Association of *SLC2A2* rs8192675 and *SLC22A1* rs72552763 polymorphisms to metformin treatment outcomes in Ethiopian patients with type 2 diabetes mellitus

A Thesis Submitted to the Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University, in partial fulfillment of the requirements for the Degree of Doctor of Philosophy (Ph.D.) in Pharmacology

By:

Abraham Degaga Abdi (B.Pharm, MSc.)

Supervisors:

Workineh Shibeshi (Ph.D., Addis Ababa University)
Professor Ephrem Engidawork (Ph.D., Addis Ababa University)
Professor Hasniza Zaman Huri (Ph.D, Universiti Malaya)
Sisay Sirgu (MD, Internist, Endocrinologist, Saint Paul Hospital Millennium Medical College)

Addis Ababa University
Addis Ababa, Ethiopia
April, 2024

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Abraham Degaga Abdi, entitled "Association of SLC2A2 rs8192675 and SLC22A1 rs72552763 polymorphisms to metformin treatment outcomes in Ethiopian patients with type 2 diabetes mellitus'' and submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Pharmacology complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by examining Committee

Advisor: Workineh Shibeshi (PhD) Signature: _____ Date: 6-28-2024

Internal Examiner: Prof. Teferra Abula Signature: _____ Date: 6-28-2024

External Examiner: Baharudin Ibrahim (PhD) Signature: _____ Date: 6-28-2024

Chair of Department or Graduate Program Coordinator

Abstract

Association of SLC2A2 rs8192675 and SLC22A1 rs72552763 polymorphisms to metformin treatment outcomes in Ethiopian patients with type 2 diabetes mellitus

Abraham Degaga Abdi

Addis Ababa University, 2024

Metformin is recommended as the first-line oral glucose-lowering agent by most clinical guidelines for people with type 2 diabetes mellitus (T2DM), although there are extensive variations in the metformin treatment outcomes, related to the differences in individual genetic profiles. Thus, in this study the association of SLC2A2 rs8192675 and SLC22A1 rs72552763 polymorphisms with metformin treatment response and SLC22A1 rs72552763 with metformin gastrointestinal intolerance were investigated in Ethiopian patients with T2DM. Recently diagnosed patients with T2DM were enrolled at St Paul's Hospital Millennium Medical College. In the metformin treatment response study, a prospective observational cohort was conducted on 86 patients receiving metformin treatment for < 1 year. The participants were classified into metformin responders and non-responders based on a cut-off value of 0.5% reduction in HbA1c levels. Genotyping of rs8192675 and rs72552763 was performed using TaqMan® Pre-Designed and Drug Metabolism Enzyme SNP Genotyping Assay, respectively. The association of each one of the polymorphisms with metformin response was assessed by measuring the change in HbA1c levels, while the association of rs8192675 polymorphism with metformin response in diabetic dyslipidemia was assessed by measuring the absolute change in lipid parameters as well as HbA1c levels. On the other hand, in the metformin gastrointestinal intolerance study, a retrospective study was conducted in 47 patients on metformin treatment for < 3 years. The association of rs72552763 polymorphism to metformin-induced gastrointestinal intolerance was assessed based on switching to a new class of glucose-lowering agents or failure to up-titrate metformin dose due to gastrointestinal intolerance. Dyslipidemia was defined in accordance with the adult treatment panel III. Chi-square, logistic regression, student's t-test, Mann-Whitney and Kruskal-Wallis statistical tests were used as applicable. A p-value of less than 0.05 was taken as statistically significant. The minor allele frequency

(MAF) of the C-allele in the T>C substitution SNP of *SLC2A2* (rs8192675) was 66.2% while the MAF of the 3-base pair (GAT) deletion mutation at rs72552763 was 9.4% in our study population. Metformin response was significantly higher in deletion_GAT (del_G) genotypes as compared to wild-type GAT_GAT (G_G) genotypes 3.675 95% CI (1.005–13.436) (p = 0.049). Furthermore, a significantly lower median treatment HbA1 level was found in del_G genotypes as compared to G_G genotypes 7% vs 8% (p=0.015). However, the association of rs72552763 with metformin response was not replicated at the allele level. Furthermore, the minor del_allele was significantly associated with good glycemic control compared to the G_allele 3.206 95% CI (1.165–8.823) (p = 0.016), though not replicated at del_G genotypes level. In contrast, the rs8192675 polymorphism showed no significant association with metformin treatment response, glycemic control and dyslipidemia. Likewise, no significant association was also observed between rs72552763 and metformin- induced gastrointestinal intolerance. The prevalence of dyslipidemia was 92.1 %. The prevalence of mixed atherogenic dyslipidemia, combined dyslipidemia and isolated dyslipidemia was 37 (29.1 %), 34 (26.8 %), and 27 (21.3%), respectively. The findings demonstrated that heterozygous carriers of the Met420del variants of SLC22A1 have an increased response to metformin. However, rs8192675 and rs72552763 genetic polymorphisms were not associated with metformin treatment response and metformin-induced gastrointestinal intolerance, respectively. The study also indicated a high prevalence of dyslipidemia in people with T2DM, with atherogenic mixed dyslipidemia being the commonest pattern.

Keywords: Type 2 diabetes mellitus, Metformin Response, SLC2A2, rs8192675, diabetic dyslipidemia, Metformin intolerance, SLC22A1, rs72552763, Ethiopia

Declaration

I, the undersigned, declare that this Ph.D. thesis is my original research work and has not been presented for a degree in any other university.

Name: Abraham Degaga Abdi

Signature: _____

Acknowledgement

I am delighted in taking this opportunity to express my earnest and sincere gratitude to all those who have made an enormous contribution to this work.

There were a lot of challenges throughout my PhD study period. However, the wise guidance and support of Dr. Workineh Shibeshi was there to keep me on my lane throughout my PhD journey starting from the inception of the tittle all the way to the final write up. I can testify that, for me, you were not only just a supervisor. You were always close by when I needed an advice. I always appreciated the rich discussions we had and your swift and critical comments in each step of my PhD work. I am delighted to express my utmost gratitude for your wisdom, encouragements and guidance throughout my study. I will never forget your exceptional support in my data collection process as well as the wise guidance in the arrangement of my 3 months research fellowship at the University of Malaya (UM). Dear Dr Workineh, I thank you from the bottommost of my heart for all the support and encouragements.

I would also like to express my earnest gratitude to Professor Ephrem Engidawork, for your constant encouragement and follow up throughout my PhD journey. You are always a true benchmark in my field, you have taught me the essence of research and science. I feel very proud and blessed for having you as my supervisor. It always amazes me how you meticulously review my manuscripts by your pin pointed eye in a very short period of time. Dear Professor Ephrem, I thank you from the bottommost of my heart for all the support and encouragements.

I would also like to express my earnest gratitude to Professor Hasniza Zaman Huri. I feel very privileged to be invited at your laboratory and able to work under your supervision. You have taught me all the skills and science in your pharmacogenetics and precision medicine laboratory. I took a great lesson from your incredible strength, intellect, and diligence. I will never forget your kindness and all the great tips while living in Kuala Lumpur. Dear Professor Hasniza, from the bottommost of my heart, thank you very much! For all what you did for me, from the very warm welcoming at UM, and ultimately making my PhD journey a success. I also thank Dr Gareth Sim Shin for teaching me hands-on

skills in pharmocegenetics and personalized medicine laboratory. I also thank Dr Navin Kumar for his support in the analysis of my pharmacogenetics data.

I would also like to thank my advisor Dr Sisay Sirgu for his constant follow up in the patient recruitment, data collection and making St Paul Hospital a very comfortable working area. Without his full and genuine support this work could have not been started. I would also like to express my earnest gratitude to all the staff members of St Paul Hospital diabetes clinic for their kindness and support during patient recruitment, interviewing and clinical data abstraction, particularly Dr Hundaol, Dr Rabira, Dr Belay, Sister Hanna, Mr. Yohannes and Sister Fistum. I am also very grateful to the hard-working St Paul Hospital laboratory staff members: sister Mistir and head of the laboratory, Mr Hailemeskel, for their support in blood withdrawal from the study participants, laboratory investigation and assisting in laboratory data abstraction.

I also thank Dr Tedla Kebede for his constructive advice on my research topic and moral support. I am also grateful for Dr Baheredin who trained me on SPSS software at UM. I also thank Dr Adem Dawud for his assistance in my data analysis.

I would like to thank Haramaya University for granting me a study leave. I also thank Addis Ababa University for giving me the opportunity to conduct this study. I am also grateful to CDT-Africa and European and Developing Countries Clinical Trials Partnership (EDCTP), for their financial support required in this study. I also thank Professor Abebaw Fekadu for all your moral support and your kind assistance in materializing my financial support through CDT-Africa for my 3-months research stay at UM.

I also owe a great deal of gratitude to Tsion Mucheye (my beloved wife), and my kids (Anna, Natan and Marcon) for their love, comfort and patience. I also thank all the study participants for their kind willingness to take part in the study.

Above all, I acknowledge the Guidance from my God, Jesus Christ, Without Your Countless Blessings and support this PhD study would not have been possible.

List of Acronyms and Abbreviations

ACC: acetyl-CoA carboxylase

ACCORD: Action to Control Cardiovascular Risk in Diabetes

AMPK: Adenosine monophosphate-activated protein kinase

ARFGEF3: ADP-ribosylation factor guanine nucleotide exchange factor 3

ARV: Antiretroviral

ATM: Ataxia-Telangiectasia Mutated

ATP6AP1: ATPase H+ transporting accessory protein 1

CAMKKβ: Ca2+/calmodulin-dependent protein kinase kinase β

CARDS: Collaborative Atorvastatin Diabetes Study

CBP: CREB binding protein

CPA6: Carboxypeptidase A6

CREB: cAMP-responsive element-binding protein 1

CRTC2: CREB-regulated transcription co-activator 2

CVD: Cardiovascular disease

DCS: Diabetes Care System West-Friesland

FBG: Fasting blood glucose

GDS: German Diabetes Study

GERA: Genetic Epidemiology of Responses to Antihypertensives studies

GLP-1: Glucagon-like peptide-1

GLUT: Glucose transporter

GoDART: Genetics of Diabetes Audit and Research Tayside

GWAS: Genome wide association study

HbA1c: Hemoglobin A1C

HDL-C: High density lipoprotein cholesterol

HNF4α: Hepatocyte nuclear factor 4α

HNF4α-P2: P2 isoform of HNF4α

HWE: Hardy–Weinberg equilibrium

IR: Immediate release

LDL-C: Low density lipoproteins cholesterol

LKB1: Liver kinase B1

MAF: Minor allele frequency

MATE: Multidrug and toxin extrusion transporters

MetGen: Metformin Genetics

NCEP: National Cholesterol Education Program

NDUFA3: NADH:ubiquinone oxidoreductase

OCT: Organic cation transporters

OGTT: Oral glucose tolerance test

PCR: Polymerase chain reaction

PEN2: Presenilin enhancer 2

PMAT: Plasma membrane monoamine transporter

PPG: Postprandial blood glucose

PRPF31*:* Pre-mRNA processing factor 31

qPCR: Real-time polymerase chain reaction

SCFA: Short-chain fatty acids

SGLT1: Sodium glucose cotransporter-1

SLC22A1: Solute carrier family 22 member 1

SLC2A2: Solute carrier family 2 member 2

SNP: Single Nucleotide polymorphism

SPHMMC: St Paul's Hospital Millennium Medical College

SSA: Sub Saharan Africa

STAT3: Signal transducer and activator of transcription 3

T2DM: Type 2 diabetes Mellitus

TC: Total cholesterol

TET3: Tet methylcytosine dioxygenase 3

TG: Triglycerides

v-ATPase: Vacuolar H+-ATPase

List of Scientific Papers

This PhD dissertation work is written based on the below four papers:

