1.838(-0.1638-7.04)	0.06503	0.9972	0.1438	0.9633
1	rs74697200	2.35	1.596(-0.7788-5.479)	0.1448	0.9591	0.991	1
8	rs1481608	2.119	1.456(-0.7339-4.972)	0.1495	0.9591	0.39	1
10	rs116245687	-0.9931	5.655(-12.08-10.09)	0.1799	0.9972	0.156	0.9625
18	rs292267	2.448	1.82(-1.12-6.015)	0.1828	0.9591	0.3344	1
2	rs34091478	-1.919	1.636(-5.133-1.294)	0.2451	0.9591	0.153	1
2	rs78325693	4.548	3.954(-3.202-12.3)	0.2533	0.9591	0.176	1
2	rs78579916	-2.12	1.868(-5.78-1.541)	0.2598	0.9591	0.1677	0.9993
9	rs62640055	-2.079	1.537(-5.092-0.9339)	0.2637	0.9591	0.1218	0.9305
9	rs78291268	-1.878	1.668(-5.148-1.392)	0.316	0.9591	0.3393	0.9994
11	rs12269875	-1.317	1.739(-4.747-2.091)	0.451	0.9972	0.8556	1
2	rs144176733	-5.802	7.866(-21.22-9.616)	0.4628	0.9972	0.3403	0.9996
15	rs620407	-1.95	1.704 (-5.289-1.389)	0.5559	0.9972	0.5456	1
7	rs1524779	-2.556	4.624(-11.62-6.507)	0.5819	0.9972	0.8167	1
4	rs115833017	2.858	5.684(-8.283-14)	0.6165	0.9972	0.9738	1
15	rs74008788	-2.125	4.545(-11.03-6.783)	0.6413	0.9972	0.9751	1
8	rs4872200	-0.9106	2.057(-4.942-3.12)	0.6592	0.9972	0.5712	1

15	rs190083650	-2.423	5.56(-13.32-8.476)	0.6642	0.9972	0.4794	1
5	rs113894396	0.9585	2.649(-11.62-6.507)	0.7183	0.9972	0.9211	1
2	rs77470226	-1.169	3.577(-8.179-5.841)	0.7445	0.9972	0.6601	1
9	rs10757831	1.607	1.593(-1.515-4.73)	0.7534	0.9591	0.8135	1
1	rs72681287	-0.9876	3.309(-7.474-5.499)	0.7661	0.9972	0.9468	1
1	rs34704551	1.048	3.663(-6.132-8.227)	0.7756	0.9972	0.0509	0.8199
11	rs7129598	0.8796	2.853(-4.713-6.472)	0.7795	0.9972	0.5	1
6	rs2753912	-0.4367	1.76(-3.887-3.013)	0.8047	0.9972	0.614	1
15	rs115988406	-1.217	5.628(-12.25-9.814)	0.8293	0.9972	0.3188	0.9982
15	rs4965150	-0.4253	2.128(-4.596-3.745)	0.8421	0.9972	0.5	1
11	rs8181574	0.8098	2.883(-4.841-6.461)	0.861	0.9972	0.524	1
1	rs34007314	-0.2358	3.592(-7.276-6.805)	0.9478	0.9591	0.709	1
16	rs116035686	-0.1988	3.624(-7.293-6.911)	0.9581	0.9972	0.7555	1
1	rs12752401	-0.1704	3.285(-6.608-6.268)	0.9588	0.9972	0.8461	1
11	rs76652740	-0.2388	5.597(-11.21-10.73)	0.9661	0.9972	0.8316	1
3	rs114680749	0.1979	5.549(-10.68-11.07)	0.9716	0.9972	0.1869	0.9898
3	rs148677019	0.1979	5.549(-10.68-11.07)	0.9716	0.9972	0.9738	1
11	rs146072506	-0.1868	5.575(-11.11-10.74)	0.9733	0.9972	0.9461	1
8	rs140593397	-1.433	4.547(-10.34-7.479)	0.9972	0.9972	0.5675	1
11	rs114622323	NA	NA	NA	NA	0.9364	1
15	rs34858683	NA	NA	NA	NA	0.4163	1

Abbreviations: Chr: chromosome, SNP: single nucleotide polymorphisms, ALT: alternative allele, REF: reference allele, SE standard error, CI: confidence interval, FDR: false discovery rate, EMP: permutation p-value

Overall, the results from the association tests did not reveal any statistically significant associations between the analysed SNPs and BMI traits in either the Nigeria or Senegal cohorts.

5.4 Discussion

Our study represents the first GWAS with SCD patients investigating genetic variants to BP in an African setting. Additionally, we performed a meta-analysis with data from African Americans with nearly replicated findings of variant, which are all African specific, with suggestive P values (3.981e-07) that were found for SBP and BMI, despite the limited small sample size of our cohort. Moreover, we also found that a targeted replication of variants for BMI, at nominal P values ($p<0.02$), in a small independent African cohort from Nigeria and Senegal. These findings suggest that new variants associated with BP are likely to be discovered in African populations, specifically if appropriately designed GWAS array for that highly genetically diverse population is used. For example, an African-specific variant was associated with type II diabetic Mellitus in multiple African populations[104]. This further highlights the need to expand this study to other SCD populations in Africa. To date, African populations represent only 2.5% of the global GWAS participants, contributing to nearly 8% of all identified associations[105]. The vast genetic diversity resulting from 300,000 years of human genome evolutionary history of African populations gives millions of genetic variants unique to or more prevalent in African populations[106].

We have used GWAS arrays developed from highly genetically diverse African genome sequences, specifically those provided by the H3Africa Consortium. This addresses the limitation identified in previous research, in which polygenic risk scores associated with quantitative traits in GWAS using UK Biobank samples performed poorly in African ancestry populations, with GWAS arrays that are developed on European ancestry Haplotype background [107]. The H3Africa array not only demonstrates the importance of studying African populations directly in detecting genetic associations, but also led to the discovery of new loci associated with low-density lipoprotein cholesterol in two large global studies. As the size of the African replication cohort increased, so did the transferability of genetic signals detected [106].

It is critical to incorporate a diverse range of African populations with SCD to increase our understanding of quantitative traits such as BP

or BMI heritability and other complex traits, specifically for this debilitating condition, with high susceptibility for cardiovascular complications with the constant major cause of SCD adult morality over the past 50 years [108, 109]. This comprehensive approach captures the substantial genetic variation found in major ethnolinguistic groups and diverse geographical regions within Africa, allowing more accurate and robust genetic association analyses. The vast genetic variation and the related complex haplotype structures in African populations are shown by the consistent discovery of millions of new variants[110–112]. However, GWASs involving African populations still face challenges. The use of different imputation panels found variations, which are most likely related to the vast genetic variation in the African genome, which cannot be fully reproduced by any single imputation panel. This underscores the need for tailored imputation panels or other techniques, such as whole-genome sequencing (WGS), in improving the accuracy of association studies in African populations. Furthermore, an improved version of the H3Africa array with a higher density of tagged SNPs obtained from across Africa might serve as a useful tool in future research[106].

Regarding the genetic associations we identified, we found genetic loci including independent and suggestive SNPs associated with SBP, the first locus was found on chromosome 11 in the *RP11-727A23.5* gene, the second locus was found on chromosome 18 in the *DLGAP1* gene. Two more SNPs were found on chromosome 11 in intergenic regions. RP11-727A23.5 is a long non-coding RNA (lncRNA) gene. RP11-727A23.5 is one of the lncRNAs that have been identified as differently expressed in thyroid cancer, and a study found that high expression of RP11-727A23.5 was associated with better disease-free survival in patients with thyroid cancer [113]. However, the function of RP11-727A23.5 remains unclear, and its association with BP has not been previously reported[113]. *DLGAP1* is a gene that encodes a protein known as disks large-associated protein 1 (*DLGAP1*), which localizes at the postsynaptic density and interacts with the postsynaptic density 95 (PSD95) protein[114, 115], is involved in signalling at neuronal postsynaptic densities and maintaining optimal brain function and development[114]. PSD95 is a scaffolding molecule in the brain that clusters postsynaptic proteins such as ion channels, receptors, and enzymes[116, 117]. Furthermore, PSD95 potentially acts as a scaffold to ensure proper expression and localization of potassium channels in smooth muscle cells [116, 117]. Disrupting the interaction between PSD95 and potassium channels results in downregulation of the channels, causing vasoconstriction [116, 117]. Consequently, PSD95 might play a role in diseases characterised by

abnormal vascular tone, including systemic and pulmonary arterial Hypertension[118]. Genetic variations in *DLGAP1* have been linked to several neurodevelopmental disorders, including schizophrenia, autism spectrum disorder, obsessive-compulsive disorder, and Alzheimer's disease [119–126], implicating these proteins in the pathogenesis of these diseases. Additionally, a recent GWAS identified an association between *DLGAP1* and resistant Hypertension in a Japanese population[127], and HIV related Fat loss in Thai patients[128]. Moreover, *DLGAP1* knockout mice exhibit alterations of postsynaptic density, with disruption of protein interactions[129]. Therefore, targeting *PSD95* and *DLGAP*1 could be a promising therapeutic strategy for restoring the normal potassium permeability of cell membranes and enhancing vasodilator responses in blood vessels.

For DBP, the only suggestive SNP we found was in an intergenic region. Additionally, we found one genetic locus associated with BMI, the locus is located on the *GPR39* gene on chromosome 2. *GPR39*, also known as G protein-coupled receptor 39, is a member of the ghrelin family of G protein-coupled receptors, which are a large group of cell surface receptors involved in signal transduction and are widely expressed throughout the body, with high levels of expression in adipose tissue, stomach, intestine, pancreas, liver, and kidney[130]. Zinc acts as a specific ligand for *GPR39*. Zinc is a potent activator of *GPR39* via several signalling pathways [131]. The potency and specificity of Zinc in activating *GPR39* suggest that it is a physiologically important agonist, controlling body weight [132] and maintaining neurovascular and energy homeostasis[130, 133–135]. For example, in pancreatic beta cells, *GPR39* activation by zinc leads to increased insulin secretion. Dysregulation of *GPR39* has been implicated in various neurovascular diseases, including stroke, Alzheimer's disease, and multiple sclerosis[130]. In the gastrointestinal tract, *GPR39* activation by zinc promotes gastric emptying and epithelial repair. In the brain, *GPR39* activation by zinc modulates neurotransmission and synaptic plasticity. In mice, *GPR39* activation leads to a prolonged antidepressant-like response in mice[131, 136]. *GPR39* knockout (KO) male mice exhibited worse brain injury, microvascular perfusion, and neurological function after experimental stroke compared with wild type (WT) mice. This implies that *GPR39* plays a sex-dependent role in re-establishing microvascular flow and limiting ischemic brain damage after stroke[137]. *GPR39* is also involved in the regulation of collagen deposition and mineralisation, and its absence leads to altered bone composition and higher mineral-to-matrix ratio[138]. *GPR39* has been shown to be involved in lipolysis via the regulation of hormone-sensitive lipase and adipose triglyceride lipase in mice. *GPR39*

KO mice have been shown to have significantly increased body weight compared with WT littermates, and this was attributed to decreased lipolytic capacity rather than increased food intake[139]. In humans, a GWAS study that investigated the contribution of rare copy number variations (CNVs) in paediatric cases of obesity identified a maternally inherited 634 kb duplication at 2q21.2 encompassing the promoter region and exon 1 of *GPR39* in a male proband 6-0210-03 (BMI+2.41 SD) with early-onset obesity and fatty liver and high triglycerides[135]. This CNV was classified as potentially clinically relevant given the known involvement of *GPR39* in energy homeostasis. The identified SNP on the *GPR39* gene suggests a potential role in BMI regulation. However, further investigations are needed to fully understand to the functional implications of the genetic variants on BMI variability and explore *GPR39*'s potential as a therapeutic target.

Identified variants in intergenic regions may play a role in specific genes regulating BP or BMI. In vitro experiments can help explain the functional consequences of suggestive variants identified in this study.

In SCD, a previous study identified suggestive candidate loci in the *DRD2* gene on chromosome 11 to be associated with SBP in SCD children [16], however, this was not significant in our study. GWAS of Hypertension and BP in 1,017 African Americans identified several significant genes associated with SBP in or near the genes: *PMS1, SLC24A4, YWHA7, IPO7,* and *CACANA1H*[140]. However, no variant reached genome wide significance for association with DBP[140]. A recent multi-ancestry GWAS investigating the gene-smoking interaction related to BP traits, specifically mean arterial pressure (MAP) and pulse pressure (PP). The study encompassed 129,913 individuals in stage 1 and further analysed an additional 480,178 individuals in stage 2. The study identified 38 novel loci associated with the BP traits emphasizing the necessity of taking gene-environment interactions into account, specifically in studies where genomic and lifestyle differences can contribute to the discovery of novel findings[141]. A recent study in Africa found that in SCD patients, long survival to be linked to deleterious mutations in *CLCN6* gene[15]. Rare, deleterious mutations in this gene have been associated with lower BP [15]. In the general population, there are limited studies that have looked at the genetics of BP and BMI in SCD patients with African ancestry. Furthermore, there are only a handful of GWASs on African-ancestry participants[85] and even fewer on continental African populations that have looked at BP[105, 142–144]. To overcome this limitation, we conducted a meta-analysis by combining Cameroon

data and the African American SITT cohort, and observed low heterogeneity in the two cohorts; however, we could not replicate our initial findings, even though a suggestive trend was found, despite the small sample size, and can be considered as an encouraging outcome to focus on functional analysis on these genes and their role in modulating BP. This may still be due to a small sample size or other potential source of heterogeneity such as genotyping methods or population characteristics. For instance, African Americans are a population that has undergone significant admixture between African and European ancestry.

None of our suggestive SNPs (rs146072506, rs58269424, rs35715722, rs12282739, rs75820288, rs9955087, rs186536474, rs79063750, rs73445228) have been reported to be associated with BP or BMI in previous GWASs. These SNPs might uniquely be associated with BP in the African population or SCD patients only, because comparative MAF found that these variants are mostly African specific. These suggestive SNPs are potential candidates for replication analysis in SCD patients or the African population with a large sample size. It is also difficult to compare our findings to previous studies because of the variations between population characteristics, phenotypic measures, and genotypic analyses could cause bias when comparing different studies.

Although our study did not uncover significant genes associated with both BP and BMI, the Venn diagram revealed common genes shared by both SBP and DBP when a cut-off 1x10-5 was used. This points to the possibility of shared pathways underlying these two components of BP. Few studies have explored SNPs, genes and pathways associated with both BMI and BP. A recent study found genetic correlations of 0.2108 for BMI-SBP, 0.2345 for BMI-DBP, and 0.6942 for SBP-DBP using a bivariate Cholesky decomposition model[145]. The genetic and environmental influences on the phenotypic correlations between BMI and BP could be estimated using this approach. But given there are still uncovered common genetic mechanism related to both cardiovascular and metabolic traits, more studies need to be performed particularly in both African populations and individuals living with SCD.

It is important to acknowledge some limitations of our study. To begin, in our replication cohorts association tests did not reveal any statistically significant associations between selected SNPs and our phenotype of interest, this may be due to the limited samples sizes, and

poor genotyping data quality, for instance the Nigeria cohort had a low genotyping rate of 62.32% after QC which indicates a substantial number of SNPs failing the QC and our replication sample size was small, with only 31 individuals passing QC, reducing the statistical power to detect meaningful associations between genetic variants and the phenotype of interest. To overcome this, using a larger sample size from multiple cohorts may be beneficial; however, it may also lead to biased results as BP measurements may vary across different groups. Second, our phenotype of interest (BP) was measured at a single time point, which might have increased some patients' likelihood of developing white-coat Hypertension. Previous studies have highlighted the importance of 24-h ambulatory BP monitoring in diagnosing masked Hypertension. Furthermore, we have not yet proved a functional analysis that supports the implication of the genes found in the pathobiology of BP and BMI variation in SCD. These could be performed in future studies in vitro experiments including expression studies, epigenetic profiling, or gene knockout experiments to provide insights into the underlying mechanisms and how these genetic variants contribute to BP and BMI abnormalities in SCD.

The results of this study provide valuable insights into the genetic factors that may play a role in BP and BMI in SCD patients from Cameroon. However, further research is required to replicate and confirm these findings through larger sample sizes, particularly among African individuals with SCD. A large sample size would provide greater insight into the genetic makeup that influences BP and BMI. Furthermore, functional studies are needed to fully understand the mechanisms by which these genetic variants influence BP and BMI. Meta-analysis of studies from diverse populations can increase the power for detecting associations across multiple populations. In addition, future studies should also consider other environmental and lifestyle factors that may interact with genetic factors to affect BP and BMI in SCD patients.

In summary, our study represents the first GWAS looking at genetic association and BP in an African setting from Cameroon in the setting of SCD. The first locus was found in the *RP11-727A23.5* on chromosome 11(rs146072506, P=4.88x10-08), and the second locus was found on the *DLGAP1* gene on chromosome 18 (rs35715722, P=3.40x10-08). Two other SNPs were found in the intergenic region. For DBP, the only significant SNP was in the intergenic region. Additionally, we found one genetic locus associated with BMI, the locus located

on the *GPR39* gene (rs186536474, p=8.90x10-09) on chromosome 2. Identified variants in intergenic regions may play a role in specific genes regulating BP or BMI. Interestingly, most significant associated variants for SBP, DBP, and BMI are African specific. The P-values for 3 variants associated were all suggestive around e-07. These results suggest that these SNPs are worthy of further investigation in larger studies and further investigation into their functional consequences. Identifying genetic variants associated with BP and BMI in African population has important implications for the prevention and treatment of RSH and Systemic Hypertension. By understanding the specific genes that contribute to RSH and Systemic Hypertension in SCD patients, researchers can develop more targeted and effective treatment strategies tailored to this specific population. Furthermore, identifying genetic risk factors for Hypertension may allow for earlier detection and intervention, potentially reducing the risk of complications such as stroke and kidney disease.

Chapter 6: Overall Discussion and conclusion

Synopsis: This chapter provide an overall conclusion of the thesis based on the manuscripts presented.

Conclusions and recommendations

This is the first in-depth investigation on the non-genetic and genetic risk factors associated with BP variation (RSH or Systemic Hypertension) in SCD patients in Cameroon, one of the rare attempts from Africa. Our analysis is based on a well-characterized homogenous study population in a resource-limited country. Therefore, these findings expand the understanding of risk factors for RSH and Systemic Hypertension in SCD beyond what has been reported from resource-limited settings. Our study includes a first meta-analysis that brings together data on BP estimates from multiple SCD studies (Chapter 3, Figure 2). Our pooled estimates (Chapter 3) illustrate that systolic BP, diastolic BP, and mean arterial pressure were lower in SCD patients. Pulse pressure and pulse rate were higher in SCD patients than in the general population, with heterogeneity ranging from low to high. Contributors to this unexplained heterogeneity may be due to various study methodologies, study settings, populations, and periods studied in our included studies. Most of the included studies were from North America, which highlights a gap in comparable studies from lower-income countries, which carry the greatest burden of SCD. All the included studies in our meta-analysis recruited study participants from SCD clinics or referral hospitals. Thus, these findings may not provide a clear prevalence of high BP in the whole SCD population.

Our analysis from this relatively large Cameroonian study provides a snapshot of the burden and risk factors for RSH and Systemic Hypertension in SCD. We found evidence of the prevalence of RSH and Hypertension in SCD patients in Cameroon. Age, male gender, and Body Mass Index were found to be independently associated factors of RSH and Hypertension in SCD patients in Cameroon (Chapter 4). These findings enable us to develop prediction models and targeted interventions for SCD patients.

Additionally, after adjusting for multiple testing, we found only suggestive loci associated with BP in Cameroonian SCD patients were identified in our GWAS (Chapter 5). Therefore, it is crucial to interpret these findings within the context of the study sample size. These findings highlight gaps in available datasets and suggest strategies for future studies in similar settings. Genetic diversity is high within African populations because of a deep evolutionary history, population admixture, and genetic drift. Therefore, it is vital for future studies to capture contributions from African studies and to understand the functional and biological relevance of associated SNPs.

Our study is without limitations, the exclusion of incomplete records with missing BP might have introduced some biases. Additionally, BP was measured at a single time point, which might have increased some patients' likelihood of developing white-coat Hypertension. Previous studies have highlighted the importance of 24-hour ambulatory BP monitoring in diagnosing masked Hypertension. Lastly, the inability to follow up the cohort as a longitudinal study is a limitation.

The major themes that arose in this study are the need to establish the optimal BP threshold above which to initiate therapy for patients with SCD and the age-specific definition of BP based on BP percentiles to facilitate comparison between studies. A need for large clinical trials and longitudinal studies to determine whether BP interventions can reduce associated complications in SCD patients as they are in the general population.

New developments in treatments such as gene therapy and CRISPR may provide promising avenues for improving patient outcomes, but further research is needed to determine their efficacy in SCD patients with RSH or Hypertension. Globalised societal trends, including lifestyle changes and access to healthcare, may affect the prevalence and incidence of high BP in SCD patients in the future. Therefore, there is a need for longitudinal studies and surveillance programs to monitor health indicators. It is also essential to collaborate with global health organisations such as the World Health Organization (WHO) to obtain data on the prevalence and incidence of high BP in SCD patients in different regions of the world. This information can assist in detecting trends and patterns and evaluating the influence of globalisation on the well-being of SCD patients.

The planned SickleInAfrica consortium cohort studies may provide important insights into the burden of RSH and Hypertension in SCD patients in different African populations and inform the development of targeted interventions for improving patient outcomes. Overall, research in this area is important for improving the health outcomes of SCD patients and reducing the burden of Hypertension in this population. Moving forward, it is critical to enhance genetic research in African communities to fill information gaps and improve the inclusivity of genetic studies. We can identify unexpected genetic associations and obtain a deeper knowledge of the genetic architecture of complex traits by including more diverse African populations and capturing the substantial genetic variation across different ethnic groups and geographic regions. Collaborative efforts including African scholars, doctors, and politicians, as well as international collaborations will be critical in accomplishing these objectives.

Recommendations:

- Personalised early interventions aimed at lowering elevated BP may improve life expectancy among SCD patients by inhibiting progression to pulmonary arterial Hypertension, advanced renal disease, and other vascular complications. However, high-quality evidence from clinical trials is needed to guide the initiation of therapy and treatment goals in SCD patients.
- Public health interventions such as population-based health education and lifestyle modification within a cultural context may play a role in preventing elevated BP in SCD patients. However, large clinical trials and longitudinal studies should be conducted to determine whether BP interventions can reduce associated complications in SCD patients as they are in the general population.
- There is a need to establish the optimal BP threshold above which to initiate therapy for patients with SCD and an age-specific definition of BP based on BP percentiles to facilitate comparison between studies.
- Genetic diversity is high within African populations; therefore, it is important for future studies to capture contributions from African studies and to understand the functional and biological relevance of associated SNPs. Improved methods need to be

developed to understand and compare heritability across populations and study participants from other parts of the African continent.

- For enhanced discovery in GWAS in Africans, future studies should aim for increased sample size for multiple BP-related traits to enhance discoverability across phenotypes, the use of Africa-centric genotyping arrays, and larger imputation reference panels that adequately represent African genetic diversity.
- In a resource-limited setting, family-based and candidate gene studies could be a practical approach to GWAS. These methods require moderate resources and smaller sample sizes and focus on specific genetic variants or families affected by SCD/BP. They offer insights into rare, highly penetrant genetic variants and specific pathways associated with these conditions. In contrast, the GWAS approach requires substantial resources and large sample sizes to detect common genetic variants associated with SCD/BP traits, which presents challenges in resource-limited African contexts.

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SBP								
No	**Genomic loci**	**unique idenfier**	**RS ID**	**Chromosome**	**pos**	**P-values**	**nSNPs**	**nGWASSNPs**
1	1	1:89361718:G:T	rs144705948	1	89361718	1.19318e-07	3	1
2	2	9:104927079:C:T	rs66504946	9	104927079	2.67556e-07	83	64
3	3	10:5698497:A:G	rs548633149	10	5698497	1.13506e-07	4	4
4	4	11:82901196:C:T	rs146072506	11	82901196	4.88915e-08	8	8
5	5	11:123787275:C:T	rs58269424	11	123787275	1.82886e-08	27	19
6	5	11:123800063:C:T	rs1453647	11	123800063	2.03881e-07	4	3
7	5	11:123802399:A:G	rs970584	11	123802399	3.935e-07	34	26
8	6	18:3534322:A:G	rs35715722	18	3534322	3.40829e-08	13	11
DBP								
No	**Genomic loci**	**unique idenfier**	**RS ID**	**Chromosome**	**pos**	**P-values**	**nSNPs**	**nGWASSNPs**
1	1	3:31357861:A:G	rs75820288	3	31357861	2.90295e-08	8	8
2	2	9:28434097:C:T	rs10968596	9	28434097	1.89432e-07	9	7
BMI								
No	**Genomic loci**	**unique idenfier**	**RS ID**	**Chromosome**	**pos**	**P-values**	**nSNPs**	**nGWASSNPs**
1	1	1:165814202:C:T	rs141022785	1	165814202	3.57535e-07	3	3
2	2	7:101510288:A:G	rs62463725	7	101510288	2.6064e-07	14	12
3	3	8:105505880:A:C	rs150821706	8	105505880	3.43664e-07	1	1
4	4	8:114989663:C:G	rs35975974	8	114989663	1.68806e-07	7	3
5	5	10:5687043:A:G	rs11256695	10	5687043	4.55372e-07	1	1
6	6	12:98209127:C:T	rs79063750	12	98209127	8.61816e-08	2	1
7	7	14:79673001:A:G	rs186913463	14	79673001	4.82081e-07	2	1
8	8	18:58243696:C:G	rs113207711	18	58243696	1.78937e-07	2	2
9	8	18:58243950:A:G	rs9955087	18	58243950	3.37879e-09	22	1

Supplementary 1: Independent significant SNPs (r2<0.6) identified from SBP GWAS.

Supplementary 2: Lead SNPs identified from independent significant SNPs of SBP GWAS.