Paper-1: Abraham Degaga, Sisay Sirgu, Hasniza Zaman Huri, Maw Shin Sim,Tedla Kebede ,Birhanemeskel Tegene , Navin Kumar Loganadan , Ephrem Engidawork, Workineh Shibeshi. Association of Met420del variant of metformin transporter gene SLC22A1 with metformin treatment response in Ethiopian Patients with Type 2 Diabetes. *Diabetes, Metabolic Syndrome and Obesity. 2023; 16: 2523-2535.*

Paper-2: Abraham Degaga, Sisay Sirgu, Hasniza Zaman Huri, Maw Shin Sim,Tedla Kebede ,Birhanemeskel Tegene , Navin Kumar Loganadan , Ephrem Engidawork, Workineh Shibeshi. Association of the reduced function Met420del polymorphism of SLC22A1 with metformin induced gastrointestinal intolerance in patients with type 2 diabetes in the Ethiopian population. *Pharmacogenomics and Personalized Medicine. 2024; 17: 183 -191.*

Paper-3: Abraham Degaga, Sisay Sirgu, Hasniza Zaman Huri, Birhanemeskel Tegene, Ephrem Engidawork, Workineh Shibeshi. The pattern of dyslipidemia and its associated factors among recently diagnosed patients with type 2 diabetes mellitus at Saint Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia: A comparative cross-sectional study (Accepted)

Paper-4: Abraham Degaga, Sisay Sirgu, Hasniza Zaman Huri, Maw Shin Sim,Tedla Kebede ,Birhanemeskel Tegene , Navin Kumar Loganadan , Ephrem Engidawork, Workineh Shibeshi. Association of rs8192675 polymorphism of *SLC2A2* with metformin treatment response in patients with type 2 diabetes in the Ethiopian population (submitted)

Table of contents

List of Tables

List of Figures

List of Appendices

1. Introduction

1.1.Background

Type 2 diabetes mellitus (T2DM) is a metabolic disease resulting from resistance to insulin and progressively declined insulin secretion, which leads to impairment of glucose utilization, dyslipidemia, hyperinsulinemia and the subsequent progressive pancreatic beta cell dysfunction (Nolan et al., 2015). It is mainly characterized by prolonged high glucose levels, which over time causes macrovascular and microvascular complications (American Diabetes Association, 2009). Over the past few decades, the burden of T2DM has increased globally, particularly in middle and low- income states (J. E. Shaw et al., 2010). Furthermore, the fastest increase in the number of patients living with T2DM is projected in the sub-Saharan Africa (SSA) region in the next two decades (Agyemang et al., 2016). In Ethiopia, recent national data on the prevalence of diabetes is lacking. However, a recent study on T2DM reported an overall pooled prevalence of 6.5%, making it one of the highest prevalence countries in SSA (Zeru et al., 2021).

The major risk of T2DM patients is related to its vascular complications, which subsequently leads to a significant morbidity and mortality (Liu et al., 2016). Diabetic microvasculature is highly predisposed for injury due to genetic predisposition and chronic hyperglycemia, leading to impairments of essential organs (Seid et al., 2021). Cardiovascular complications are also the major complications of T2DM that lead to premature death (MacDonald et al., 2008). Furthermore, dyslipidemia often coexists with T2DM (Mithal et al., 2014; Taskinen, 2002), which in turn significantly increases the cardiovascular risk (Wilson et al., 2005). As a result, studies showed that about 70–80% of people with diabetes would die of cardiovascular diseases (CVD) (Feher, 2004; Huang, 2009).

Maintaining blood glucose level within the ideal blood glucose control target is the most effective way in preventing complications of diabetes (Reaven et al., 2019). Thus, one of the key goals of T2DM management is achieving glycemic control in order to delay or prevent diabetes complications. However, despite the recent advancement in the

management of T2DM, approximately 50% of patients do not achieve glycemic control worldwide (Pastakia et al., 2017). Furthermore, according to the recent combined regional estimates on glycemic control, only 37% of the patients with T2DM attained an HbA1C of < 7.0% in the Middle East and North Africa (Al-Ma'aitah et al., 2022). Moreover, studies have also indicated that glycemic control rates are generally poor in SSA (Kirkman et al., 2018). Indeed, a recent meta-analysis conducted on glycemic control rates based on HbA1C measurements showed that 66.8% of Ethiopian patients with diabetes do not achieve adequate glycemic control (Gebreyohannes et al., 2019). In addition, a recent study in northeast Ethiopia found that approximately 60% of diabetic patients were affected by one or more of diabetic complications (Abejew et al., 2015). Thus, poor glycemic control among people with diabetes constitutes a major public health problem and a significant risk for complications associated with diabetes (Carls et al., 2017). Moreover, most of the recent studies on T2DM patients associated long duration of diabetes, inadequate physical exercise, lower level of education, dyslipidemia and complexity of medications with poor glycemic control (Milo & Connelly, 2019; Shan et al., 2017). However, one of the most important yet not well studied factor, particularly in Ethiopian patients with T2DM is the role of pharmacogenetics; the study that is focused on the variation of human gene and its effect on the individual drug response and adverse effect.

Metformin is the first-line agent for the treatment of people with T2DM as recommended by most clinical guidelines (Davies et al., 2018), which are supported by prospective studies ("United Kingdom Prospective Diabetes 34)," 1998; "United Kingdom Prospective Diabetes Study 24," 1998), and recent meta-analyses (Maruthur et al., 2016; Palmer et al., 2016). The guidelines are founded on the improved glycemic profile and cardiovascular mortality reduction by metformin treatment, without the risk of hypoglycemia and weight gains that are linked with other glucose lowering agents ("United Kingdom Prospective Diabetes 34," 1998; "United Kingdom Prospective Diabetes Study 24," 1998; Palmer et al., 2016; Sanchez-Rangel & Inzucchi, 2017). Furthermore, metformin is also the favored glucose lowering agent because of its minimal cost and relative safety profile (Howlett & Bailey, 1999).

However, despite its favored profile in the treatment of patients with T2DM, there is a substantial variation in response to metformin therapy ranging from up to 4% improvement in HbA1C to worsening of HbA1C following treatment (Rena & Sakamoto, 2012) and estimates of about 35% metformin monotherapy failure rate (Cook et al., 2007). Thus, in patients receiving metformin as their initial treatment for T2DM, only less than two-third achieve acceptable glycemic target or a target HbA1c of <7.0% (Hermann et al., 1994; Kahn et al., 2006). In addition, metformin therapy is linked with more gastrointestinal symptoms than most other oral glucose lowering agents (DeFronzo, 1999; Haupt et al., 1991). Furthermore, previous studies reported the inter-subject variability in metformin treatment response (K. Zhou et al., 2014) and occurrence of side effects related to differences in individual genetic profiles (Pawlyk et al., 2014; Todd & Florez, 2014). Thus, recently precision medicine is getting attention regarding metformin pharmacotherapy in patients with T2DM. As part of an effort to personalize metformin treatment among patients with T2DM, several pharmacogenomics studies particularly indicated genetic polymorphisms of hepatic glucose transporter and drug transporters in association with the observed variations in metformin treatment outcomes.

In terms of pharmacodynamics, glucose transporter 2 (GLUT2) encoded by solute carrier family 2 member 2 *(SLC2A2)* gene, is reported to be an excellent biological candidate for metformin's pharmacogenetic studies (Dupuis et al., 2010), as it is the main transporter of glucose output from the liver (Seyer et al., 2013; Burcelin et al., 2000) and metformin has a major pharmacologic effect of reducing glucose output from the liver (Madiraju et al., 2014; Foretz et al., 2010). Furthermore, a genome-wide association study (GWAS) conducted in European patients with T2DM supported this notion indicating that the minor C-allele (rs8192675) in the intron of *SLC2A2* gene is associated with a greater reduction in HbA1c (K. Zhou, Yee, et al., 2016) although not replicated in other studies (Rotroff et al., 2018). Moreover, the minor C-allele of *SLC2A2* might have particular importance in the African population as it was reported to have a higher frequency of 79% (*rs8192675 (SNP) - Population Genetics - Homo_sapiens - Ensembl Genome Browser*).

Similarly, studies conducted in diverse population have suggested that solute carrier family 22 member 1(*SLC22A1)* genetic polymorphisms, encoding for one of the main metformin

transporters, the organic cation transporter-1 (OCT1) (Becker et al., 2009; Takane et al., 2008), could affect metformin treatment responses by altering its pharmacokinetics (Dujic et al., 2017; Seitz et al., 2015). However, there is no agreement about its precise effect since inconsistent and contrasting findings have been reported (Becker et al., 2009; Dujic et al., 2015, 2017; Tarasova et al., 2012; Y. Zhou et al., 2015). In addition, a more recent study indicated that the role of *SLC22A1* polymorphisms in individual responses to metformin treatment is population-specific (Mofo Mato et al., 2018).

Furthermore, variations in the *SLC22A1* gene were also reported to be associated with metformin induced gastrointestinal adverse effects (Tarasova et al., 2012). Although the biological mechanism for metformin-induced gastrointestinal intolerance remains poorly understood (K. Zhou, Pedersen, et al., 2016), the accumulation of metformin in the gut as a result of the expression of the reduced function variants of *SLC22A1* (rs628031 and rs36056065) in enterocytes can partially explain the intolerance (Tarasova et al., 2012). However, there are inconsistent reports on the association between the role of individual *SLC22A1* variants and gastrointestinal side effects (Tarasova et al., 2012). Furthermore, the reduced function variant of *SLC22A1* (rs72552763), methionine deletion at codon 420 (M*et420del*), was selected in this study as it is the most studied variant of *SLC22A1* for its influence on metformin pharmacokinetics and metformin treatment outcomes (both in response and gastrointestinal intolerance) though there were inconsistent reports based on an extensive survey of recent literature. In addition, the Met420 deletion variant of *SLC22A1* gene is quite common in African descent (Goswami et al., 2014) and across Africa (Schaller & Lauschke, 2019). Moreover, this variant is generally observed in combination with a rare OCT1 loss of function polymorphism, *Cys88Arg* (rs55918055), that causes its improper membrane localization and the associated metformin induced gastrointestinal intolerance (Dujic et al., 2015).