SBP								
No	**Genomic loci**	**Unique Identifier**	**RS Id**	**Chromo-some**	**Position**	**P-values**	**nInd-SigSNPs**	**IndSigSNPs**
1	1	1:89361718:G:T	rs144705948	1	89361718	1.19318e-07	1	rs144705948
2	2	9:104927079:C:T	rs66504946	9	104927079	2.67556e-07	1	rs66504946
3	3	10:5698497:A:G	rs548633149	10	5698497	1.13506e-07	1	rs548633149
4	4	11:82901196:C:T	rs146072506	11	82901196	4.88915e-08	1	rs146072506
5	5	11:123787275:C:T	rs58269424	11	123787275	1.82886e-08	2	rs58269424;rs970584
6	5	11:123800063:C:T	rs1453647	11	123800063	2.03881e-07	2	rs1453647;rs970584
7	6	18:3534322:A:G	rs35715722	18	3534322	3.40829e-08	1	rs35715722
DBP								
No	**Genomic loci**	**Unique Identifier**	**RS Id**	**Chromo-some**	**Position**	**P-values**	**nInd-SigSNPs**	**IndSigSNPs**
1	1	3:31357861:A:G	rs75820288	3	31357861	2.90295e-08	1	rs75820288
2	2	9:28434097:C:T	rs10968596	9	28434097	1.89432e-07	1	rs10968596
BMI								
No	**Genomic loci**	**Unique Identifier**	**RS Id**	**Chromo-some**	**Position**	**P-values**	**nInd-SigSNPs**	**IndSigSNPs**
1	1	1:8055457:A:T	rs184568557	1	8055457	3.89418e-07	1	rs184568557

2	2	1:40287191:G:GGAAAAGAAAA-GAAAA	rs149530974	1	40287191	1.37066e-07	1	rs149530974
3	3	1:165814202:C:T	rs141022785	1	165814202	2.82142e-07	1	rs141022785
4	4	2:133337559:C:T	rs186536474	2	133337559	8.90144e-09	1	rs186536474
5	5	6:69223424:A:G	rs9346211	6	69223424	4.49117e-07	1	rs9346211
6	6	8:114989663:C:G	rs35975974	8	114989663	3.53729e-08	1	rs35975974
7	7	10:80636009:A:G	rs499918	10	80636009	4.01851e-07	1	rs499918
8	8	12:98209127:C:T	rs79063750	12	98209127	2.32577e-07	1	rs79063750
9	9	16:55173276:C:T	rs8063222	16	55173276	3.09847e-07	1	rs8063222
10	10	18:58260605:C:T	rs73445228	18	58260605	1.27271e-08	1	rs73445228

Supplementary 3: Prioritized genes from oral ulcers GWAS by functional mapping.

SBP																			
Ensg	Symbol	Chro-mosomes	Start	End	Strand	Type	entrezID	HUGO	pLI	ncRVIS	posMap-SNPs	posMapMaxCADD	eqtlMap-SNPs	eqtlMap-minP	eqtlMapminQ	eqtlMapts	eqtlDirection	minGwasP	IndSigSNPs	Genomic loci
ENSG00001379477	GTF2B	1	89318615	89357627	-1	protein_coding	2959	GTF2B	0.489078694212667	-0.36516047	1	4.482	0	NA	NA	NA	NA	1.19318e-07	rs144705948	1
ENSG0000136881	BAAT	9	104122699	104145801	-1	protein_coding	570	BAAT	7.963750420417 63e-06	1.24585 4727	0	0	1	1.60996e-05	0.0452424	GTEx/v7/Pan-creas	+	5.46069e-06	rs66504946	2
ENSG00001963 72	ASB13	10	56808300	57085558	-1	protein_coding	7975 4	ASB13	0.257156363 6997	3.20907 8603	4	7.393	0	NA	NA	NA	NA	1.13506e-07	rs548633149	3
ENSG00001654 94	PCF11	11	82868030	82898493	1	protein_coding	5158 5	PCF11	0.999999873982097	0.23526 4814	1	0.246	0	NA	NA	NA	NA	4.88915e-08	rs146072506	4

ENSG00001374944	ANKRD42	11	82904781	82971736	1	protein_coding	33	ANKRD42	2.600393e-08	-0.18132389161	3	1.302	0	NA	NA	NA	NA	4.8915e-08	rs146072506	4
ENSG00001662557	SCN3B	11	123499589	123525952	-1	protein_coding	55	SCN3B	0.3472979421	-0.4129321477	0	0	1	5.9755e-07	0.0035063	GTEx/v7/Heart_Atrial_Appendage	-	3.935e-07	rs970584	5
ENSG00001662612	ZNF202	11	123594884	123612538	-1	protein_coding	77	ZNF202	0.1387432978747912	-0.6023090922	0	0	39	8.1072e-08	5.54404e-29	GTEx/v8/Pancreas:GTEx/v8/Stomach:GTEx/v8/Testis:GTEx/v7/Pancreas	+	1.8286e-08	rs58269424;rs970584	5
ENSG00002043300	TMEM225	11	123753637	123756349	-1	protein_coding	33	TMEM225	0.5410945573884445	0.4829319547	0	0	40	9.6002e-08	3.28419e-47	GTEx/v8/Testis	-	1.8286e-08	rs58269424;rs970584	5
ENSG00001815188	OR8D4	11	123737710	123781863	1	protein_coding	33	OR8D4	0.0504863379478637861	0.2274321338	8	8.15	0	NA	NA	NA	NA	1.8286e-08	rs58269424;rs970584	5
ENSG00001710145	OR4D5	11	123810259	123811344	1	protein_coding	21	OR4D5	0.0018675711430402796	0.04155967598	25	10.11	0	NA	NA	NA	NA	8.6325e-08	rs1453647;rs970584;rs58269424	5
ENSG00001016144	OR6T1	11	123813813	123814184	-1	protein_coding	74	OR6T1	7.9619444418434	0.677734	17	7.26	16	7.3714	0.0123422	GTEx/v8/Testis	+	8.6325e-08	rs970584;rs58269424	5

81499			492	580		ding			611e-05	3787				3e-06				e-08		
ENSG00001826334	OR10G7	11	123908773	123909717	-1	protein_coding	390265	OR10G7	0.00712210899720655	-0.10399385	0	0	2	0.000440054	0.02975032483899 86	PsychENCODE_eQTLs	-	2.03881e-07	rs1453647	5
ENSG00001100 02	VWA5A	11	123986069	124018428	1	protein_coding	4013	VWA5A	1.886236820 85499e-15	1.775706905	0	0	1	1.0793e-06	0.00329744279 946164	eQTLGen_cis_eQTLs	-	3.935e-07	rs970584	5
ENSG00001705 79	DLGAP1	18	34960330	44553335	-1	protein_coding	9229	DLGAP1	0.9930455 1428 3556	-0.68600 5455	13	10.5	0	NA	NA	NA	NA	3.40829e-08	rs35715722	6
DBP																				
Ensg	**Symbol**	**Chro-mosomes**	**Start**	**End**	**Strand**	**Type**	**entrezID**	**HUGO**	**pLI**	**ncRVIS**	**posMap-SNPs**	**posMapMaxCADD**	**eqtlMap-SNPs**	**eqtlMap-minP**	**eqtlMapminQ**	**eqtlMapts**	**eqtlDirection**	**minGwasP**	**IndSigSNPs**	**Genomic loci**
ENSG00001744 82	LINGO2	9	27948076	28670283	-1	protein_coding	158038	LINGO2	0.8071673 0386 1535	-0.93573 1919	9	11.85	0	NA	NA	NA	NA	1.89432e-07	rs10968596	2
BMI																				
Ensg	**Symbol**	**Chro-moso**	**Start**	**End**	**Stra**	**Type**	**entre**	**HUGO**	**pLI**	**ncRVIS**	**posM**	**posMapMax**	**eqtlM**	**eqtlM**	**eqtlMapminQ**	**eqtlMapts**	**eqtlDirec**	**minGw**	**IndSigSNPs**	**Genomic**

		mes			nd		zID				ap-SNPs	CADD	ap-SNPs	ap-minP			tion	asP		loci
ENSG00000049249	TNFRSF9	1	7979907	8000926	-1	protein_coding	3604	TNFRSF9	0.253050500972917	-0.271623752	5	4.669	0	NA	NA	NA	NA	NA	rs184568557	1
ENSG00000116288	PARK7	1	8014351	8045565	1	protein_coding	11315	PARK7	0.903949397411231	0.0858881436	16	12.29	0	NA	NA	NA	NA	3.89418e-07	rs184568557	1
ENSG00000116285	ERRFI1	1	8064464	8086368	-1	protein_coding	54206	ERRFI1	0.046551582399882	0.149372713	7	12.29	0	NA	NA	NA	NA	3.89418e-07	rs184568557	1
ENSG00000143179	UCK2	1	165796768	165880855	1	protein_coding	7371	UCK2	0.962082764358181	NA	3	4.776	0	NA	NA	NA	NA	2.82142e-07	rs141022785	3
ENSG00000183840	GPR39	2	133174147	133404132	1	protein_coding	2863	GPR39	1.54608229502499e-12	-0.108893739	1	8.266	0	NA	NA	NA	NA	8.90144e-09	rs186536474	4
ENSG00000108175	ZMIZ1	10	80828792	81076276	1	protein_coding	57178	ZMIZ1	0.999743700872505	-0.003623491	0	0	6	5.3967e-08	8.03746e-07	GTEx/v8/Thyroid:GTEx/v7/Thyroid:GTEx/v6/Thyroid	+	4.01851e-07	rs499918	7

ENSG00001768442	IRX5	16	54964774	54968397	1	protein_coding	1026 5	IRX5	NA	NA	0	0	2	7.0278 4e-17	3.51392e-13	eQTLcatalogue/GENCORD_ge_fibroblast	+	3.0984 7e-07	rs8063222	9

Supplementary 4: Enriched gene sets identified by prioritized genes from SBP GWAS.

Category	GeneSet	N_genes	N_overlap	P-values	adjP	genes	link
GWAS catalog	Serum uric acid levels in response to allopurinol in gout	27	4	3.11122761117386 87e-09	5.64687811428057 2e-06	TMEM225:OR8D4:OR4D5:OR6T1	
GWAS catalog	Response to citalopram or escitalopram in depression	10	2	2.22781715238048 2e-05	0.02021744065785 2876	SCN3B:ZNF202	
Positional_gene_sets	chr11q24	88	8	4.22829626692334 2e-16	1.26426058381007 94e-13	SCN3B:ZNF202:TMEM225:OR8D4:OR4D5:OR6T1:OR10G7:VWA5A	http://www.gsea-msigdb.org/gsea/msigdb/cards/chr11q24
KEGG	KEGG_OLFACTORY_TRANSDUCTION	383	4	0.00013472113465 994864	0.02505813104675 0445	OR8D4:OR4D5:OR6T1:OR10G7	http://www.gsea-msigdb.org/gsea/msigdb/cards/KEGG_OLFACTORY_TRANSDUCTION
DBP (no significant gene set)							
Category	**GeneSet**	**N_genes**	**N_overlap**	**P-values**	**adjP**	**genes**	**link**

NA	NA	NA	NA	NA	NA	NA	NA
BMI							
Cate-gory	**GeneSet**	**N_ genes**	**N_ overlap**	**P-values**	**adjP**	**genes**	**link**
GWAS catalog	Response to platinum-based chemotherapy in non-small-cell lung cancer	10	2	2.45429902875589e-06	0.00445455273719 1948	NRXN3:LRP12	

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ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/ierr20

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To cite this article: Arthemon Nguweneza, Chandré Oosterwyk, Kambe Banda, Victoria Nembaware, Gaston Mazandu, Andre P Kengne & Ambroise Wonkam (2022) Factors associated with blood pressure variation in sickle cell disease patients: a systematic review and meta-analyses, Expert Review of Hematology, 15:4, 359-368, DOI: 10.1080/17474086.2022.2043743

To link to this article: https://doi.org/10.1080/17474086.2022.2043743

Published online: 28 Feb 2022.

EXPERT REVIEW OF HEMATOLOGY
2022, VOL. 15, NO. 4, 359–368
https://doi.org/10.1080/17474086.2022.2043743

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META-ANALYSIS

Factors associated with blood pressure variation in sickle cell disease patients: a systematic review and meta-analyses

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ABSTRACT

Objectives: Blood pressure (BP) values ≥120/70 mmHg considerably increase the risk of pulmonary hypertension and renal dysfunction in Sickle Cell Disease (CSD) patients and ultimately increased morbidity and mortality. This has led to the development of the term relative systemic hypertension (RSH). RSH was defined as Systolic BP 120–139 mm Hg or diastolic BP 70–89 mm Hg, whereas systemic hypertension is defined as Systolic BP ≥ 140 mm Hg or diastolic BP ≥ 90 mm Hg. Systematic identification of BP variations and risk factors in SCD patients could promote effective management. This review aimed to identify factors associated with BP variation among SCD patients.

Methods: We searched PubMed, Scopus, Web of Science, and Google Scholar up to December 2020 with no geographical or language restrictions. Two reviewers independently screened and summarized data from eligible studies.

Results: Advancing age, gender, higher body weight, hemoglobin, eGFR, triglycerides, greater hematocrit, higher blood viscosity, history of blood transfusion, and targeted variants in *DRD2* and *MIR4301* genes were independently associated with the risk of hypertension in SCD patients.

Conclusion: Interventions that consider these risk factors may potentially contribute to lower BP pressure in SCD patients and prevent the development of severe complications.

ARTICLE HISTORY
Received 9 June 2021
Accepted 15 February 2022

KEYWORDS
Relative hypertension; risk factors; sickle cell; blood pressure; systolic; diastolic

1. Introduction

Sickle cell disease (SCD) is an inherited, autosomal, recessive condition caused by several mutations in the β-globin gene (*HBB*). SCD encompasses any one of the syndromes, which arise from the mutation that leads to substitution of valine for glutamic acid in a single nucleotide polymorphism (SNP) in the sixth codon of the *HBB* gene on chromosome 11. This mutation leads to the production of sickle hemoglobin (HbS), with altered physical properties of hemoglobin (Hb) [1–3]. According to a systematic review by the Global Burden of Disease Study, 3.2 million people live with SCD, 43 million people have sickle cell trait (i.e. are carriers of the mutation), and 176,000 people die due to SCD-related complications per year [4]. The most common and the most severe form of SCD is homozygous HbSS (sickle cell anemia), resulting from the inheritance of β^S gene from both parents. The HbSS genotype accounts for 70% of the cases of SCD in patients of African ancestry. Other forms of SCD include the inheritance of the β^s gene in combination with hemoglobin C or β-Thalassemia mutations, resulting in HbSC or HbSβThal genotypes, respectively [1,2].

SCD patients show substantial heterogeneity in the presentation and clinical course of the disease [2]. Typically, the pathobiology of SCD is a vicious cycle of four major processes. First, under deoxygenation conditions, HbS molecules polymerize to form bundles. The polymer bundles form long fibers that impair the erythrocyte membrane, leading to erythrocyte sickling. Second, the normally freely flowing cytosol of the erythrocyte becomes viscous making the cell much less deformable. This leads to the blockage of blood vessels, disrupting the blood supply, referred to as vaso-occlusion. Thirdly, the polymer bundles also promote hemolysis causing chronic anemia with Hb levels ranging from 6 to 11 g/dl. The severity of this anemia is dependent on primary genotype and genetic modifiers of fetal hemoglobin levels, the strongest known modifier of the SCD clinical expression. Patients with higher rates of hemolysis are more likely to develop vascular injury and multi-organ dysfunction. Finally, hemolysis by-products such as erythrocyte damage-associated molecular patterns (eDAMPS) along with ischemia-reperfusion injury caused by vaso-oclussion, promote sterile inflammation and oxidative stress. These events further amplify vaso-occlusion through a feedback loop by promoting adhesion of neutrophils, platelets, and endothelial cells. These molecular, cellular, and biophysical processes work in synergy to promote acute and chronic pain, ischemic reperfusion injury, and infarctions of vital organs, such as spleen, heart, kidneys, liver, muscle, brain, lung, and bone.

While in sub-Saharan Africa, without treatment, most babies born with SCD rarely live beyond 5 years of age [5,6] the life

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expectancy has improved drastically in high-income countries; This is mainly due to the widely implemented new-born screening, followed by a comprehensive care, including penicillin prophylaxis, pneumococcal immunization, availability of red cell transfusions, and use of the fetal hemoglobin-inducing therapy Hydroxyurea. Some patients have the potential to live well into their 7th decade [7]. However, with improved life expectancy, cardiovascular complications become more common, due to persistent intravascular hemolysis [8]. Cardiovascular complications such as proliferative systemic vasculopathy, left ventricular diastolic dysfunction and pulmonary hypertension have been reported. These factors are known predictors of mortality in SCD patients [7,9].

SCD patients commonly have lower diastolic, systolic, and mean blood pressure in comparison to the general population. Notably, the incidence of pulmonary hypertension and renal insufficiency at relatively normal BP (≥120/70 mmHg) have been reported in some SCD patients [10]. Previous studies in SCD patients have labeled this phenomenon 'relative systemic hypertension' (RSH) [10]. RSH was defined as Systolic BP (SBP) 120–139 mm Hg or diastolic BP (DBP) 70–89 mm Hg and systemic hypertension as SBP 140 mm Hg or DBP 90 mm Hg or higher.

BP is a potential modulator of clinical severity in SCD patients, recurrent deleterious and loss of function mutation with genes associated with lowering BP has been recently associated with long survival in SCD in Africa [11]. Identifying factors associated with BP variation is key to controlling BP, as well as preventing associated causes of mortality in SCD patients. Herein, we present a systematic review of risk factors associated with BP variation in SCD patients.

2. Patients and methods

2.1. Protocol registration

This review was registered with the International Prospective Register of Systematic Reviews (PROSPERO), registration number CRD42020168798.

2.2. Search strategy and eligibility criteria

The Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) served as a template for reporting the present review [12].

Two reviewers (AN and CO) with the help of an expert llibrariandeveloped a comprehensive literature search strategy (Figure 1) in the following databases:

- MEDLINE/PubMed.
- Scopus.
- Web of Science.

The PubMed database search syntaxes are presented in Appendix 1. This syntax is a combination of MeSH terms, keywords, and tags and was adapted for other databases. AN and CO searched for studies using the following three concepts: SCD patients, risk factors, and blood pressure. The Boolean operators 'OR' were used with synonyms within each concept and then the search results for different concepts were combined with the 'AND' operator. In addition, AN and CO searched other sources using Google Scholar search engine and checked the reference lists of relevant studies. AN and CO completed the search process by manually searching Google. All the identified articles were imported into Mendeley (reference manager) software. Duplicate studies from the different electronic databases were removed through the Mendeley reference manager. Independently, AN and CO screened titles and abstracts of the search results for potentially eligible studies. Full texts of the remaining articles were retrieved and further assessed independently by AN and CO using eligibility criteria. Any discrepancies were resolved through consensus.

The following exclusion and inclusion eligibility criteria were used to screen full text:

- Only observational studies addressing the risk factors of blood pressure variation among SCD patients were included.
- Risk factors included but not limited to age, sex, demographic and genetic variants, epigenetic, socio-economic, psychological, anthropometric, biological, and disease-related factors.
- We excluded review articles without original data, case reports and expert opinion commentaries, and studies whose main outcome was not BP in SCD patients.
- Eligible studies were included regardless of language, year of publication or geographical location.

2.3. Assessment of the methodological quality

The Newcastle-Ottawa Scale (NOS) and Q-Genie were used to evaluate the methodological quality of observational and genetic studies included in this review, respectively. Since there was no validation study to provide a cutoff score for rating low-quality studies, we considered 0–4, 5–7, and 8–10 stars as indicative of high, moderate, and low risk of bias, respectively. Two investigators (AN and CO) independently assessed the study quality and disagreements were resolved by consensus.

2.4. Data extraction

The study inclusion criteria were the study population, the study design, the country, the outcomes. Relevant data were independently extracted by the two reviewers (AN and CO) using a standardized extraction form in Microsoft Excel. The data extracted included the citation, language of the paper, study period, study location, study objectives, study design, study period, characteristics of participants, sample size, and sampling technique, explanatory and outcome variables, and the major findings. Specific data such as demographic and socio-economic characteristics, any anthropometric measurements, and blood pressure measurement procedures were also extracted. The risk factors that are associated with BP variations were categorized into demographic, anthropometrical, biological, and genetic.

2.5. Data synthesis

We conducted as a metanalysis and a narrative synthesis where a meta-analysis was not possible and descriptive synthesis. The descriptive synthesis included (a) PRISMA Flow; (b) a quality assessment; (c) a description of the study characteristics and findings (Table 2); and (d) a narrative synthesis. Furthermore, the results of meta-analysis are presented as a forest plot (Figure 2). Ninety-five-percent confidence intervals (CI) and the standardized mean difference (std Mean difference) were calculated for each study and used for the meta-analysis. We use inverse-variance weighted random effects meta-analysis to pool the study-specific estimates. The choice of a random effects model was made *a priori* to allow for inter-study heterogeneity of results. The inter-study heterogeneity was evaluated by the X^2 test on Cochrane's Q statistic and its magnitude estimated using the I^2 values. These values represent a percentage of variation in effect size between studies that can be attributed to genuine variability rather than chance. The I^2 values of 25%, 50%, and 75% represent low, medium, and high heterogeneity, respectively. Quantitative data was pooled in a statistical meta-analysis using Review Manager (RevMan) software.

3. Results

3.1. The review process

The database search yielded 2477 citations and further 30 articles were identified by manual screening of citations (Figure 1). After the elimination of duplicates, 2420 records remained. After screening titles and abstracts, we found that 2325 records were irrelevant and excluded them. After assessing the full texts of the remaining 95 papers for eligibility, 77 of them were excluded. Sixteen studies were part of the qualitative synthesis, and seven studies were included in the meta-analysis (Figure 1).

3.2. Assessment of risk of bias

All 16 studies included in the review were considered for critical appraisal. The results of the critical appraisal were used to generate a description of the risk of bias for each included study (Table 1). Studies were classified as having low (n = 2), moderate (n = 12) and high (n = 2) risk of bias. In most cases, the selection of non-representative samples was the source bias.

3.3. Description of study characteristics

The studies' sample sizes ranged from 38 to 3317 participants. Study participants' mean age ranged from 6 to 81 years. Included studies were published between 1981 and 2018. Studies had a cross-sectional (n = 9), retrospective cohort (n = 6), and genome-wide meta-analysis (n = 1) design. The study settings included outpatient or in-hospital SCD participants. None of the studies included community-based recruitment of SCD participants. Studies were carried out in North America (n = 12), in Africa (=3) and Europe (n = 1). Details on the characteristics of the 16 included studies are reported in Table 2. Studies have described BP and risk factors associated with either SCD patients versus healthy controls or SCD patients only.

3.4. Meta-analysis

Due to limited data, we were only able to conduct a meta-analysis on seven studies that looked at BP in SCD patients versus controls. The meta-analysis results are presented in Figure 2. From the pooled results systolic, diastolic, and mean arterial pressure were lower in SCD patients compared to control groups, whereas and pulse rate and pulse pressure were higher in SCD patients compared to control groups. Heterogeneity (I^2) ranged from 20% to 96%, indicating substantial heterogeneity between studies (Figure 2c). In the sensitivity analysis, we identified outlier [14]. The study substantially affected the heterogeneity of the pooled results. After excluding the study, the heterogeneity decreased from 96% to 60%. Furthermore, mean arterial pressure remained lower in SCD patients than in controls. We were unable to conduct a sensitivity/ subgroup analysis for pulse rate and pulse pressure, due to having fewer studies. The results that were not included in the meta-analysis are summarized in the narrative synthesis below.

Table 1. Risk of bias in the included studies.

Reference	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Risk of bias score	Risk of bias
Genetic data											
Bhatnagar	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	7	Moderate
Descriptive/Cross-sectional studies											
Campbell	No	Yes	Yes	Yes	Yes	NA	No	Yes	Yes	6	Moderate
Desai	No	No	No	Yes	Yes	NA	No	Yes	Yes	4	High
Johnson	No	Yes	Yes	Yes	Yes	NA	No	Yes	Yes	6	Moderate
Strumph	No	Yes	Yes	Yes	Yes	NA	No	Yes	Yes	6	Moderate
Lamarre	No	Yes	Yes	Yes	Yes	NA	No	Yes	Yes	6	Moderate
Lemonne	No	Yes	Yes	Yes	Yes	NA	No	Yes	Yes	6	Moderate
Oguanobi	No	No	Yes	Yes	Yes	NA	No	Yes	Yes	5	High
Pegelow	No	Yes	Yes	Yes	Yes	NA	No	Yes	Yes	6	Moderate
Pikilidou	No	Yes	Yes	Yes	Yes	NA	No	Yes	Yes	6	Moderate
Cohort/Case control studies											
Gordeuk	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes	7	Moderate
Grell	No	Yes	Yes	Unclear	Yes	Yes	Unclear	Yes	Yes	6	Moderate
Homi	No	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	7	Moderate
Kuma	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	8	Low
Makubi	No	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	7	Moderate
Novelli	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	8	Low

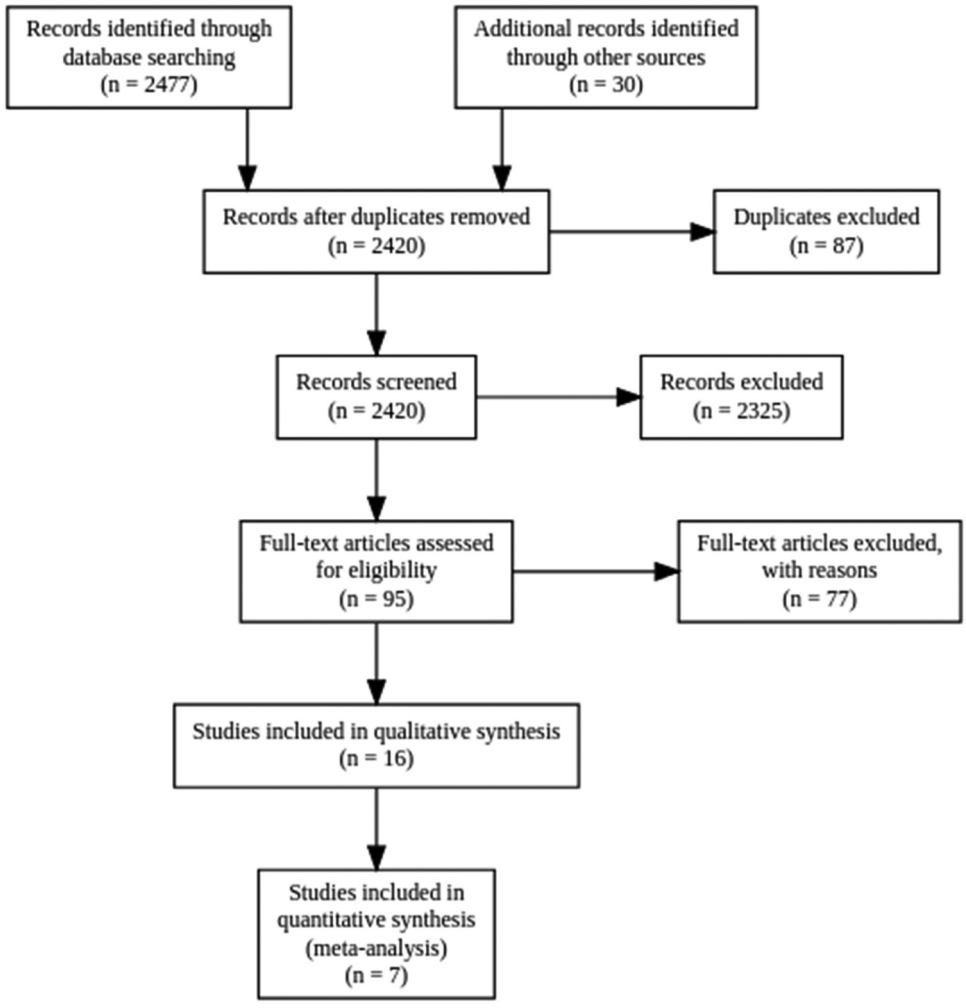

Figure 1. Flow chart of study selection.