1.2. Statement of the problem

In clinical practice, the inter-individual variation in response to the first line oral glucose lowering agent, metformin, is very high among T2DM patients (Cook et al., 2007; van Leeuwen et al., 2013; Florez, 2011). Some of the variations can be explained by differences

in dosage and adherence (Donnelly et al., 2006). However, others do not (Rena et al., 2017; R. Song, 2016), suggesting that variations in metformin pharmacogenetics could be a factor (Brunetti et al., 2017). Thus, although metformin is still the recommended first-line oral glucose lowering agent for patients with T2DM (Overbeek et al., 2017; Tanabe et al., 2017), there are increasing reports demanding more personalized approaches in its use (Holt, 2016; Pearson, 2016), as populations of different ethnic background may exhibit marked variability in the metformin treatment outcomes. In addition, few or no studies have addressed the role of pharmacogenetics on metformin treatment outcome in Ethiopian patients despite an increasing number of patients living with diabetes (Gebreyes et al., 2018). Consequently, there is a need to assess the potential role of candidate genetic polymorphisms, in terms of metformin treatment outcomes in Ethiopian patients with T2DM, given the high prevalence of poor glycemic control (Abera et al., 2022; Abdissa & Hirpa, 2022).

To date, although there are several studies, it's been difficult to designate the exact relationship between *SLC2A2* rs8192675 and *SLC22A1* rs72552763 polymorphisms and metformin treatment outcomes in patients with T2DM in ethnically diverse populations. Besides, although higher frequencies were reported for these polymorphisms in African population, most the studies on these polymorphisms regarding its association with metformin treatment outcomes in T2DM primarily focused on Caucasian, Asian and European populations (I. S. Song et al., 2008). Thus, similar studies are essential to be conducted in African populations (Campbell & Tishkoff, 2008), including Ethiopia.

Furthermore, reports emanating from Ethiopian studies indicated a presence of a vast number of T2DM patients with dyslipidemia (Abdosh et al., 2019; Gebreyesus et al., 2022; Haile & Timerga, 2020). Moreover, although proper management is essential (Dagnew et al., 2021), lipid lowering agents are expensive in resource-limited settings like Ethiopia. In addition, although dyslipidemia is a well-established devastating cardiovascular risk factor in T2DM, different populations might exhibit different changes in their blood lipid profiles, owing to their genetic and environmental factors (Sert et al., 2019). Hence, measuring the prevalence, pattern and determinants of dyslipidemia and directing the scarce resource in its prevention and tailored management is the best solution. However, there is no

comprehensive study carried out in Ethiopia to determine the prevalence, pattern and determinants of dyslipidemia among patients with diabetes, particularly in T2DM (Bekele et al., 2017).

1.3. Research questions and hypothesis

In this study, we hypothesized that metformin treatment outcomes might be influenced by pharmacogenetics in Ethiopian patients with T2DM. Thus, primarily two research questions related to metformin pharmacogenetics were addressed in our study: I) Does metformin treatment response influenced by *SLC2A2* rs8192675 and *SLC22A1* rs72552763 polymorphisms in Ethiopian patients with T2DM? II) Does metformin- induced gastrointestinal intolerance influenced by *SLC22A1* rs72552763 polymorphism in Ethiopian patients with T2DM?

1.4 Objectives

1.4.1 General objective

To investigate the association of *SLC2A2* rs8192675 and *SLC22A1* rs72552763 polymorphisms with metformin treatment response and *SLC22A1* rs72552763 with metformin induced gastrointestinal intolerance in Ethiopian patients with T2DM.

1.4.2 Specific objectives

- To determine the allele frequencies of rs8192675 polymorphism of *SLC2A2* and Met420del variant of *SLC22A1* (rs72552763) in patients with T2DM at SPHMMC.
- To investigate the association of rs8192675 polymorphism of *SLC2A2* with metformin treatment response in patients with T2DM at SPHMMC.
- To investigate the association of rs8192675 polymorphism of *SLC2A2* with diabetic dyslipidemia in patients with T2DM at SPHMMC.
- To investigate the association of Met420del variants of *SLC22A1* with metformin treatment response in patients with T2DM at SPHMMC.
- To investigate the association of Met420del variants of *SLC22A1* with metformin induced gastrointestinal intolerance in patients with T2DM at SPHMMC.

1.5 Significance of the study

Understanding genetic predictors of the variability in metformin treatment outcome, both in terms of its response and intolerance, is important in the rational utilization of metformin pharmacotherapy in patients with T2DM. Accordingly, the finding of our study indicated that genotyping for the Met420del variant of *SLC22A1* gene may contribute to timely identification of metformin treatment responders to non-responders before the start of pharmacological therapy if our finding is replicated in the study of larger sample size. Furthermore, the findings in our study regarding the association of *SLC2A2* (rs8192675) and *SLC22A1* (rs72552763) polymorphisms with metformin treatment outcomes might contribute to knowledge synthesis for the Ethiopian and African genetic diversity in terms of metformin treatment response and gastrointestinal intolerance. In addition, this study generated evidence on the prevalence, pattern and determinants of dyslipidemia among patients with T2DM so as early detection and control of blood lipid levels could be possible, thereby reducing adverse events of cardiovascular origin in patients with T2DM.

1.6 Operational definitions

Related patient: Having at least one-first-degree relative with diabetes (Zhang et al., 2015) who has follow up in the diabetic clinic of SPHMMC.

Unrelated patient: Patient who do not have a related patient in the diabetic clinic of SPHMMC.

Metformin responders: responders were patients showing $\geq$ 0.5% reduction in HbA1c levels from baseline within 3 months of metformin monotherapy and remained low for at least another 3 months (Park et al., 2018; Shikata et al., 2007).

Metformin non-responders: non-responders were patients with < 0.5% reduction in HbA1c levels from baseline within 3 months of metformin monotherapy and/or those for whom either another hypoglycemic drug was added or replaced because of unsatisfactory reduction (Park et al., 2018; Shikata et al., 2007).

Glycemic control: Attainment of HbA1c < 7.0% were defined as good glycemic control and poor glycemic control when ≥7.0% (American Diabetes Association, 2018).

Physical activity: Individuals who performed at least 150 minutes of moderate-intensity exercise per week (3 days) were regarded as active or inactive if performed less than 150 minutes per week in the last seven days (American Diabetes Association, 2022).

Adherence to diet: Individuals who adjust their lifestyle (diet) as recommended for at least 4 days in the last seven consecutive days (American Diabetes Association, 2022).

Adherence to metformin treatment: A four-item Self-Reported Measure of Adherence scale were used to assess medication adherence and individuals who responded "NO' for all the four questions were considered adherent to medication (Lim et al., 2021).

Metformin daily dose: In the response study, metformin dose per day was defined as the average dose in the first three months of metformin treatment (Park et al., 2018; Shikata et al., 2007). Metformin dose per day was considered as the last prescribed dose for intolerant patients while it was defined as an average dose in the first 6 months (after satisfying tolerance definition) of treatment for tolerant patients (Dujic et al., 2015).

Metformin gastrointestinal intolerance: Patients meeting the following were taken as intolerant: I) Patients on metformin monotherapy and stopped taking metformin immediate release (IR) preparation in the first 6 months of metformin treatment and switched to another oral glucose lowering agent (including slow-release formulations of metformin) within six months of last metformin IR prescription and reported GI adverse effects as the reason for switching (Dujic et al., 2015, 2016); II) Patients on metformin monotherapy and could not increase their metformin IR dose > 500 mg per day despite an HbA1c > 7.0% because of the reported GI adverse effects (Dawed et al., 2019).

Metformin gastrointestinal tolerance: Tolerant individuals were defined as those treated with ≥ 2,000 mg of metformin per day for more than 6 months (excluding modified-release preparations of metformin) and reported no gastrointestinal adverse effects (Dujic et al., 2015).

Dyslipidemia: defined according to the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (adult treatment panel III) as the presence of one or more of the following lipid abnormalities: TC ≥ 200 mg/dl, LDL-C levels ≥ 100 mg/dl, TG ≥ 150 mg/dl, or HDL-C levels < 50 mg/dl or < 40 mg/dl in females and males, respectively (NCEP, 2001).

Diabetic dyslipidemia: defined as triglyceride levels of ≥ 150 mg/dL and/or HDL levels of < 40 mg/dL and < 50 mg/dL in men and women respectively (American Diabetes Association, 2022).

Elevated non-HDL-C: defined as non-HDL-C levels ≥ 130 mg/dl (Grundy et al., 2019).

Pattern of dyslipidemia: Isolated single-parameter dyslipidemia was defined as derangement of only one lipid parameter, combined-parameter dyslipidemia was defined as two abnormal lipid parameters while mixed-parameter dyslipidemia was defined as elevations in TG and LDL-C combined with decreased levels of HDL-C (Daya et al., 2017).

BMI classification: BMI values of 18–24.9 kg/m^2 was defined as normal weight, while values of 25–29.9 kg/m^2 was considered overweight and obese when ≥ 30.0 kg/m^2 ("Obesity," 2000).

2. Literature review

2.1. Epidemiology of type 2 diabetes mellitus

T2DM has reached epidemic proportions globally as the International Diabetes Federation (IDF) estimated 463 million people living with T2DM in 2019 and this figure is projected to rise to 700.2 million by 2045 (Saeedi et al., 2019). Furthermore, evident disparities in the projected regional increases were reported, in which SSA countries are expected to register the highest growth rates (Saeedi et al., 2019). Thus, T2DM, which was once considered rare in SSA, has begun to become an important cause of morbidity and mortality (Motala et al., 2022). Furthermore, in contrast to the evidence coming from the Western world, numerous reports show that the majority of people diagnosed with diabetes in SSA show a distinct diabetes phenotypes in which pancreatic beta cell secretory dysfunction predominates rather than peripheral insulin resistance (G. Gill et al., 2010; G. V. Gill et al., 2011; Kibirige et al., 2019; Mbanya et al., 2010). In support of this notion, a recent GWAS of 5231 African patients with T2DM identified a novel locus called *ZRANB3* (encoding zinc finger RANBP2-type containing 3). *ZRANB3* gene product, via apoptotic events, leads to the reduction of pancreatic beta cell mass (Adeyemo et al., 2019). In addition, the gene that encodes transcription factor 7- like 2 (TCF7L2), well-known to affect pancreatic secretory function, is also identified in African populations with T2DM (Adeyemo et al., 2015).

Furthermore, several studies (Osei et al., 1997), showed both lower postprandial and basal insulin clearance in people of African descent compared to Europeans. Furthermore, studies proposed the reduction in hepatic insulin clearance as a compensatory mechanism to the body insulin resistance in order to preserve beta cell function (Mittelman et al., 2000), based on experimental models. In addition, a 2-year follow-up study in a multi-ethnic cohort showed that lower insulin clearance by the liver was an independent determinant of the reduced beta-cell function (Galderisi et al., 2019). In agreement with this observation, a recent study comparing men of west African descent recently diagnosed with T2DM to their European counterpart showed that African men had lower insulin secretion in

response to oral glucose with concomitant lower insulin clearance rate by the liver (Mohandas et al., 2018).

Thus, although the conventional paradigm that defines the pathogenesis of T2DM places insulin resistance as the primary defect that results in compensatory hyperinsulinemia and beta cell exhaustion (Petersen & Shulman, 2018), there is increasing evidence for the theory that hyperinsulinemia may be the distinct initial event in the pathogenesis of T2DM in Black Africans (Goedecke & Olsson, 2020). Moreover, in contrast to the widely believed notion, in some studies circulating TG levels were reported to be lower among Black Africans and not associated with the reduction of insulin sensitivity (Knight et al., 2011). In addition, studies reported that, there is a huge number of microvascular complications from T2DM in SSA compared with other regions of the world (Hayfron-Benjamin et al., 2019). Indeed, this could in part justified by the late diagnosis of T2DM, lack of specialized diabetes care and poor metabolic control (Hayfron-Benjamin et al., 2019). Overall, recent literatures underlines the need to comprehensively understand and describe the unique phenotype of diabetes in SSA. Hence, these evidences support the need for a much tailored approach across ethnicities in defining and managing T2DM (Pearson, 2019).