3.5. Narrative synthesis

Although BP is a multifactorial condition, we present our results in categories of risk factors, namely demographic, anthropometric, biological, and genetic.

3.5.1. Demographic risk factors

Age was assessed in four studies. Studies have reported a lower BP in SCD patients than age- and sex-matched controls [15–18]. However, a common finding was that age is associated with BP in SCD and that the BP increases rapidly with advancing age in SCD patients starting in their early twenties. Gender was assessed in five studies [16,18–21]. One study reported a significantly lower SBP in male compared to females SCD patients, but after adjusting for weight this difference disappeared (P = 0.050) [21]. The expected DBP rise with advancing age was not present in male participants [18]. Pulse pressure was higher in males of all ages [6,16,22].

3.5.2. Anthropometric risk factors

BMI was assessed in five studies [15,17,19,20,23]. Studies have found that, after controlling for other covariates, SBP was significantly associated with BMI (P < 0.0001) [15]. BMI is independently associated with hypertension [17,20,23]. In addition, SCD patients with elevated BP had significantly higher body weight, waist, and neck circumference (p < 0.050) [19].

3.5.3. Biological risk factors

After controlling for other covariates, three studies found that DBP was independently associated with the level of hemoglobin [15,17,23,24]. DBP showed a positive correlation with age, body mass index in those over 17, hemoglobin concentration in women, blood urea nitrogen in males under 18, and a negative correlation with estimated glomerular filtration rate (eGFR) in patients under-age 18, SBP was negatively correlated with fetal hemoglobin in males over 17 [23]. Triglycerides increased hematocrit and higher blood viscosity were independent risk factors for RSH in SCD patients [24,25]. Furthermore, pulse pressure and a history of blood transfusion were independently associated with systemic hypertension in SCD patients [17]. Markers of hemolysis are associated with a higher pulse pressure in SCD patients [25]. Augmentation index, a measure of arterial stiffness, was significantly higher in SCD patients compared with healthy controls (p < 0.050) [22]. In the multivariate analysis, there was a significant correlation between SBP and age, BMI, history of hypertension, and absolute neutrophil count (estimate: 21.18, P = 0.023) [15].

Table 2. Characteristics of included studies.

Studies	Year	Countries	Study settings	Sample size	Methodology	Charateristics of study participants	Outcomes	Identified factors
Bhatnagar [26]	2013	United States (USA)	SCD cohorts of African American ancestry	1,617 patients	Genome-wide meta-analysis	This study includes two African American ancestry SCD cohorts. Gender: 843 males, 774 females. Average age was 8.96 from one cohort and 9.57 years from the second cohort	Systolic blood pressure (SBP)	Genetic factors
Campbell [13]	2007	Jamaica	SCD clinic	51 subjects with HbSC and 88 subjects with HbSS	Cross-sectional	51 subjects with HbSC (29 males, 22 females) and 88 subjects with HbSS (43 males, 45 females) Age: Age ranged from 23.1 to 31.6 years.	Blood pressure (BP) and BP by genotype	Anthropometric, hematological and renal functional factors
Desai [15]	2012	USA	SCD patients cohort at University of North Carolina (UNC) clinic	156 SCD patients in theUNC cohort	Cross-sectional	Age: Mean age was 36 years (range 27–47) Gender: 97 (62%) females Ethinicity: 154 (99%) african american	SBP and diastolic blood pressure (DBP)	Clinical and laboratory factors
Gordeuk [10]	2008	USA	Patients from Sickle Cell Pulmonary Hypertension Screening Study	163 SCD patients	Retrospective cohort cohort	Adults with hemoglobin SS or Sβ0 thalassemia patients	Relative systemic hypertension (RSH) (i.e. SBP 120–139 mm Hg or DBP 70–89 mm Hg)	Complications associated
Grell [14]	1981	Jamaica	SCDclinic of the University Hospital in Jamaica.	70 SCD patients	Retrospective cohort	Participants were aged over 30 years. Gender: 29 males and 41 females. Age: age ranged from 30 to 69 years.	SBP and DBP	Age and gender
Homi [21]	1993	Jamaica	SCD clinic of the University Hospital of the West Indies, Kingston, Jamaica.	342 SCD patients	Retrospective cohort	Adults aged 9.5 to 18.5 years.220 with homozygous SS and 122 with hemoglobin C	BP	Weight
Johnson [18]	1981	USA	SCD clinics at the Los Angeles County-University of Southern California Medical Center and The Martin Luther King, Jnr General Hospital.	187 SCD patients	Cross-sectional	Gender: 81 males, 106 females Age: mean age was 29 years (range 18–67). 130 sickle cell anemia, 43 sickle cell-hemoglobin C, 15 had sickle cell thalassemia	Prevalence and severity of hypertension in SCD	Hemoglobinopathy, age and advancing age
Strumph [28]	2021	USA	Children's hospital	100 patients with SCD	Cross-sectional	100 SCD patients with age ranging from 5 to 21	Nocturnal hypertension	Complication associated
Kuma [16]	2018	Ghana	The Ghana Institute of Clinical Genetics	875 with homozygous or compound heterozygous SCD	Retrospective cohort	Participants were adults aged >18 years. Gender: 63% were females. Age: Median age was 31 (range 23–44)	Prevalence of RSH	Complications associated
Lamarre [20]	2013	(Guadeloupe, French West Indies)	SCD Center at the Academic Hospital of Pointe-a`-Pitre.	97 SCD patients	Cross-sectional	Adults aged ≥18 years. Gender: 43 male and 54 females	RSH and systematic hypertension	Biological factors
Lemonne [24]	2014	(Guadeloupe, French West Indies).	SCD Center at the Academic Hospital of Pointe-a`-Pitre.	89 SCD patients (sickle cell-hemoglobin C)	Cross-sectional	Gender: 40 males, 49 females Age: mean age was 38 ± 13 years	RSH	Blood Viscosity
Makubi [17]	2017	Tanzania	Patient were identified at pediatric or hematology clinics or during hospitalization	1013 SCD patients	Retrospective cohort	Gender: 56% were females Age: Median age was 17 (range15–22)	RSH and systematic hypertension	Any risk factors
Novelli [25]	2014	USA	Multicentre international sickle cell trial (USA and United Kingdom)	661 SCD patients	Retrospective cohort	Participants were adults: 500 HbSS and 161 HbSC, with mean age34 (range 25–45) and 41 (range 28–51)	Pulse pressure	Markers of hemolysis
Oguanobi [19]	2010	Nigeria	Outpatient sickle cell clinics and the cardiac center of the University of Nigeria Teaching Hospital, Enugu, Nigeria	62 SCD patients	Cross-sectional	Participants were adults. Age: Mean age was 28.27 (range 18–44)	Arterial BP	Age, gender, and anthropometric data
Pegelow [23]	1997	USA	Cooperative Study of Sickle Cell Disease (CSSCD) at at 23 clinical centers, USA	3,317 SCD patients	Cross-sectional	Participants included were children and adults (2 years old or older)	BP	Age, gender, anthropometric, clinical and laboratory data
Pikilidou [22]	2015	Greece	SCD center	45 SCD patients	Cross-sectional	Participants were adults. Age: Mean age was 43 years (range 26–65) Body mass index (BMI):(24.1 ± 3.6 kg/m2 Gender: 30 males and 15 females	BP	Arterial stiffness

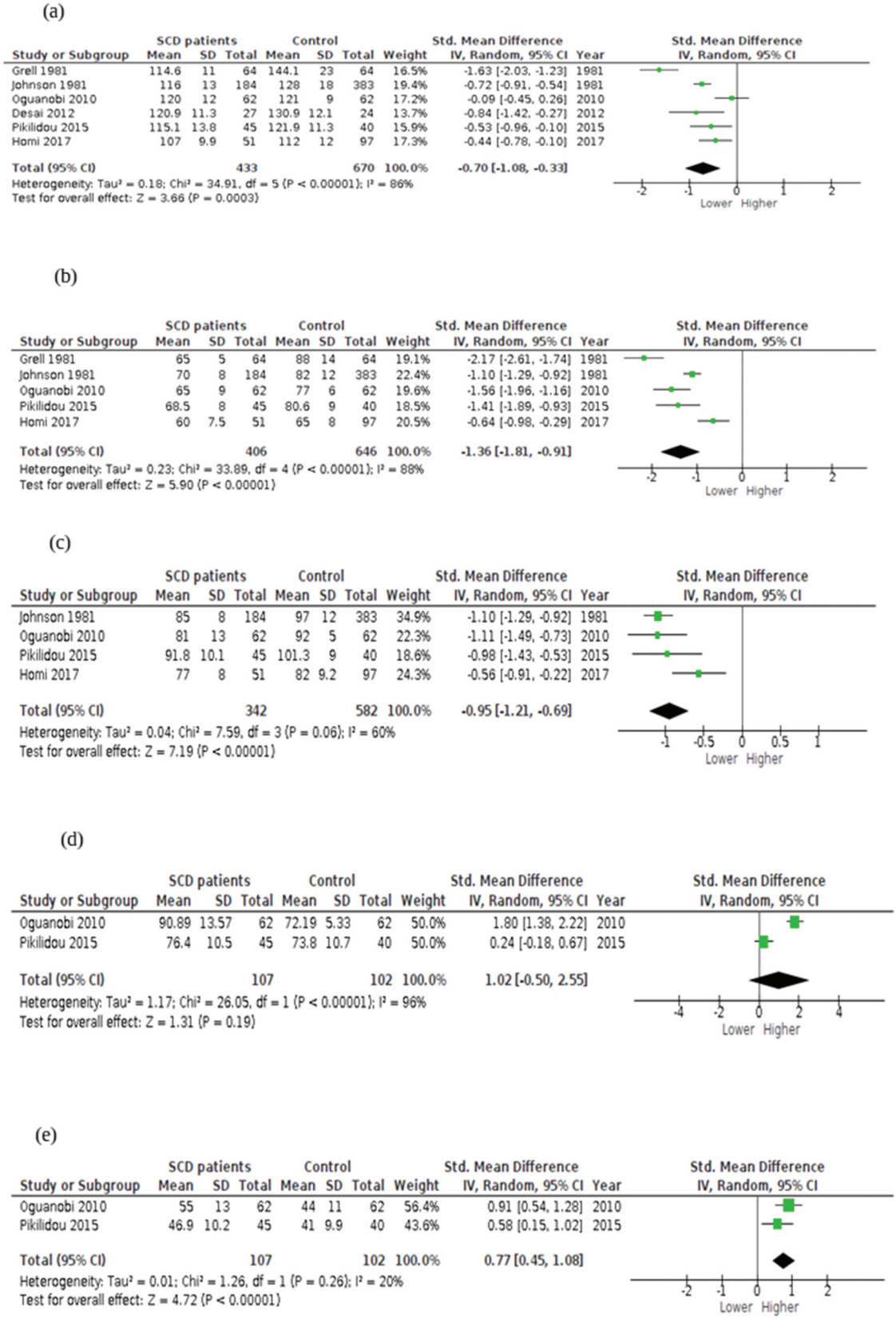

Figure 2. BP indices in SCD patients versus controls, (a) = systolic BP, (b) = diastolic BP, (c) = mean arterial pressure; (d) = Pulse rate, (e) = Pulse pressure.

3.5.4. Genetic risk factors

We found limited data investigating the underlying genetic factors contributing to blood pressure variation, particularly in SCD patients. We found one study, which was a single genome-wide meta-analysis of SBP. The study included 1617 African-American SCD children and identified a suggestive candidate locus at rs7952106 in *DRD2* gene and in *MIR4301* gene [26].

3.5.5. Clinical complications and blood pressure

Gordeuk *et al.* found that patients with SBP > 120 or DBP > 70 mm Hg may represent relative hypertension in SCD patients and identify patients at increased risk for pulmonary arterial hypertension and renal dysfunction. When they stratified patients by tricuspid regurgitant jet velocity (TRV) and serum creatinine concentration according to three blood pressure categories (Normal, RSH, systemic hypertension), TRV was 2.5 m/sec in 27% of the patients with normal BP, in 37% of the patients with RSH, and in 93% of the systemic patients with hypertension. Serum creatinine concentration was 1.0 mg/dL or higher in 7% of the patients with normal BP, in 17% with the patients with RSH and in 50% of the patients with systemic hypertension. During the two-year follow-up evaluation, systemic hypertension was observed in (16%) of those with RSH during the initial evaluation. Over 2 years of follow-up, there were trends for more frequent progression to elevated TRV or creatinine values in RSH and systemic hypertension groups [10]. DeBaun et al., reported that higher baseline systolic blood pressure (P = 0.018) and male sex (P = 0.030) were statistically significantly associated with an increased risk of silent cerebral infarcts [27]. Contrary to similar studies, Kuma et al., found a high prevalence of RSH and hypertension with a relatively low frequency of renal insufficiency, and no association between RSH and stroke [16]. Novelli et al., found that higher pulse pressure was associated with markers of hemolysis, elevated serum creatinine and with proteinuria in SCD patients [25]. Additionally, Kuma et al., found a higher pulse pressure in males of all ages compared to females [10]. A recent cross-sectional study looking at the association between twenty-four-hour ambulatory BP monitoring and cerebrovascular outcomes found an association between nocturnal hypertension and a higher prevalence of silent cerebral infarcts (SCI), and stroke in children with SCD [28].

4. Discussion

To our knowledge, this is the first reported systematic review and meta-analysis that provides information on a wide range of risk factors associated with BP variation among SCD patients. Advancing age, gender, BMI, higher body weight, waist, and neck circumference, hemoglobin, BUN, eGFR, triglycerides, greater hematocrit, higher blood viscosity, absolute neutrophil count, history of blood transfusion, and potentially variant in MIR4301 gene were independently associated with the risk of RSH or systemic hypertension in SCD patients. Furthermore, our pooled estimates illustrate that SBP, DBP, and mean arterial pressure were lower in SCD patients. Pulse pressure and pulse rate were higher in SCD patients compared to the general population, with heterogeneity ranging from low to high.

Previous studies have explained that lower BP in SCD patients may potentially be due to renal tubular defects or hyposthenuria, increased Sodium and water loss [18,23], lower BMI, and alterations in peripheral vascular resistance and vasodilation [21] which affects cardiac parameters including cardiac output, cardiac index, ejection fraction, and stroke volume. It is well documented that SCD patients with RSH or Systemic hypertension are at increased risk of renal insufficiency, pulmonary hypertension, and silent cerebral infarction or stroke [10,16,25,27]. It has also been reported that the risk of occlusive stroke increases with SBP. No significant relationship between DBP and occlusive stroke was observed. No association was found between BP and a history of painful vaso-occlusive crisis. However, an association between increased triglycerides (a known risk factor for RSH and systemic hypertension) and the occurrence of vaso-occlusive crises was observed [20]. In addition, pulse rate and pulse pressure being higher in SCD patients is a concern because pulse pressure is a well-documented predictor of sudden death [29,30]. Akingbola et al., reported that the history of stroke was associated with higher SBP and BMI on univariate analyze ($p < 0.006$) [31]; however, Kuma et al., found a high prevalence of RSH and hypertension with a relatively low frequency of renal insufficiency, and no association between RSH and stroke. The study attributed these differences mostly to selection bias and under documentation of complications in their study design [16].

In SCD patients, elevated pulse pressure has been reported to be associated with hemolysis, proteinuria, and chronic kidney disease [16,25]. Elevated TRV and serum creatinine concentrations in RSH and systemic hypertension in SCD [10] reinforces the importance of systemic blood pressure as a predictor of kidney deterioration in SCD and pulmonary arterial hypertension.

This review also highlights a wide range of risk factors associated with BP variation in SCD patients and subsequently leads to complications described above. Previous studies show that the risk of RSH and systemic hypertension in SCD patients increases with advancing age, with an onset in the early twenties [23,32]. In addition, gender-dependent factors, such as nitric oxide production, which is higher in females compared to males, may explain the higher pulse pressure observed among SCD male patients [33]. Nitric oxide is thought to be important in maintaining vasomotor tone, limiting platelet aggregation, inhibiting ischemia-reperfusion injury, and modulating endothelial adhesion molecule expression [34]. Therefore, being an adult and/or male SCD patient could potentially increase the risk of developing RSH and Systemic Hypertension. Furthermore, SCD patients with elevated BP have higher waist, neck circumference, elevated BMI, history of blood transfusion, and prior diagnosis [17,19,35]. Elevated BP has been reported to be associated with a history of stroke [31], higher prevalence of silent cerebral infarcts (SCI), and stroke in children with SCD [28]. Therefore, the ability to identify individuals prior to the development of cardiovascular complications is of paramount importance, particularly in SCD patients [35].

Further evidence shows a correlation between biological factors (hemoglobin, hematocrit, and triglycerides) and BP variation. However, this correlation varies among age and sex subgroups in SCD patients. In the RSH and systemic hypertension group, there is an increase in blood viscosity compared to SCD patients in the normal group [20]. Increased blood viscosity in the RSH and systemic hypertension groups may be due to lower hemolytic rate, which leads

to elevation of hematocrit and hemoglobin levels [20]. As the viscosity increases, there is a negative impact on blood flow, which may worsen cardiovascular function [36]. Interventions such as hydroxyurea known to modulate hemolytic rate and increase levels of hemoglobin do not increase blood viscosity in SCD patients and positively impacts the rheology properties of sickle red blood cells (RBC) [2,20]. However, these findings are generalizable to patients having a hemoglobin increase of around 1 g per deciliter (g/dl) under hydroxyurea [2]. Therefore, blood flow may be an important factor for the diagnosis and management of RSH and systemic hypertension in SCD patients [36]. The importance of rheology and vascular complications will require to be further investigated. Additionally, alpha-thalassemia can also modulate the hemolytic rate by decreasing the tendency of HbS to polymerize, which results in increased hemoglobin concentrations [36]. The protective effect of alpha-thalassemia varies from reduced occurrence of stroke, gallstones, leg ulcers, and priapism [2]. However, elevated hematocrit and blood viscosity may be associated with increased frequency of pain in patients with alpha-thalassemia [2,20,37].

The findings on genetic factors relating to BP in SCD patients are consistent with a previous review [38]. Since genetic factors were only investigated in one study, no solid inference can be drawn on whether the same genetic variants influence BP across different human populations. This highlights the urgent need for studies aimed at identifying specific SNPs associated with BP and cardiovascular complications in SCD patient populations, particularly in Africa.

Even though we conducted a comprehensive search and most of our included observation studies were low to moderate risk of bias, this review is not without limitations. When we pooled point estimates in a meta-analysis, we observed heterogeneity ranging from low to high, thus limiting our interpretation of pooled estimates. Contributors to this unexplained heterogeneity may be due to a wide range of study methodologies, study settings, populations, and periods studied in our included studies. Most of the included studies were from North America, which highlights a gap in comparable studies from lower-income countries, which carry the greatest burden of SCD. All the included studies recruited their study participants from SCD clinics or referral hospitals. Thus, these findings may not be generalizable to the whole SCD population. Nevertheless, the study still provides a comprehensive evaluation of risk factors associated with BP in an SCD clinic or hospital setting.

The study provides a comprehensive evaluation of risk factors associated with BP in an SCD clinic or hospital setting. Despite study limitations, our study updates current research on a range of factors that may contribute to the risk of RSH and systematic hypertension in SCD patients. Consequently, personalized early interventions aimed at lowering elevated BP may improve life expectancy among SCD patients by inhibiting progression to pulmonary arterial hypertension, advanced renal disease, and other vascular complications. However, high-quality evidence from clinical trials is needed to guide initiation of therapy and treatment goals in SCD patients. Similarly, public health interventions, such as population-based health education and lifestyle modification, within the cultural context, may play a role in preventing elevated BP in SCD patients. However, clinical trials and longitudinal studies should be conducted to confirm these hypotheses. Our systematic review identified methodological limitations and gaps in knowledge that should be addressed in future studies. Limited genetic studies have been done to identify SNPs associated with BP and cardiovascular complications in SCD patients, particularly in Africa. There is a gap in comparable studies from lower-income countries, which carry the greatest burden of SCD. Most importantly, there is a need for clinical trials that aim to determine whether BP interventions can reduce associated complications in SCD patients as they are in the general population. Lastly, the current proposed values for RSH are defined as Systolic BP 120–139 mm Hg or diastolic BP 70–89 mm Hg, whereas systemic hypertension is defined as Systolic BP ≥ 140 mm Hg or diastolic BP ≥ 90 mm Hg in all SCD patients. However, providing age-specific values for RSH and systemic hypertension would make it easier for readers and allow comparison across studies.

A combination of genetic and large longitudinal cohort studies may be able to identify SCD patients who are at highest risk of cardiovascular complications. Over the next 5 years, we will hopefully have more genetic studies looking at SNPs associated with BP and larger longitudinal cohort studies with scientifically rigorous methods for understanding risk factors associated with BP in SCD patients. The establishment of the Sickle In Africa consortium, which aims to facilitate research in SCD, may provide more prospective observational studies on BP and SCD, particularly in Africa. Additionally, there is a need to establish the optimal BP threshold above which to initiate therapy for patients with SCD and age-specific definition of BP based on BP centiles to facilitate comparison between SCD studies, as inclusion of children aged <18 years could have contributed to dilute some of the statistics reported.

5. Conclusions

Blood pressure is lower in SCD patients compared to the general population; however, SCD patients with RSH or systemic hypertension are at increased risk of morbidity and mortality. In addition, risk factors, such as age, gender, triglycerides, blood transfusion, and hemoglobin level are determinants of BP variation in SCD patients. Interventions that consider these risk factors may potentially lower BP pressure in SCD patients and prevent the development of severe complications.

Acknowledgments

Gill Morgan, Health Science Library, University of Cape Town, who assisted with developing the search strategy.

Disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or conflict with the subject matter or materials discussed in this manuscript apart from those disclosed.

Reviewer disclosures

Peer reviewers in this manuscript have no relevant financial or other relationships to disclose.

Funding

This work was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health (Award Numbers U24HL135600 and 1U24HL135881).

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Appendix 1

The PubMed database search strategy

Search ((((((((((((('risk factors'[MeSH Terms] OR risk factors[Text Word])) OR ((determinant OR determinants OR exposure OR exposures)))) OR ('genome-wide association study'[MeSH Terms] OR genome wide association studies[Text Word])) OR (('Genetic Loci'[Mesh]) OR 'Genetic Association Studies'[Mesh] OR genetic loci[Text Word] OR Genetic Association Studies[Text Word])) OR ('epidemiology'[Subheading] OR 'epidemiology'[MeSH Terms] OR epidemiology[Text Word])) OR ('epigenomics'[MeSH Terms] OR Epigenetics[Text Word])) OR ('dna copy number variations'[MeSH Terms] OR copy number variation[Text Word])) OR ('polymorphism, genetic'[MeSH Terms] OR polymorphism[Text Word])))) OR ('polymorphism, single nucleotide'[MeSH Terms] OR snps[Text Word])))) AND ((((('blood pressure'[MeSH Terms] OR 'blood pressure determination'[MeSH Terms] OR 'arterial pressure'[MeSH Terms] OR blood pressure[Text Word])) OR ((pulse pressure OR diastolic OR systolic))) OR ((variation OR variations OR irregularity OR irregularities OR variability OR variabilities OR difference OR differences)))) AND ((('anemia, sickle cell'[MeSH Terms] OR sickle cell disease[Text Word])) OR ((hemoglobin s disease OR hemoglobin s diseases OR hbs disease OR hbs diseases)))

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 19 July 2022
DOI 10.3389/fmed.2022.924722

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Hematology, a section of the journal Frontiers in Medicine

RECEIVED 20 April 2022
ACCEPTED 30 June 2022
PUBLISHED 19 July 2022

CITATION
Nguweneza A, Ngo Bitoungui VJ, Mnika K, Mazandu G, Nembaware V, Kengne AP and Wonkam A (2022) Clinical characteristics and risk factors of relative systemic hypertension and hypertension among sickle cell patients in Cameroon. *Front. Med.* 9:924722. doi: 10.3389/fmed.2022.924722

Clinical characteristics and risk factors of relative systemic hypertension and hypertension among sickle cell patients in Cameroon

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Increased blood pressure (BP) has been associated with higher risk of stroke and mortality in Sickle Cell Disease (SCD). We investigated risk factors associated with Relative Systemic Hypertension (RSH) or systemic hypertension in SCD patients in Cameroon. Using R, Multivariate multinomial logistic regression modeling was used to examine the effects of the demographic, anthropometric, clinical, and laboratory factors to determine risk factors. A total of 815 individuals with SCD, including 380 (46.6%) males were analyzed. At baseline, the median age [interquartile range] was 18.0 [12.0–25.0] years, ranging from 3 to 66 years. Approximately three-quarters of the patients ($n = 645$; 79.1%) had normal BP, 151 (18.5%) had RSH and 19 (2.3%) had hypertension. Age ($P < 0.001$) and gender ($P = 0.022$) were significantly different across the BP categories. Weight ($P < 0.001$), height ($P < 0.001$), BMI ($P < 0.001$), pulse pressure ($P = 0.020$), history of stroke ($P = 0.012$), hemoglobin level ($P = 0.002$), red blood cell count ($P = 0.031$), creatinine ($P < 0.001$), and (estimated glomerular filtration rate) eGFR ($P = 0.002$) was also significantly different across the three BP categories. After adjustment, the significantly associated factors of RSH in the SCD patients were age [OR = 1.03, (95% CI = 1.01–1.06), $P < 0.010$], male gender [OR = 1.54, (95% CI = 1.04–2.27), $P = 0.029$], BMI [OR = 1.10, (95% CI = 1.04–1.17), $P = 0.001$]. After adjustment, the independent variables significantly associated factors of Hypertension in the SCD patients were age [OR = 1.05, (95% CI = 1.01–1.10), $P = 0.034$], male gender [OR = 3.31, (95% CI = 1.04–10.52), $P = 0.042$], BMI [OR = 1.14, (95% CI = 1.01–1.29), $P = 0.027$]. Creatinine was significantly associated with RSH [OR =1.31 (1.05–1.63), $P = 0.016$]. SCD patients with RSH or hypertension maybe at increased risk of renal dysfunction. We found relatively high prevalence of RSH and hypertension (20.8%) in SCD patients in Cameroon. Tailored Interventions that consider major risk factors (age, gender, and BMI) may lower BP pressure and prevent severe complications.