2.2 Metformin therapy in type 2 diabetes mellitus

To date, the efforts in the T2DM management focus to control blood glucose, improve insulin resistance, enhance insulin sensitivity, improve symptoms and reduce the risk of macro- and microvascular complications (K. Wu et al., 2020). In order to attain these goals, metformin is recommended as the first line oral medication in addition to lifestyle adjustment, according to most clinical guidelines (Buse et al., 2020; Dodds, 2017). Although metformin is considered as first choice oral glucose-lowering agent and has been utilized for the treatment of T2DM for more than half a century, the exact molecular mechanism of its antidiabetic effect still remains poorly understood (Foretz et al., 2019).

Indeed, over the past decades several studies identified multiple cellular targets of metformin on its glucose lowering effect, among which, the adenosine monophosphate (AMP)-activated kinase (AMPK) pathway has been widely considered the primary mechanism (Zhu et al., 2023). It is widely believed that metformin inhibits complex I of

the hepatic mitochondrial electron transport chain (Foretz et al., 2019; Yoval-Sánchez et al., 2022). Subsequently, hepatic production of adenosine triphosphate (ATP) decreases, thereby increasing AMP levels in the hepatocytes. AMPK is a trimeric complex which is allosterically regulated by AMP binding, as AMP-bound AMPK promote its phosphorylation at Thr172 either by Ca2+/calmodulin-dependent protein kinase kinase β (CAMKKβ) or liver kinase B1 (LKB1) (Hardie, 2014). Activation of AMPK is proposed to induce disassembly of the cAMP-responsive element-binding protein 1 (CREB) - CREB-binding protein (CBP) - CREB-regulated transcription co-activator 2 (CRTC2) complex which transcriptionally regulates gluconeogenic gene expression and thereby results in the reduction of hepatic glucose production (Petersen et al., 2017; Lin & Accili, 2011). It is worth mentioning that CREB is often utilized as gluconeogenic regulation readout and the CREB-CBP-CRTC2 transcriptional complex enhances expression of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase1, the two gluconeogenic genes (Petersen et al., 2017; Lin & Accili, 2011). In addition, activation of the pathway is proposed to induce phosphorylation of acetyl-CoA carboxylase 1 (ACC1) and ACC2, which inhibits lipogenesis and enhances hepatic mitochondrial oxidation, respectively (Savage et al., 2006), resulting in the reduction of liver diacylglycerol content and increased hepatic insulin sensitivity (Fullerton et al., 2013; R. J. Shaw et al., 2005; G. Zhou et al., 2001). Furthermore, since gluconeogenesis is an ATP-dependent pathway, the decrement in cellular energy charge might account for the reduction in hepatic gluconeogenic flux (Foretz et al., 2010). In addition, the increased intracellular levels of AMP might lead to the inhibition of AMP regulated hepatic gluconeogenic enzymes such as adenylate cyclase and fructose-1,6-bisphosphatase, which contributes to the decreased hepatic glucose production (Foretz et al., 2010; Miller et al., 2013; Hunter et al., 2018).

However, the AMPK pathway was once challenged when studies showed that metformin could inhibit liver gluconeogenesis in mice lacking either LKB1 or AMPKα1α2 catalytic isoforms (Foretz et al., 2010). Foretz et al., found that the glucose lowering effect of metformin was not altered in hepatic AMPK deficient mice, as compared to the wildtype (Foretz et al., 2010). Consistently, metformin induced suppression of the gluconeogenic genes expression in the liver was comparable in LKB1 deficient, wild-type and

AMPKα1α2 deficient hepatocytes, confirming that neither LKB1 nor AMPK are required for metformin-induced suppression of gluconeogenesis in the liver (Foretz et al., 2010). Indeed, subsequent studies questioned the supra-pharmacological concentrations of metformin in the previous study and confirmed that low concentrations of metformin inhibits gluconeogenesis via an AMPK dependent mechanism (Cao et al., 2014). Therefore, it could be speculated that the cellular mechanisms of metformin-induced suppression of glucose production might be associated with its dose (He & Wondisford, 2015).

On the other hand, studies showed that clinically relevant (micro-molar) metformin concentrations suppress hepatic glucose production independent of any detectable changes in cellular energy charge (Alshawi & Agius, 2019; Madiraju et al., 2014; Cao et al., 2014). However, sometimes measuring the small and transient physiological changes at the adenine nucleotide levels might be impeded by technical limitations. Furthermore, using AMPK as a sensitive probe in order to detect subtle changes on the AMP to ATP ratio had shown metformin mediated changes in energy charge that are not measureable with other methods (Hawley et al., 2010). Thus, mitochondrial complex I inhibition mediated reduction in energy charge cannot be ruled out as a reason behind the reduced hepatic glucose production in response to low doses of metformin.

Furthermore, using clinically relevant doses of metformin, one recent study demonstrated that metformin mediated activation of AMPK occurs via inhibition of the lysosomal proton pump vacuolar -ATPase (Ma et al., 2022) instead of the mitochondrial complex I. Ma et al. further identified the membrane protein presenilin enhancer 2 (PEN2; a subunit of γ-secretase complex), as the binding partner of metformin. Moreover, they showed that the binding of metformin to PEN2 forms a complex with ATPase H+ transporting accessory protein 1 (ATP6AP1), subunit of the vacuolar H+-ATPase (v-ATPase), which leads to the inhibition of v-ATPase and activation of AMPK (Ma et al., 2022). In addition, using in vivo models, they demonstrated that liver-specific PEN2 knockout abolishes metformin-induced reduction of fat in the liver and glucose tolerance, whereas intestine-specific PEN2 knockout impairs the glucose-lowering effects of metformin that is associated with GLP1

secretion (Ma et al., 2022). Furthermore, the identification of PEN2-mediated AMPK activation in the gut by Ma et al., as a mechanism for metformin's glucose lowering effect is in agreement with an early study that indicated activation of duodenal AMPK pathway as a mechanism for metformin's suppression of hepatic glucose production in rats (Duca et al., 2015). In addition, the activation of AMPK by metformin is also proposed to increase glucose uptake by enhancing glucose transporter 4 translocation in the skeletal muscle cells (Griffin et al., 2017; Polianskyte-Prause et al., 2019).

More recently, Xie and associates have demonstrated that, metformin upregulates microRNA let-7, to downregulate Tet methylcytosine dioxygenase 3 (TET3) and suppresses the TET3/ hepatocyte nuclear factor 4α (HNF4α)-P2 axis at clinically relevant doses, which in turn leads to decreased hepatic glucose production (Xie et al., 2022). In addition, recent studies on patients with T2DM implicated metformin associated alteration in the gut microbiome composition as its potential mechanism of glucose lowering action (H. Wu et al., 2017; Forslund et al., 2015). Some of the well-documented metformin–induced gut microbiota alteration is the shift towards short-chain fatty acids (SCFA)-producing bacteria and increased fecal concentrations of both propionate and butyrate in patients with obesity and T2DM (Forslund et al., 2015; Mueller et al., 2021; H. Wu et al., 2017). The beneficial effects of SCFAs on glucose metabolism occurs mainly via modulation of intestinal gluconeogenesis and stimulation of peptide YY and GLP1 release from enteroendocrine L cells (De Vadder et al., 2014; Holst et al., 2021). Moreover, metformin is also reported to upregulate the expression of sodium glucose cotransporter-1 (SGLT1) from the upper small intestine, partly through increasing the abundance of Lactobacillus species (Bauer et al., 2018). Furthermore, metformin modulates intestinal bile acid pool by reducing *Bacteroides fragilis* abundance and decreasing its bile salt hydrolase activity (Sun et al., 2018). The increased bile acid pool increases bile acid glycoursodeoxycholic acid levels, which improves glucose homeostasis via inhibition of intestinal farnesoid X receptor signalling through AMPK-independent mechanism of increased GLP1 levels (Sansome et al., 2020; Sun et al., 2018). Furthermore, this notion is supported by several studies, which showed that exogenous bile acids increase circulating GLP-1 (Adrian et al., 1993; Hansen et al., 2016; Thomas et al., 2009; T. Wu et

al., 2013). Interestingly, previous studies also shown that metformin increases the luminal concentration of bile acids in the colon and ileum by inhibiting the apical sodium-dependent bile acid transporter (ASBT) in the ileum (Scarpello et al., 1998).

2.3 Pharmacogenetics of metformin in type 2 diabetes mellitus

Despite the popularity of metformin in the treatment of T2DM, there are significant inter-individual differences in the glucose lowering effect of metformin, while more than 30% of patients do not reach the target glycemic level after treatment (Cook et al., 2007). Furthermore, approximately 30% of patients had significant metformin associated gastrointestinal intolerance after taking normal doses (Ieiri et al., 2006). This variation in metformin treatment outcome may reflect phenotypic differences and the variation in drug distribution or action. However, several studies have shown no or little clinical effect of phenotypic parameters such as sex, age, or BMI on metformin treatment response (DeFronzo & Goodman, 1995; Donnelly et al., 2006; Hermann, Scherstén, Bitzén, et al., 1994), suggesting genetic variation in pharmacodynamics or pharmacokinetics of metformin at molecular level may be important.

To date, four GWAS have been published in different cohorts on metformin, reporting on HbA1c change in people with T2DM, with additional GWAS reporting the genetic interaction with diabetes prevention and metformin (J. H. Li, Perry, et al., 2023) and acute treatment response to metformin in non-diabetic people (J. H. Li, Brenner, et al., 2023).

The Genetics of Diabetes and Audit Research Tayside Study (GoDARTS) research group has pioneered GWAS for metformin response by enrolling 1024 Scottish patients with T2DM in 2011. In GoDARTS study, an association signal was detected for metformin response around ataxia-telangiectasia mutated kinase (ATM) gene. The strongest detected association was at SNP rs11212617. According to the study the C-allele of rs11212617 was associated with treatment success (K. Zhou et al., 2011). In addition, a significant association between rs11212617 and metformin treatment response was replicated in 2 independent cohorts, UKPDS (UK Prospective Diabetes) cohort of 1113 patients and GoDARTS cohort of 1783 patients (K. Zhou et al., 2011). ATM belongs to the

phosphatidylinositol 3-kinases which phosphorylate key substrates involved in cell cycle control and/or DNA repair (Abraham, 1998; Gatti et al., 1988).