KEYWORDS

relative hypertension, hypertension, risk factors, sickle cell disease, Cameroon, Africa

Introduction

Sickle cell disease (SCD) patients, generally, have lower systolic, diastolic, and mean blood pressure compared to age and sex-matched controls (1, 2). There are no specific recommendations proposed regarding the defining criteria (and management) of hypertension in SCD patients. The lack of recommendations is a major concern since increased BP has been associated with higher risk of stroke and mortality in SCD patients, even in a range of systolic and diastolic BPs (SBP, DBP) that are considered relatively normal for the general population (i.e., lower than 140 mmHg) (2).

Blood pressure is a potential modulator of clinical severity in SCD patients, recent studies showed that relative systematic hypertension (RSH), defined as BP 120–139/70–89 mmHg, and Systemic Hypertension (BP >140/>90), considerably increased the risk of pulmonary hypertension and renal dysfunction (2). Previous studies have reported demographic, biological, anthropometric, and genetic factors to be associate with blood pressure in SCD patients (3–8). Blood pressure is a heritable trait with estimates of heritability indicating that 30–70% of the trait variance is attributable to genetic variation and a recurrent deleterious and loss of functions mutation with genes associated with lowering BP has been recently associate with long survival in SCD in Africa (9).

Identification of risk factors associated with BP variation in different populations is key to controlling BP, as well as preventing associated causes of mortality in SCD patients. We investigated risk factors associated with RSH or systemic hypertension in SCD patients in Cameroon to gain insight into the pathophysiology of BP variation in this disease in an African setting.

Patients and methods

Ethical approval

A proposal was submitted to the University of Cape Town, Faculty of Health Sciences Human Research Ethics Committee, Cape Town, South Africa (HREC/REF: R015/2018). All patients older than 18 years signed consent forms, while informed consent was given by the parents or guardians for participants younger than 18 years old, in accordance with the Declaration of Helsinki. This study was approved by the National Ethical Committee of the Ministry of Public Health of Cameroon (No 193/CNE/SE/15).

Written and signed informed consent forms were obtained from adult participants and parents/guardians of minor patients. An assent was also obtained from the participants of more than 7 years old.

Participants' recruitment

All SCD patients with complete socio-demographic, clinical, laboratory variables, and complete systolic and diastolic blood pressure measurements were included in the study. The data were obtained from a cross-sectional study conducted in Cameroon from May 2016 to July 2018. The data were collected from nine hospitals from five cities in Cameroon, including Yaoundé, Douala, Bafoussam, Bertoua, and Maroua. Patients who have not experienced a painful crisis a month before, and who had not received a blood transfusion in the past 6 months, were recruited irrespective of age and gender.

Use of variables

Dependent variables

Sickle cell disease patients with a SBP within the range of 120–139 mmHg and/or DBP within the range of 80–89 mmHg is defined as having RSH. Systemic hypertension is further defined as SBPs greater than 140 mmHg or DBPs greater than 90 mmHg. Participants who had incomplete/out of range blood pressure readings were excluded from the analysis.

Independent variables

Information on demographics, including age, residential location, sex, ethnicity, educational level, marital status, and household income status, was collected using a standard questionnaire involving the household and individual levels. Clinical information and laboratory information were also collected. Those who had incomplete/out of range relevant information such as age, gender, BMI, demographic, clinical, laboratory information were also excluded from the dataset.

Statistical analysis

All our analysis was analyzed using R (version 4.0.2). Continuous variables were presented as median and interquartile range (IQR) and categorical variables as percentages (%).

Categorical variables were compared using X^2-test or Fisher exact test if the expected count in a cell was less than five while continuous variables were compared according to BP category with the Kruskal–Wallis test.

Multivariate multinomial logistic regression modeling was used to examine the effects of the demographic, anthropometric, clinical, and laboratory factors to determine the potential independent risk factors for RSH and Systemic hypertension.

A final model was created that included all the predictors and interactions that were significantly associated at the level of $P <$

TABLE 1 Baseline demographic, anthropometric, clinical and laboratory characteristics of Cameroonian SCD patients by BP levels.

Characteristics	All (*n*/*N*, %)	Normal (*n* =, %) or median (IQR)	RSH (*n* = , %) or median (IQR)	Hypertension (*n* = , %) or median (IQR)	*P*-value
Demographics					
Age, years	815/815 (100.0)	17.0 (11.0–24.0)	22.0 (18.0–28.0)	24.0 (18.0–40.5)	<0.001
Aged less than 18	373/815 (45.8)	336/645 (52.0)	33/151 (22.0)	4/19 (21.1)	<0.001
Aged older or equal 18	442/815 (54.2)	309/645 (48.0)	118/151 (78.0)	15/19 (78.9)	
Gender, male	380/815 (46.6)	289/645 (44.7)	77/151 (51.3)	14/19 (73.7)	0.022
Anthropometric and clinical					
Weight	815	46.0 (30.0–55.0)	56.0 (50.0–62.0)	60.0 (50.0–68.0)	<0.001
Height	815	1.58 (1.37–1.67)	1.67 (1.60–1.73)	1.64 (1.58–1.77)	<0.001
Body mass index (BMI)	815	18.0 (16.0–20.0)	20.0 (18.0–22.0)	21.0 (19.5–23.0)	<0.001
Pulse pressure[a]	789	91.0 (81.3–101)	88.0 (80.3–96.8)	84.0 (76.0–92.5)	0.020
History of stroke[a]	28/803 (3.5)	20/637 (3.1)	5/147 (3.4)	3/19 (15.8)	0.012
History of kidney disease[a]	82/807 (10.2)	69/640 (10.8)	10/148 (6.8)	3/19 (15.8)	0.246
History of Pulmonary hypertension[a]	68/807 (8.4)	52/640 (8.1)	13/148 (8.8)	3/19 (15.8)	0.4888
History of transfusion[a]	634/813 (78.0)	504/644 (78.3)	118/150 (78.7)	12/19 (63.2)	0.286
Hydroxuria[a]	72 /807 (8.9)	58/640 (9.1)	14/148 (9.5)	0/19 (0.0)	0.381
Biological data					
Hemoglobin (g/dl)	797/815	7.60 (6.80–8.50)	8.00 (7.10–8.90)	8.10 (7.80–10.8)	0.0020
Hemoglobin F (%)	794/815	6.40 (3.80–11.6)	6.30 (3.40–11.5)	8.35 (4.73–12.3)	0.459
White blood cell count (10^9/L)	797/815	10.3 (7.80–13.0)	9.80 (7.77–12.6)	9.45 (8.30–11.3)	0.637
Mean corpuscular volume (fl)	798/815	88.0 (82.0–95.0)	89.0 (83.0–95.0)	85.0 (77.5–92.0)	0.360
Red blood cell count	794/815	2.70 (2.30–3.13)	2.83 (2.40–3.19)	2.87 (2.63–3.38)	0.031
Creatinine (mg/dl)	770/815	0.45 (0.37–0.60)	0.60 (0.40–0.78)	0.65 (0.50–0.87)	<0.001
(Estimated glomerular filtration rate) eGFR	760/815	175 (151–204)	158 (136–178)	150 (119–182)	0.002

[a]Total number of children may differ because of missing data; IQR, interquartile range.

0.05. The findings presented as crude and adjusted odds ratios with their 95% confidence intervals (CI).

Results

Baseline characteristics

Table 1 Shows the demographic, anthropometric, clinical and laboratory characteristics of the BP categories. Our analysis included 815 individuals with SCD, of whom 645 (79%) had normal BP, 151 (19%) had RSH, 19 (2%) had systemic hypertension. 380 (46.6%) were males. At baseline, the median age [interquartile range] was 18.0 [12.0–25.0] years, ranging from 3 to 66 years. Approximately three-quarters of the patients (645 or 79.1%) were normal BP, 151 (18.5%) had relative hypertension and 19 (2.3%) had hypertension. Age ($P < 0.001$) and gender ($P = 0.022$) were significantly different across the BP categories, with age increasing with BP. Weight ($P < 0.001$), height ($P < 0.001$), BMI ($P < 0.001$), pulse pressure ($P = 0.020$), history of stroke ($P = 0.012$), hemoglobin ($P = 0.002$), red blood cell count ($P = 0.031$), creatinine ($P < 0.001$), and eGFR ($P = 0.002$) were also significantly different across the three BP categories.

Univariate and multivariate analysis

The normal BP group vs. RSH group

Among SCD patients, univariate analyses indicated that these variables were significantly more common risk factors for higher BP values among patients with RSH than those with normal BP: Age ($P < 0.001$), patients >18 years ($P < 0.001$), weight ($P < 0.001$), height ($P < 0.001$), BMI ($P < 0.001$), pulse pressure ($P = 0.046$), creatinine ($P < 0.001$), eGFR ($P < 0.001$) and hemoglobin ($P = 0.020$) (Table 2). Multivariate analyses found that age [OR = 1.02, (95% CI = 1.01–1.05), $P = 0.021$], creatinine [OR = 1.310, 95% CI = 1.05–1.63, $P = 0.016$], BMI [OR = 1.09, (95% CI = 1.03–1.16), $P = 0.002$] were independent risk factors for high BP values in SCD patients with RSH compared with SCD patients with normal BP values (Table 3).

TABLE 2 Univariate multinomial logistic regression analyses of factors associated with RSV and hypertension among SCD patients in Cameroon (reference: Normal BP).

Factors	RSH vs. normal		Hypertension vs. normal BP	
	cOR (95% CI)	*P*-value	cOR (95% CI)	*P*-value
Demographics				
Age*	1.05 (1.03–1.06)	<0.001	1.08 (1.04–1.12)	<0.001
Aged less than 18 (ref.)				
Aged older or equal 18*	3.89 (2.56–5.89)	<0.001	4.07 (1.34–12.41)	0.013
Gender, female (ref.)				
Gender, male*	1.28 (0.89–1.83)	0.170	3.45 (1.22–9.69)	0.019
Anthropometric and clinical				
Weight* (kg)	1.05 (1.04–1.07)	<0.001	1.07 (1.03–1.11)	<0.001
Height (m)*	76.1 (21.77–265.89)	<0.001	15.9 (0.96–264.38)	0.054
Body mass index*	1.16 (1.10–1.23)	<0.001	1.22 (1.11–1.35)	<0.001
Pulse pressure*	0.98 (0.97–1.00)	0.046	0.95 (0.92–1.00)	0.019
History of stroke* vs. No (ref)	1.03 (0.38–2.77)	0.957	5.59 (1.51–20.66)	0.010
History of kidney disease vs. No (ref)	0.60 (030–1.19)	0.145	1.57 (0.44–5.54)	0.480
History of pulmonary hypertension vs. No (ref)	1.11 (058–2.10)	0.749	2.20 (0.26–7.78)	0.223
History of transfusion vs. No (ref)	1.00 (0.65–1.54)	0.985	0.47 (0.18–1.23)	0.128
Hydroxuria	1.00 (0.53–1.89)	0.992	0.00 (−7.07–1.7)	0.762
Biological data				
Hemoglobin (g/dl)*	1.12 (1.01–1.23)	0.020	1.36 (1.14–1.61)	<0.001
Hemoglobin F (%)	0.99 (0.97–1.03)	0.746	1.04 (0.97–1.11)	0.226
White blood cell count (10^9/L)	0.99 (0.95–1.09)	0.782	0.95 (0.85.1.08)	0.485
Mean corpuscular volume (fl)	1.00 (1.00–1.02)	0.528	1.00 (0.94–1.01)	0.062
Red blood cell count*	1.23 (1.00–1.53)	0.061	1.9 (1.24–2.94)	0.03
Creatinine (mg/dl)	1.24 (1.15–1.34)	<0.001	1.38 (1.17–1.62)	<0.001
eGFR	1.00 (0.98–1.00)	<0.001	0.99 (0.98–1.00)	0.007

cOR, Crude odds ratio; CI, Confidence interval; *denotes significant at the 5% level.