Furthermore, the association of rs11212617 with metformin response was replicated on numerous independent cohorts such as the Chinese Han population and the population of Western Saudi Arabia (Altall et al., 2019; Y. Zhou et al., 2014). Similarly, in a meta-analysis involving users of metformin selected from the Rotterdam Study, the Diabetes Care System West-Friesland (DCS), and the Collaborative Atorvastatin Diabetes Study (CARDS) Trial shown that rs11212617 was associated with treatment success (van Leeuwen et al., 2012). In contrast, no significant association was observed between rs11212617 and response to metformin in Indian and Iranian patients with T2DM (Shokri et al., 2016; Vilvanathan et al., 2014). Furthermore, functional validation of the possible role of ATM in metformin treatment outcome remains a subject of controversy. Although the investigators presented in vitro data, in cell cultures, that showed ATM was involved in metformin mediated activation of AMPK (K. Zhou et al., 2011), it has since been known that the small molecule utilized to inhibit ATM in cell culture studies is actually an OCT1 inhibitor (Yee et al., 2012). Thus, it greatly complicated the interpretation of the experiments, in order to determine the mechanistic role of ATM in metformin response.

Metformin Genetics (MetGen) Consortium performed the second GWAS on metformin treatment response in 2016, using participants with a harmonized measure of response to metformin. Thus, a genome-wide significant association was detected at rs8192675, in an intron of *SLC2A2* that codes for GLUT2. According to the research, T2DM patients with the C-allele of the intronic variant of *SLC2A2* (rs8192675) had increased before treatment HbA1c levels and had greater on treatment HbA1c reduction after metformin therapy compared to individuals having T-allele at this locus (K. Zhou, Yee, et al., 2016). In same study, the C-allele of rs8192675 was shown to result in the reduced expression of *SLC2A2* (K. Zhou, Yee, et al., 2016). Thus, underpinning these genetic observations, a very recent study conducted to confirm the direct mechanistic role of *SLC2A2* used pre-clinical mouse models of diabetes with reduced whole-body *Slc2a2* levels. Accordingly, the result of the study showed that reduced whole-body *Slc2a2* have improved metformin mediated glucose uptake into the gut from the circulation. In this Slc2a2+/- mice, reduced *Slc2a2* expression

resulted a rise in the blood glucose levels that was reversed by metformin treatment. Furthermore, they observed a reduction in the *Slc2a2* expression in the gut of *Slc2a2+/-* mice exposed to chronic metformin (Morrice et al., 2023). Although it seems counterintuitive that decreasing a glucose transporter would improve glucose transport, there is evidence in the role of other glucose transporters (like SGLT1) in the metformin mediated glucose uptake from the lumen (Morrice et al., 2023). Thus, it is plausible to assume the impact of the other transporters is enhanced in the face of reduced GLUT2 levels.

Furthermore, the association of metformin response with rs8192675 has been replicated in an independent cohort from the German Diabetes Study (GDS) (Rathmann et al., 2019). However, another recent study (Abrahams-October et al., 2021), in the indigenous SSA patients with T2DM found no association between the rs8192675 polymorphism of *SLC2A2* and metformin treatment response. GLUT2, the *SLC2A2* gene product, is a low affinity facilitative transporter of glucose that is expressed in cells involved in glucose homeostasis, i.e., intestine, kidney, liver, and pancreatic cells (Thorens et al., 1990). Thus, defects in *SLC2A2* gene could potentially affect glucose homeostasis at any of these sites (K. Zhou et al., 2014). The *SLC2A2* rs8192675 in addition to its association with metformin treatment response, other studies indicated several potentially interesting associations with various metabolic phenotypes, including HDL and TG levels. Interestingly, data collected from the discovery cohort, Genetic Epidemiology of Responses to Antihypertensives studies (GERA I and II) showed that *SLC2A2* rs8192675 was associated with decreased HDL levels and increased TG levels after adjusting for covariates (Le et al., 2013).

The third GWAS on 1312 white and black participants used a large cohort of patients with T2DM in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study, and showed that the variants in carboxypeptidase A6 *(CPA6)*, pre-mRNA processing factor 31 *(PRPF31)* and signal transducer and activator of transcription 3 *(STAT3)* were associated with metformin response (Rotroff et al., 2018). Variants rs2162145 in *CPA6* and rs254271 in *PRPF31* were associated with better and worse metformin response, respectively. Interestingly, rs254271 is an expression quantitative trait locus for PRPF31 and nearby genes, including NADH:ubiquinone oxidoreductase (NDUFA3), which codes for a subunit

of the complex I of the respiratory chain (Lonsdale et al., 2013; Owen et al., 2000; Zeller et al., 2010). Meta-analysis in independent cohorts also displayed similar associations with metformin treatment response (Rotroff et al., 2018). Additionally, the rare variant STAT3 achieved statistical significance with worse metformin response (Rotroff et al., 2018). However, the findings from the previous GWAS which have identified the association of rs8192675 in *SLC2A2* and rs11212617 in the ATM gene with metformin treatment response were not replicated in the ACCORD study (Rotroff et al., 2018).

The recent and fourth discovery GWAS utilized data from 447 African Americans, while replication study was undertaken in 466 European Americans and 353 African Americans (B. Wu et al., 2024). The most significant allele associated with metformin response and replicated was rs143276236, an intronic SNP of ADP-ribosylation factor guanine nucleotide exchange factor 3 (ARFGEF3), which has a plausible connection with glucose metabolism, since it is expressed in β-cells and α-cells and its knockout in mice increased insulin granule content and its secretion (H. Li et al., 2014, 2015). The genome-wide variant, rs143276236, was not replicated European American population while it was replicated in an independent cohort of African Americans. While the mechanistic relationship between metformin treatment response and ARFGEF3/BIG3 remain to be determined, the gene itself is a biologically plausible mediator of blood glucose level (B. Wu et al., 2024).

On the other hand, most candidate gene association studies as well as several experimental studies demonstrated that drug transporter gene polymorphisms may alter metformin disposition in the body, given metformin virtually exists as a monoprotonated cation at physiological pH and its transport across biological membranes involves uptake via specific transporters (Hardie, 2014).

As such, metformin uptake from the intestinal lumen is primarily mediated by plasma membrane monoamine transporter (PMAT) and OCT3 which are localized in the apical membrane of enterocytes. The drug is subsequently transported from the enterocytes into the bloodstream by the basolateral OCT1 (Liang & Giacomini, 2017). The liver uptake of metformin is mediated by OCT1 and possibly OCT3, whereas multidrug and toxin

extruders-1 (MATE1) contributes to its hepatic elimination (Liang & Giacomini, 2017), while its transport into the renal epithelial cells depends on OCT2 and its urinary excretion is mediated by MATE1/2 (Liang & Giacomini, 2017; Phate et al., 2020). Among which, OCT1 is the most studied transporter protein regarding the association of its genetic variants with metformin treatment outcome, as it is implicated in the intestinal absorption and hepatic distribution of metformin (Shu et al., 2007). OCT1 is a member of polyspecific OCT family expressed in the intestine, kidney and liver (Gründemann et al., 1994). It is electrogenic, facilitative transporter that function bidirectionally and it is independent of chloride and sodium ions (Koepsell & Endou, 2004; Wagner et al., 2016). However, there are inconsistent reports in associating metformin treatment outcome with genetic variants of OCT1 (Nies et al., 2009). In addition, the *SLC22A1* gene is highly polymorphic and several variants of the gene affect transporter function and expression (Florez, 2017; Umamaheswaran et al., 2015). Thus, the importance of OCT1 and the potential role of *SLC22A1* in metformin pharmacogenetics suggest that further studies are required to scrutinize its relationship with metformin treatment outcome in different ethnic populations.

2.3.1 SLC22A1 polymorphisms on metformin treatment response

In recent years, the association of *SLC22A1* genetic variations with metformin treatment response has been extensively studied but the results were contradictory (Mofo Mato et al., 2018). Indeed, studies on the potential role of *SLC22A1* polymorphisms in metformin therapeutic responses demonstrated both positive and negative effects (Mofo Mato et al., 2018). Thus, the inconsistent outcomes might be attributed to the differences in the frequency of the genetic variants and/or due to population differences that could also be genetic or environmental (Bachtiar & Lee, 2013).

A recent meta-analysis specifically on the studies involved Chinese population showed that T2DM patients homozygous for the rs628031, reduced function variant of *SLC22A1*, exhibited greater reductions in FBG level compared with variant heterozygous and wild homozygous after metformin monotherapy (Peng et al., 2023). The rs628031 (1222A>G) polymorphism causes a missense mutation in the OCT1 functional protein that changes

methionine by valine at position 408 (Met408Val) (Becker et al., 2009), which causes decreased OCT1 mRNA expression in the enterocytes that decreases intestinal metformin absorption and its plasma concentration (Semiz et al., 2013). In addition, the pooled result from the same study showed that *SLC22A1* rs622342 was associated to the glucose lowering action of metformin. The rs622342 polymorphism does not change the amino acid sequence of OCT1 functional protein, although it may affect its gene expression (Peng et al., 2023). Similarly, research conducted on 33 individuals in Japan associated the Met408Val variant of *SLC22A1* as positive predictor of metformin efficacy (Shikata et al., 2007). Likewise, a study conducted on healthy volunteers from the Danish population showed Gly401Ser reduced function variant of *SLC22A1* was associated with a significantly better absolute decrease in HbA1c levels (Christensen et al., 2015b). Glycine to serine substitution at codon 401 (Gly401Ser), caused by 17857 G>A polymorphism at rs34130495, results a general impairment of OCT1 transport activity without affecting its membrane localization (Seitz et al., 2015). In addition, although the true effect of rs4646272 (-43T>G) intronic variant of *SLC22A1* remains unknown, it exhibited a greater reduction of HbA1c and FBG in Han Chinese population (Y. Zhou et al., 2015).

In contrast, a Danish study on T2DM patients found out that the rs72552763, rs34059508 and rs12208357 decreased function variants of *SLC22A1* were associated with lower trough metformin concentrations and a reduction in the absolute decrease in HbA1c following metformin therapy, although the association of number of reduced function alleles with a reduction in the absolute decrease in HbA1c was no longer found to be statistically significant after adjustment for baseline HbA1c (Christensen et al., 2011). Similarly, Shu and colleagues conducted a series of experiments to investigate whether reduced function variants rs12208357 (R61C), rs72552763 (Met420del), rs34130495 (G401S) and rs34059508 (G465R) of SLC22A1 contribute to the reduction in therapeutic response to metformin. The study by Shu et al showed that healthy volunteers carrying at least one of the four reduced function variants had higher plasma glucose levels in the oral glucose tolerance test (OGTT) compared to those having only reference alleles (Shu et al., 2007). Furthermore, the intronic variant rs4646272 (-43T>G) of *SLC22A1* was also reported to be a negative predictor of metformin efficacy in a Japanese population (L. Chen

et al., 2010). Moreover, the Rotterdam Study involving T2DM participants, reported a reduced glucose lowering action of metformin in association with the reduced function rs622342 variant of *SLC22A1* (Becker et al., 2009).

On the other hand, the study involving 1531 patients with T2DM found that the two most common *SLC22A1* polymorphisms, rs72552763 (Met420del) and *SLC22A1* rs12208357 (R61C), were not associated with glycemic response to metformin. The finding of the study reported that both R61C and Met420del variants of *SLC22A1* did not affect the initial and long-term HbA1c reduction and the chance of reaching a treatment target (K. Zhou et al., 2009). Furthermore, a study in healthy volunteers indicated that the different genotypes of *SLC22A1* had no impact both on glucose utilization and metformin steady-state pharmacokinetics (Christensen et al., 2015b, 2015a).

Besides, although several studies demonstrated that *SLC22A1* genetic variants may alter metformin pharmacokinetics, pooled analysis indicated that these alteration on metformin dispositions might not be significant enough to affect the clinical metformin effectiveness. However, the analysis involved only T2DM patients with European ancestry (Dujic et al., 2017).

2.3.2 SLC22A1 polymorphisms on metformin induced gastrointestinal intolerance

Several studies postulated the decreased transport of metformin through OCT1 might raise local metformin concentration in the intestine, hence leading to metformin's gastrointestinal side effects (Dujic et al., 2015; Khatami et al., 2019; Wilcock & Bailey, 1994). In support of this assumption, the Met408Val variant of *SLC22A1* in cohort of T2DM patients from Latvia shown to predispose to metformin associated gastrointestinal intolerance. Furthermore, the authors of the study reported that the rs628031 and rs36056065 reduced function variants of *SLC22A1* were independently associated with the occurrence of metformin associated gastrointestinal adverse effects, although reported no association with rs72552763 variants (Tarasova et al., 2012). Similarly, in another study involving participants from Bosnia and Herzegovina, the rs12208357 and rs72552763 reduced function variants of *SLC22A1* were associated with metformin associated gastrointestinal side effects (Dujic et al., 2016). In addition, a large cohort of GoDART

Study, showed that two reduced function *SLC22A1* alleles (rs12208357 and rs72552763) were associated with metformin associated gastrointestinal intolerance (Dujic et al., 2015). The 181C>T polymorphism at rs12208357 changes arginine to cysteine at position 61 (Arg61Cys) in the OCT1 functional protein that leads to a decreased OCT1-mediated metformin uptake by more than 70% (Seitz et al., 2015). The rs72552763 which is the most common functional variant of OCT1, constitutes a 3 base pair deletion at position 420 (Met420del). Although it does not change the membrane localization of OCT1 (Koepsell et al., 2007), Met420del decreases OCT1 mediated metformin uptake by more than 60% (Seitz et al., 2015). Moreover, individuals with two *SLC22A1* reduced-function alleles receiving treatment with known OCT1 inhibitors exhibited a nearly four times more gastrointestinal intolerance in the GoDART study (Dujic et al., 2015). Thus, inadequate OCT1 transport appears to be the major contributor in the metformin associated gastrointestinal intolerance, although there is lack of consistency in the association between individual reduced function *SLC22A1* variants and metformin induced gastrointestinal intolerance.

2.4 Dyslipidemia in type 2 diabetes mellitus

In T2DM, insulin resistance and/or deficiency trigger a cascade of metabolic derangements, that leads to a characteristic lipoproteins abnormality termed as diabetic dyslipidemia, which is characterized by elevated TG, low levels of HDL-C and slightly elevated LDL-C, with higher proportion of small dense LDL-C particles (Bekele et al., 2017; Grundy, 1997; Macho-González et al., 2019). Thus, an important phenotype of diabetic dyslipidemia is the elevation in the proportion of highly atherogenic small dense LDL-C particles rather than the absolute increase in LDL-C level (Schofield et al., 2016).

In patients with T2DM, the etiology leading to hypertriglyceridemia directly relates to hyperglycemia and insulin resistance, which results in hepatic overproduction of triglyceride-rich lipoproteins while decreasing its clearance and, in some cases, via alteration of postprandial lipoprotein metabolism (L. Wu & Parhofer, 2014). In T2DM, insulin resistance causes reduced inhibition of the hormone-sensitive lipase which leads to an increased lipolysis in adipose tissue, and thereby increased portal flux of free fatty acid

(FFA) to the liver. Furthermore, the elevated FFAs can directly disrupt lipoprotein lipase activity by causing its detachment from the endothelial surface (Hirano, 2018; Schofield et al., 2016). Consequently, the increased availability of FFAs in the liver causes in the decreased degradation of apoB, which leads to an overproduction of very-low-density lipoprotein in the face of insulin resistance. Moreover, an increase in the triglyceride-rich lipoproteins is associated with an increased small dense LDL-C and a reduction in HDL-C levels (Goldberg, 2001).

The global burden of dyslipidemia is continuously increasing, particularly in diabetic patients due to urbanization, obesity, reduced physical activity and increased consumption of unhealthy diets (Goldberg, 2001; Kiplagat et al., 2017). Ethiopia is also amongst the rapidly growing countries with rapid urbanization and lifestyle change where the prevalence of T2DM and its associated complications is also increasing promptly (Guariguata et al., 2014). Furthermore, a recent study in eastern Ethiopia reported dyslipidemia as a public health problem, reporting overall prevalence of 59.6% (Motuma et al., 2023), while a prevalence rate of 91% reported in northern Ethiopia among patients with T2DM (Gebreyesus et al., 2022).

Indeed, early identification and treatment of dyslipidemia can reduce the risk of atherogenic CVD in patients with T2DM (Dixit et al., 2014). However, even in individuals on statins and lifestyle therapy, and achieved the LDL-C management target, there is an increased residual cardiovascular risk (Cannon et al., 2004; Fitchett et al., 2006), indicating that there is more to attaining optimal LDL-C levels. Interestingly, the cardiovascular risk rate reduction with LDL-C-lowering therapies is only 30–40%, suggesting the presence of residual risk associated with low HDL-C, hypertriglyceridemia and small dense or highly oxidized LDL-C particles (Z. Chen et al., 2020).

3. Materials and Methods

3.1. Study setting

The participants for this study were recruited from a tertiary referral Hospital, St Paul's Hospital Millennium Medical College (SPHMMC), which is located in Addis Ababa, Ethiopia. Addis Ababa is both the capital and biggest city of Ethiopia, having a total of 12 public hospitals and 92 health centers. SPHMMC, the second largest hospital in Ethiopia, was selected because it serves a large number of people with diabetes referred from the surrounding health centers in its catchment area. In SPHMMC, the regular schedule for the outpatient diabetic clinic was three days a week. On these days, new cases diagnosed inside the hospital and those diagnosed somewhere else and referred to the hospital were accepted and enrolled in the follow up care. On average, 70 to 80 people were receiving diabetes care in a given service day. People with uncomplicated diabetes were appointed every three months. The diabetes clinic was staffed by two endocrinologists, five residents and four nurses working full time.

3.2. Study design and duration

In this observational study, we reviewed medical charts of 568 recently diagnosed T2DM patients throughout the study period, between March 2021 and March 2022, from the diabetes clinic of SPHMMC to identify eligible study participants for the metformin treatment response study, metformin induced gastrointestinal study and the pattern of dyslipidemia study. A prospective observational cohort study design was used in the metformin treatment response study while a retrospective observational cohort study was conducted in the metformin-induced gastrointestinal intolerance study. Likewise, a comparative cross-sectional study design was employed in the pattern of dyslipidemia study.

3.3. Study subjects

3.3.1. Source population

All T2DM patients who were on metformin as their initial oral antidiabetic drug therapy at SPHMMC outpatient department diabetes clinic during the study period.

3.3.2. Study population

Recently diagnosed unrelated T2DM patients who were on metformin treatment at SPHMMC outpatient department diabetes clinic during the study period.

3.3.2.1 Eligibility criteria

Inclusion criteria

- Age 18-65
- Recently diagnosed (less than 1 year) unrelated patients with T2DM who were on metformin treatment for the response study
- Recently diagnosed (less than 3 years) unrelated patients with T2DM who were on metformin treatment for gastrointestinal intolerance study
- Recently diagnosed (less than 3 years) patients with T2DM who had a fully documented hemoglobin A1C, fasting blood glucose and full lipid panel test in the first 3 months of diagnosis for the pattern of dyslipidemia study
- Those willing to give informed consent to participate in the study

Exclusion criteria

- Pregnant or lactating women
- Patients with renal or hepatic insufficiency (serum creatinine >1.2mg/dl for renal insufficiency; serum glutamic-pyruvic transaminase or serum glutamic-oxaloacetic transaminase > 40IU/L for hepatic insufficiency)
- Active smokers (An adult who has smoked even one puff during the past seven days).

- Patients with malignancies or thyroid disorders or depression depending on the documented medical history of the patient
- Patients not adherent to medications
- Patients who have plans to change their follow-up center before the end of six months for the response study
- Patients not adherent to diet for the response and pattern of dyslipidemia studies
- Patients with chronic gastrointestinal disorders (including gastroduodenal ulcer, cholelithiasis, chronic pancreatitis, chronic liver disease and inflammatory bowel disease) for the gastrointestinal intolerance study
- Patients receiving medications that modify lipid pattern (mainly steroids and antiretrovirals) for the response and pattern of dyslipidemia studies
- Patients not recall being on metformin treatment or having the gastrointestinal adverse effects for gastrointestinal intolerance study
- Patients with microvascular and/or macrovascular complications

3.4. Determination of sample size and sampling

The sample size was estimated using a comparison of two proportion formula (Wang & Chow, 2007), to see whether difference in the rs8192675 and rs72552763 polymorphisms exist between the metformin responder and non-responder groups, considering the following assumptions: $p_1 = 0.35$ (Cook et al., 2007), 95% CI, 5% margin of error and a power of 80%. Therefore, the calculated sample size was set at 40 for each group. Likewise, the same formula was used to see whether difference in the rs72552763 polymorphism of *SLC22A1* exists between metformin tolerant and intolerant groups, considering the following assumptions: $p_1 = 0.30$ (Kirpichnikov et al., 2002), 95% CI, 5% margin of error and a power of 80%. Accordingly, the calculated sample size was set at 21 for each group. Adding a 10% to account for potential dropouts, the final sample size was 88 (44 for each group) for the response study and 47 (24 for tolerant and 23 for the intolerant group) for the intolerance study. A purposive sampling method was used to recruit study participants. In the pattern of dyslipidemia study, all the eligible participants in the study period satisfying the inclusion criteria where recruited.

3.5. Data collection

A written informed consent was obtained from each one of the study participants before collection of data. Data were collected by trained nurses working in the diabetes follow-up clinic after the participants agreed to provide written informed consent. Training was given for the data collectors and supervisor on the data collection tools and procedures, eligible study subjects, interview methods and research objective. Data was collected using a chart review and an interviewer administered questionnaire. All patients were diagnosed according to the World Health Organization (WHO) criteria (Bennett, 1999), and information about medical history, comorbidities, renal and liver function tests (Karita et al., 2009), biochemical parameters (HbA1c, FBG, TC, TG, LDL and HDL), metformin gastrointestinal intolerance and medication use were obtained from medical charts. Socio-demographic data, life style (level of exercise, adherence to diet and smoking habit), metformin gastrointestinal intolerance, and medication adherence were obtained from questionnaire interview. Information on gastrointestinal intolerance was obtained both from medical charts as well as through interview as the information was not available in the charts for some participants and also to confirm for those available.

For the response study cohort, biochemical measurements of the study participants were prospectively collected from their medical charts at diagnosis and/or first contact at the diabetes clinic (M1), at 3 months (M2), and at 6 months (M3). The participants were then categorized into metformin responders and non-responders. Classification was performed based on a cut-off value (0.5% reduction in HbA1c), as suggested elsewhere (Shikata et al., 2007; Park et al., 2018).