The normal BP group vs. systemic hypertension group

Among SCD patients, univariate analyses indicated that these variables were significantly more common risk factors for higher BP values among SCD patients with hypertension than those SCD patients with normal BP: Age ($P < 0.001$), patients >18 years ($P = 0.013$), male gender ($P = 0.019$), weight ($P < 0.001$), BMI ($P < 0.001$), pulse pressure ($P = 0.019$), hemoglobin ($P < 0.001$), creatinine ($P < 0.001$) and Red blood cell count ($P = 0.03$) (Table 2). Multivariate analyses found that age [OR = 1.05, (95% CI = 1.01–1.10), $P = 0.034$], male gender [OR = 3.31, (95% CI = 1.04–10.52), $P = 0.042$], BMI [OR = 1.14, (95% CI = 1.01–1.29), $P = 0.027$] were independent risk factors for higher BP values in SCD patients with hypertension compared with SCD patients with normal BP values (Table 3).

Additionally, Figure 1 Illustrates the relationship between BP and age, gender, BMI. As age increases the probability of SCD patients having RSH or hypertension increases (Figure 1A). Secondly, Males have a higher probability of having RSH or hypertension than females among SCD patients (Figure 1B). For every increase in BMI units, the probability of having RSH or Hypertension increases among SCD patients (Figure 1C). Lastly, for every increase in creatine units, the probability of having RSH or Hypertension increases among SCD patients (Figure 1D).

Discussion

This study determined the role of demographic, anthropometric, clinical and laboratory factors associated with RSH and hypertension among SCD patients in Cameroon, one of the rare attempts from Africa. The main findings, from this relatively large dataset are as follows. Approximately one quarter of our 815 SCD patients were classified in either RSH or systemic hypertension group. At baseline, we observed statistically significant differences in age, gender, weight, height, BMI, pulse pressure, a history of stroke, hemoglobin, and red blood cell count across our three BP groups (normal BP, RSH, hypertension). We found that age, BMI, creatinine, and

TABLE 3 Multivariate multinomial logistic regression analyses of factors associated with RSV and hypertension among SCD patients in Cameroon (reference: Normal BP).

Factors	RSH vs. normal BP		Hypertension vs. normal BP	
	aOR (95% CI)	*P*-value	aOR (95% CI)	*P*-value
Age, years*	1.02 (1.01–1.05)	0.021	1.05 (1.01–1.10)	0.034
Gender, male vs. female (ref.)*	1.20 (1.10–1.80)	0.372	3.31 (1.04–10.52)	0.042
Body mass index (BMI)*	1.09 (1.03–1.10)	0.002	1.14 (1.01–1.29)	0.027
Pulse pressure	1.00 (0.98–1.01)	0.679	0.98 (0.93–1.01)	0.289
History of stroke vs. No (ref)	0.89 (0.30–2.56	0.830	2.24 (0.42–11.79)	0.339
Creatinine (mg/dl)	1.31 (1.05–1.63)	0.016	1.26 (0.75–2.09)	0.373
Hemoglobin (g/dl)	1.00 (0.70–1.40)	0.234	0.88 (0.35–2.47)	0.572
Red blood cell count	1.02 (0.87–1.13)	0.684	1.15 (0.81–2.13)	0.145

aOR, Adjusted odds ratio; CI, Confidence interval; *denotes significant at the 5% level.

male gender were independently associated with an increased risk of RSH and systemic hypertension after adjusting for other variables.

The nearly 19% prevalence of RSH reported in this study was similar to that of 17% reported in studies from North America by Becker et al. (10) and Bodas et al. (11). In similar setting as our study, RSH was lower to that of 45% reported by Benneh Akwasi Kuma et al. (12) and 44% found by Makubi et al. (13), the participants in these studies were adult patients, whereas our study included both pediatric and adult patients. The 2% prevalence of systemic hypertension reported in this study from Cameroon also agrees with previous reports from both high and low incomes settings, which have reported the prevalence of systemic hypertension in SCD patients to be lower than that of the general population (2–8% vs. 28%, respectively) (13–15). Potential explanations of low prevalence of RSH and systemic hypertension in SCD patients include Sodium and water wasting due to the medullary defect, (16) systemic vasodilatation compensating for microcirculatory flow disturbances, (14) increased production of prostaglandins and nitric oxide, (17) reduced vascular reactivity, (16) and premature deaths that remove those individuals whose BP might reach hypertensive levels in middle adulthood (13).

Unsurprisingly, this study also found that age was significantly associated with BP in SCD patients, SCD patients with RSH and systemic hypertension were older than SCD patients with normal BP values suggesting that advancing age contributed to their higher BP values. This finding corroborates with previous reports in developed countries; (1, 8) and in Africa (18), that reported that BP rapidly increases with advancing age in SCD patients starting in the early twenties. With the improved survival of patients with SCD patients, the incidence of RSH or systemic hypertension is expected to rise, thus screening and awareness are necessary to prevent the expected complications, in all part of the world. Indeed, mortality in adult with SCD in the USA and other high-income countries have not changed over the past four decades, mostly dues to debilitating and severe cardiovascular complications (19). Most of the previous data is from 18-year olds. However, younger patients may already show elevated BP and risk for complications.

Pegelow et al. (1) demonstrated that BP values were higher in males than in females, which is consistent with our results showing that male gender is independently associated with RSH and systemic hypertension in SCD patients. This gender disparity in BP is likely due to gender-related differences in SCD biology or health-seeking behavior between genders (20, 21). For instance, older males with elevated BP relative to the SCD population are at increased risk of stroke than age-matched females (12). SCD males have higher pulse pressure, a predictor of all-cause mortality, than age-matched SCD female patients (12, 22) which further highlights the risk of adverse outcomes associated with RSH and systemic hypertension in males. Another study suggests that regular medical visits are critical for improving hypertension awareness among young adults and reducing gender disparities in cardiovascular health (21).

Consistent with previous studies, Oguanobi et al. (23) in Nigeria, and Pegelow et al. (1) who reported that BMI correlates positively with SBP and DBP and Homi et al. who reported that low weight is a risk factor for low BP. In this study, we found that BMI correlates positively with BP, and BMI was independently associated with RSH and systemic hypertension among SCD patients. Suggesting that a higher BMI in SCD patients with RSH or systemic hypertension may contribute to their higher BP values compared to the SCD patients with normal BP. SCD patients have lower BMI compare to general population but increased BMI in SCD patients has potential to modulate BP (8). In addition, the prevalence of obesity in patients with SCD seems to be on the increase. Obesity is a risk factor for other diseases, including, but not limited to, type 2 diabetes, hypertension, sleep apnea, cardiovascular disease (24). These diseases, in turn,

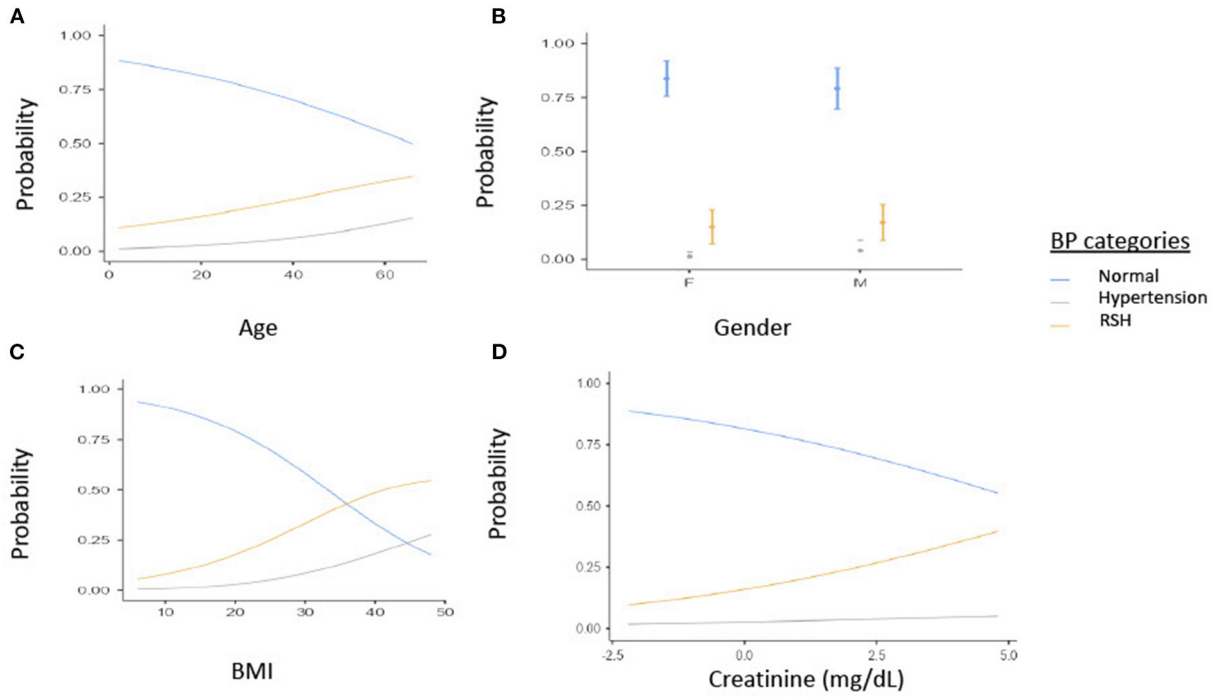

FIGURE 1
Estimated marginal means of **(A)** Age, **(B)** Gender, **(C)** BMI by BP categories, **(D)** creatine (mg/dl). BP, Blood pressure; BMI, Body mass index; RSH, Relative systemic hypertension; F, Females; M, Males.

worsen the clinical picture of SCD and increase the frequency of vaso-occlusive crises (VOCs) (24). Because of the clinical importance as well as public health importance of RSH or systemic hypertension, the ability to identify otherwise normal BMI is of paramount importance, particularly in SCD patients.

Furthermore, measuring BMI alone, in SCD, is sufficient to screen for adiposity and obesity. Previous reports show the body composition of SCD patients with normal mean BMI (22.6 kg/m^2), showed a 32.6% fat composition, indicating high levels of adiposity. Since fat accumulation and adipocyte secretion are responsible for many hormonal changes playing a role in the development of vascular dysfunction and hypertension in the general population, this could be the case in SCD patients too, even if BMI values are normal. Therefore, further studies are needed to better understand the relationship between BMI; hormonal status and BP in SCD.

Previous studies reported that SCD patients with SBP 120–139 mm Hg or DBP 70–89 mm Hg had elevated levels of creatinine compared to SCD patients with SBP <120 mm Hg and DBP <70 mm Hg (2). In this study we found that creatinine was independently associated with RSH. Additionally, SCD patients in RSH and systemic hypertension group had a higher creatinine compared to SCD patients in the normal group. Suggesting that SCD patients with RSH or hypertension are at increased risk of renal dysfunction. Longitudinal studies are needed to better understand temporal relationship between renal dysfunction and RSH.

Previous studies found Increasing hemoglobin, blood viscosity and blood transfusion to be independent risk factors for RSH or hypertension in SCD patients. However, in this study we did not find these factors to be significantly associated with BP among SCD patients (25). These observed differences may be explained by differences in study design, patient's clinical characteristics and thresholds used to define RSH or systemic hypertension.

- Our participants were recruited form referral hospitals. Thus, the findings may not be representative of RSH or systemic hypertension seen in a community. Nevertheless, our analysis is based on the large and well-characterized homozygous study population in a resource-limited country. Therefore, these findings expand the understanding of risk factors for RSH and systemic hypertension in SCD beyond what has been reported from resource-limited settings.
- The exclusion of incomplete records with missing BP might have introduced some bias. Additionally, BP was measured

at single time point which might have increased some patients' likelihood of developing white coat hypertension. Previous studies have highlighted the importance of 24-h ambulatory blood pressure monitoring in diagnosing masked hypertension (26).

- The inability to follow up the cohort as a longitudinal study is a limitation.

In conclusion, this study found evidence of the prevalence of RSH and hypertension in the SCD patients in Cameroon. Age, male gender, BMI was found to be independently associated factors of RSH and hypertension in the SCD patients in Cameroon. Tailored Interventions that consider these risk factors have potential to lower BP pressure in SCD patients and prevent developing severe complications.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

A proposal was submitted to the University of Cape Town, Faculty of Health Sciences Human Research Ethics Committee, Cape Town, South Africa (HREC/REF: R015/2018). All patients older than 18 years signed consent forms, while informed consent was given by the parents or guardians for participants younger than 18 years old, in accordance with the Declaration of Helsinki. This study was approved by the National Ethical Committee of the Ministry of Public Health of Cameroon (No 193/CNE/SE/15). Written and signed informed consent forms were obtained from adult participants and parents/guardians of minor patients. An assent was also obtained from the participants of more than 7 years old. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

AW conceived the study. AN, VN, GM, and AW made substantial contributions to the conception, design of the work, methodology, analysis, data interpretation, and wrote the final manuscript. AN and GM analyzed and interpreted the data. AN issued the first draft of the paper. AN, VN, KM, GM, VN, AK, and AW critically revised successive drafts of the manuscript. VN, GM, AK, and AW supervised the project and compiled the revisions. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health (Award Number U24HL135600) and (Award Number 1U24HL135881).

Acknowledgments

Sickle Africa Data Coordinating Center (SADaCC) team, Human genetics Division, Department of Pathology, Health Science Faculty, University of Cape Town.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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