For the intolerance study, a documented clinical report was recorded from their medical charts and confirmed through oral interview whether gastrointestinal intolerance was a reason for discontinuation over the first 6 months of therapy and/or not increasing metformin dose. The participants were then categorized into metformin tolerant and intolerants depending on the operational definition in this study (Dujic et al., 2015, 2016; Dawed et al., 2019).

The weight of each study participant was measured using a digital scale to the nearest 0.1 kg while their height is measured using a mounted stadiometer to the nearest 0.1 cm, with participants wearing minimal clothing. Blood assays for fasting blood glucose (FBG), glycated hemoglobin (HbA1c), and lipid panel (total cholesterol, TC; triglyceride, TG; high-density lipoprotein cholesterol, HDL-C; low-density lipoprotein cholesterol, LDL-C) were conducted at SPHMMC Laboratory, Addis Ababa, Ethiopia, in accordance with standard protocols.

3.6. DNA Isolation

Venous blood (3 mL) was collected in vials containing EDTA for DNA extraction and stored at -80 ^{0}C until extraction. DNA was isolated from whole blood using a Blood Genomic DNA Extraction Mini Kit (ALPHAGEN Biotech Ltd., Taiwan) according to the manufacturer's instructions. Briefly, 200 µL of whole blood was incubated at 60°C with 20 µL proteinase K and 200 µL BG Buffer for 15 min by vortexing the sample every 5 min. Ethanol (200 µL, 99.5%) was added, transferred to a BG mini-column, centrifuged, and the mini-column placed in a new collection tube. The column was washed once with 200 µL GW Buffer and then with 750 µL Wash Buffer. After drying, the DNA was eluted in 100 µL of preheated Elution Buffer by centrifugation of the BG mini-column at full speed. DNA quantification was done using NanoDrop 2000/2000c UV/VIS Spectrophotometer (ThermoScientific™). The purity of DNA was assessed by dividing the absorbance at 260 nm (A_{260}) to the absorbance at 280 nm (A_{280}). A_{260}/A_{280} ratio of 1.7 to 2.0 was considered good-quality DNA devoid of proteins. Extracted DNA samples were stored at -20 ^{0}C until genotyping.

3.7. Genotyping

Genotyping was conducted at the Pharmacogenomics & Precision Medicine Laboratory in the Faculty of Pharmacy, University of Malaya, Kuala Lumpur, Malaysia. Genotyping of the substitution SNP of *SLC2A2* (rs8192675) and the deletion polymorphism on *SLC22A1* (rs72552763) were carried out using real-time PCR (qPCR), in accordance with the manufacturer's protocol (*SNP Genotyping Analysis Using TaqMan Assays*). The allelic

discrimination plots were analyzed to determine the genotypes and alleles of the polymorphism for each of the study subjects.

Genotyping of the rs8192675 polymorphism was performed using TaqMan® Pre-Designed SNP Genotyping Assay (Assay ID: C__3142140_10) from Applied Biosystems (Carlsbad, CA, USA) and ChamQ Geno-SNP Probe Master Mix (Vazyme Biotechnology, Singapore). Briefly, 10 ng of genomic DNA was added to the reconstituted 2X TaqMan® Master Mix, 20X Assay Working Stock and Nuclease-free water to achieve a final reaction volume of 20 µl. The qPCR was conducted using the StepOnePlus™ Real-Time PCR System, employing the standard ramp speed. The PCR conditions for amplification consisted of initial denaturation at 95°C for 40 seconds followed by PCR thermal condition of 45 cycles: 10 seconds for denaturation at 95 °C and 60 seconds for the DNA extension and terminal signal detection at 60 °C.

Genotyping of the rs72552763 polymorphism was performed using the TaqMan® Drug Metabolism Enzyme Genotyping Assay (Assay ID: C__34211613_10) from Applied Biosystems (Carlsbad, CA, USA) and ChamQ Geno-SNP Probe Master Mix (Vazyme Biotechnology, Singapore). Briefly, 10 ng of genomic DNA was added to the reconstituted 2X TaqMan® Master Mix, 20X Assay Working Stock and Nuclease-free water to achieve a final reaction volume of 20 µl. The qPCR was conducted using the StepOnePlus™ Real-Time PCR System, employing the standard ramp speed. The PCR conditions for amplification consisted of initial denaturation at 95°C for 40 seconds followed by PCR thermal condition of 45 cycles: 10 seconds for denaturation at 95 °C and 90 seconds for DNA extension and terminal signal detection at 60 °C.

3.8. Data analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) Version 26 for Windows (IBM Corp., Armonk, NY, USA). Kolmogorov-Smirnov and Shapiro-Wilk tests were performed to determine the normality of continuous variables. Normally distributed continuous variables were presented as mean $\pm$ standard deviation (SD). Non-normally distributed continuous variables were presented as median and interquartile range (IQR). Categorical variables were reported as percentages. For non-

normally distributed continuous dependent variables, associations were assessed by Independent-Samples Kruskal-Wallis test and Independent-Samples Mann-Whitney U test. For normally distributed continuous dependent variables, associations were assessed by student's t - test. The association between binary categorical variables were assessed by a Chi-square test and 95% confidence interval. Logistic regression model analysis was also performed for categorical dependent variables. After performing univariate binary logistic regression model analysis, independent variables ($p < 0.2$) were included to the multivariate logistic regression model. Univariate logistic regression model analysis results were presented as crude odds ratios (COR) while multivariate logistic regression model analysis were expressed as adjusted odds ratios (AOR) at 95% confidence intervals. Hosmer-Lemeshow goodness-of-fit test was used to assess the model fit (Hosmer-Lemeshow statistic ≥ 0.05). Variable-selection was done using backward elimination (likelihood ratio) method. A p-value < 0.05 was considered statistically significant. The minor allele frequency (MAF) was calculated using Excel, and the Hardy–Weinberg equilibrium (HWE) test was performed using Gene-Calc software which uses the Chi-square test to assess whether or not observed genotype frequencies are consistent with Hardy-Weinberg expectations (Miks, 2018).

In order to evaluate the association of rs8192675 and rs72552763 polymorphisms with biochemical parameters, the difference between the biochemical values obtained after 3 months of treatment and values obtained at baseline were noted, in the response study group. In contrast, the biochemical parameters measurement on the six months was not used in the association analysis as it was confounded by the use of new class of glucose lowering medication(s) in the non-respondent group. The association between the polymorphisms, rs8192675 and rs72552763, and the number of patients reaching a treatment goal of HbAlc < 7.0% was also noted. Furthermore, in the metformin response study, in the subset of study participants with comorbid diabetic dyslipidemia and receiving daily statins, we assessed whether response to statins was associated with participants' response to metformin. This was assessed by comparing the absolute reduction in triglyceride and/or absolute improvement in HDL cholesterol between metformin responder and non-responder study participants. The association between rs72552763

polymorphism and the number of study participants with metformin induced gastrointestinal intolerance was also assessed.

3.9. Ethical considerations

The study was approved by the Institutional Review Board (IRB) of the College of Health Sciences, Addis Ababa University (protocol number:118/20/SoP) and IRB of SPHMMC (RN: DM23/576) and conducted in accordance with the Declaration of Helsinki. National Ethical approval was granted by the National Research Ethics Review Committee, Ministry of Education (MOE), Ethiopia (RN:02/246/572/22). Furthermore, each one of the study participants was well informed about the objective and procedure of the study in their local language (Amharic) and a written informed consent was obtained prior to the data collection from all the study participants. Confidentiality and anonymity were ensured by limiting data access and removing the identifiers of study participants.

4. Results

4.1. Characteristics of the study participants

In metformin treatment response study, no significant difference was observed in the median values of baseline HbA1c, FBG, TG, HDL, BMI, metformin daily doses, and age at diagnosis between the metformin responders and non-responders. The median daily dose of metformin was1000 mg per day in both groups. Furthermore, no significant difference was observed in sex or level of physical activity between metformin responders and non-responders (Table 1, Paper 1).

In the metformin gastrointestinal intolerance study, the median daily dose of metformin was 1000 mg in metformin intolerant group, while it was 2000 mg in the tolerant group (Table 1, Paper 2). No significant difference was observed in the median values of BMI and age at diagnosis between tolerant and intolerant groups (Table 3, Paper 2). Furthermore, the female gender (OR=4.019, 95%CI (1.048-15.416), $p<0.05$) and physical inactivity (OR=5.455, 95%CI (1.414-21.035), $p<0.05$) were found to be significantly associated with increased metformin intolerance (Table 3, Paper 2).

In the pattern of dyslipidemia study, the prevalence of dyslipidemia was 92.1 % (n = 117). There was no statistically significant difference in the prevalence of overall dyslipidemia across age groups, BMI, type of glucose lowering agents, being on statin treatment, presence of comorbid hypertension, baseline HbA1c, and physical activity (Table 2, Paper 3), although the prevalence of individual lipid abnormality varied with gender (Table 3, Paper 3). The prevalence of high LDL-C and low HDL-C were significantly higher among females as compared to males, although the gender difference for high TC failed to reach statistical significance (Table 3, Paper 3). The overall prevalence of high (TC, TG, LDL-C) and low HDL-C was 28.35%, 52.76%, 69.29% and 64.57% respectively.

The prevalence of mixed atherogenic dyslipidemia, combined dyslipidemia and isolated dyslipidemia was 37 (29.1 %), 34 (26.8 %), and 27 (21.3%), respectively. The co-occurrence of low HDL-C and high LDL-C was the predominant combined dyslipidemia

(16, 12.6 %), while low HDL-C was the most common isolated dyslipidemia (13, 10.2%) (Table 4, Paper 3).

4.2 Determinants of dyslipidemia among patients with type 2 diabetes mellitus

Female sex was found to be an independent predictor of higher TC (AOR (95%CI) 3.617 (13.42 – 9.743)) (Table 5, Paper 3), low HDL-C (AOR (95%CI) 3.703 (1.663 – 8.244)) and high LDL-C (Table 7, Paper 3). Age of less than 50 years (AOR (95%CI) 0.23 (0.054 – 0.831)) was found as independent predictors of higher TC (Table 5, Paper 3). Not being on statin treatment was found as an independent predictor of higher TC (AOR (95%CI) 3.22 (1.306 – 7.935)) (Table 5, Paper 3) and low HDL-C (AOR (95%CI) 2.743 (1.186 – 6.341)) (Table 7, Paper 3). Likewise, being physically inactive (AOR (95%CI) 4.091 (1.546 – 10.824)), not hypertensive (AOR (95%CI) 5.975 (2.479 – 14.401)) and being on metformin + insulin treatment (AOR (95%CI) 0.129 (0.033 – 0.503)) as compared to metformin monotherapy or metformin sulfonylurea combination were found to be independent predictors of high TG (Table 6, Paper 3).

4.3 Diabetic dyslipidemia and metformin response

In the subgroup of the study participants with diabetic dyslipidemia receiving daily statin, we found that comorbid diabetic dyslipidemia significantly reduced metformin response (COR=0.2, 95%CI (0.044-0.913), $p<0.05$). Furthermore, in multivariate logistic regression model adjusted for age and baseline TG, a greater absolute reduction in TG was significantly associated with better metformin response (AOR=1.018, 95%CI (1.003-1.032), $p<0.05$) (Table 1).

Table 1: Logistic regression analysis for a predictor of metformin response among study participants with comorbid diabetic dyslipidemia receiving statin therapy (n=38)

Predictor Factors	Univariate analysis		Multivariate analysis	
	OR(95%CI)	p-value	OR(95%CI)	p-value
Comorbid diabetic dyslipidemia	0.2 (0.044-0.913)	0.038		
Baseline triglyceride (TG)	1.005(0.999 -1.011)	0.130		
Absolute reduction in TG	1.011(0.999-1.023)	0.066	1.018(1.003-1.032)	0.014
Age	1.112(1.012-1.222)	0.027	1.194(1.049-1.360)	0.007

OR: odds ratio

4.4 Genotype frequency

The Single Nucleotide polymorphism (SNPs) of interest in this study, rs8192675 and rs72552763, were in line with the principles of the Hardy–Weinberg equilibrium (HWE) with p-values of 0.68967 and 0.984, respectively. The Minor allele frequency (MAF) of the C-allele in the T>C substitution SNP of *SLC2A2* gene (rs8192675) was 66.2%, while the wild-type T allele was 33.8%. Likewise, the MAF of the 3-base pair (GAT) deletion mutation at rs72552763 was 9.4% whereas that of the wild-type GAT (G) allele was 90.6% in our study. No patient was homozygous for the rs72552763 deletion (del) allele in the intolerance study group. However, one patient was homozygous for the rs72552763 del allele in the response study group (Table 2).

Table 2: Genotypic and allelic frequency distribution of the rs8192675 and rs72552763 polymorphisms across response and intolerance study participants (n = 133)

Polymorphisms of interest	Genotypes and alleles of the respective polymorphism	Response study group		Intolerance study group		Allele/genotype frequency (%)
		Non responder(N)	Responder(N)	Tolerant(N)	Non tolerant(N)	
(T>C) substitution polymorphism of SLC2A2 (rs8192675)	CT genotype	21	19	11	13	48.12
	CC genotype	20	17	10	9	42.11
	TT genotype	3	6	3	1	9.77
	C-allele	61	53	31	31	66.2
	T-allele	27	31	17	15	33.8
Met420del polymorphism of SLC22A1 (rs72552763)	del_del genotype	1	0	0	0	0.75
	del_G genotype	4	10	5	4	17.29
	G_G genotype	39	32	19	19	81.95
	del-allele	6	10	5	4	9.4
	G-allele	82	74	43	42	90.6

N=frequency; del_del:deletion_deletion; G_G:GAT_GAT; del_G: deletion_GAT

4.5 Association of the genotypes/alleles with metformin treatment response

The metformin response cohort comprised 48.8% (n = 42) of the metformin responders and 51.2% (n = 44) of the metformin non-responders. In multivariate logistic regression analysis adjusted for baseline FBG, age and BMI, metformin response was significantly higher in del_G genotypes than in the wild-type G_G genotypes (AOR =3.675 and 95% CI (1.005-13.436), p= 0.049) (Table 3, Paper 1). Furthermore, no significant difference was observed between metformin responders and non-responders in the median baseline HbA1c (p = 0.907) (not included in Table 3, Paper 1 as univariate analysis p > 0.2). In addition, a significantly lower median treatment HbA1c level was found in the del_G genotype (7.0%) than in the wild-type G_G genotype (8.0%) (p = 0.015) (Figure 2, Paper 1). In contrast, the absolute reductions in HbA1c and FBG levels did not significantly vary depending on the del_G and G_G genotypes (Figure 1, Paper 1). Furthermore, there was no significant difference among the genotypes of rs72552763 polymorphism in the median baseline HbA1c and FBG (Figure 1 and 2). On the other hand, no significant intergroup difference between the responder and non-responder groups was observed regarding the

genotypes of the SNP rs8192675 (Table 2, Paper 4). Furthermore, there was no significant difference among the three genotypes of the SNP rs8192675 in the median baseline HbA1c and FBG (Figure 1, Paper 4). In addition, the median values of absolute reduction in HbA1c and FBG as well as the treatment values of HbA1c and FBG after 3 months of metformin therapy were not significantly associated with the genotypes of rs8192675 (Figure 2 and 3, Paper 4). On other hand, there was no association between the alleles of both SNPs, (rs72552763 (OR=1.746, 95%CI, 0.664-4.592, p=0.251), Paper 1; rs8192675 (OR = 0.910, 95%CI, 0.734-1.128, p=0.388), Table 3, Paper 4) and metformin response.

On the other hand, 59.3% of the participants had poor glycemic control. BMI (AOR= 0.847; 95%CI (0.740-0.970), p = 0.016) and baseline HbA1c level (AOR=0.478, 95%CI (0.305-0.751), p= 0.001) were significantly associated with poor glycemic control (Table 4, Paper 1). Glycemic control was nearly significantly higher in the del_G genotypes than in the wild-type G_G genotypes (COR =3.312; 95%CI (1.001-10.961), p = 0.05) (Table 4, Paper 1). In addition, the minor del_allele was significantly associated with good glycemic control compared with the wild-type G_allele (OR=3.206, 95%CI (1.165-8.823), p = 0.016, Paper 1). In contrast, no significant association was observed between the genotypes and alleles of rs8192675 and glycemic control (Table 2 and 3, Paper 4).

4.6 Association of *SLC2A2* rs8192675 with diabetic dyslipidemia

Our study found no significant intergroup differences in genotypes of *SLC2A2* rs8192675 between the study participants with and without comorbid diabetic dyslipidemia (Table 4, Paper 4). Moreover, it was found that there were no significant difference among the three genotypes in the median values of baseline TG and HDL levels (Figure 4, Paper 4).

4.7 Association of the *Met420 deletion (rs72552763)* polymorphism with metformin intolerance

Logistic regression analyses indicated no significant association of metformin induced gastrointestinal intolerance with the genotypes of the SNP rs72552763 (Table 3, Paper 2). Likewise, chi-square test analyses indicated that the alleles of rs72552763 polymorphism

were not significantly associated with metformin induced gastrointestinal intolerance (Table 4, Paper 2).

p = 0.303

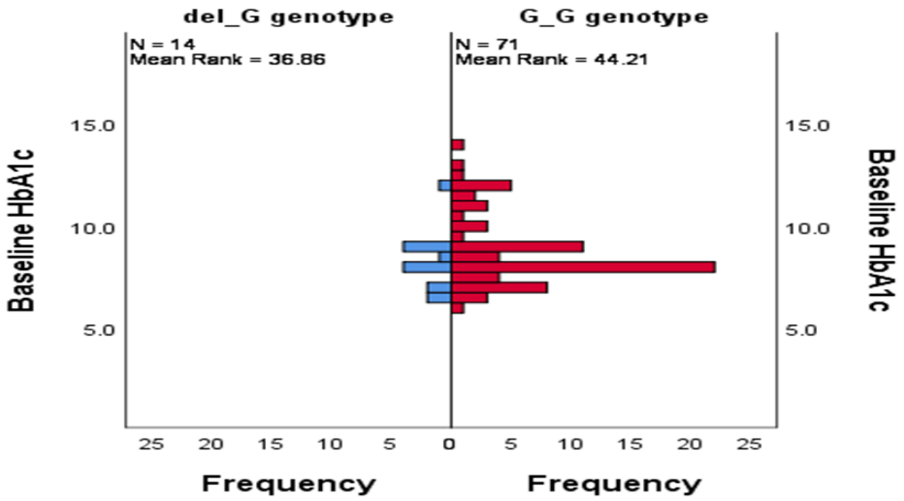

Figure 1: Independent Samples Mann–Whitney test between genotypes of Met420del variant of SLC22A1gene (rs72552763) and the median baseline glycated hemoglobin levels.

p = 0.118

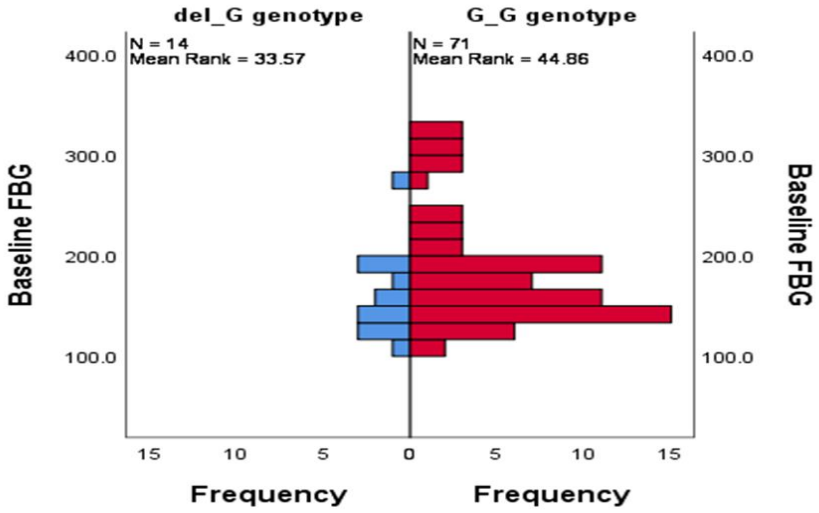

Figure 2: Independent Samples Mann–Whitney test between genotypes of Met420del variant of SLC22A1gene (rs72552763) and the median baseline fasting blood glucose levels.

5. Discussion

The main goal of T2DM management is ensuring optimal glycemic control ("United Kingdom Prospective Diabetes Study 33," 1998). However, reports emanating from Ethiopian studies indicated a high prevalence of inadequate glycemic control (Abebe et al., 2015; Alebachew Woldu & Diriba Wami, 2014; Kassahun et al., 2016). Furthermore, a significant inter-individual variation in treatment outcomes to the first line agent (Davies et al., 2018), metformin, in the management of T2DM (in terms of treatment response (K. Zhou et al., 2014) and adverse effects (Pawlyk et al., 2014; Todd & Florez, 2014)) is reported in the literature owing to differences in individual genetic profiles.

Thus, in the current study, we first prospectively determined the response to metformin across the study participants and then examined the association of *Met420del* variant of *SLC22A1* with metformin treatment response (Paper 1). In addition to metformin response, we have also assessed the level of glycemic control and its association with the *Met420del* variant of *SLC22A1*. These two outcomes provide complementary, but not redundant information. The metformin response outcome assessed whether patients showed $\geq$ 0.5% reduction in HbA1c levels from baseline within 3 months of metformin monotherapy and remained low for at least another 3 months, while in glycemic control, we assessed whether patients had achieved the optimal glycemic level (measured by attainment of HbA1c < 7%). We also assessed the association of *Met420del* variant of *SLC22A1* with metformin-induced gastrointestinal intolerance (Paper 2) as well as the prevalence, pattern and determinants of dyslipidemia in the study participants (Paper 3). In addition, the association of rs8192675 polymorphism of *SLC2A2* with metformin treatment response and diabetic dyslipidemia was also investigated (Paper 4).

In our study, 59.3% of the study participants had poor glycemic control, which is in line with previous studies that reported approximately two-third of the Ethiopian T2DM patients had poor glycemic control (Abdissa & Hirpa, 2022). However, the percentage of T2DM patients with poor glycemic control in the present study is slightly lower than the previous studies in Ethiopia, as the current study involved only recently diagnosed T2DM patients. In addition, in this study, we involved only patients who were adherent to diet and

medication. The percentage of T2DM patients with HbA1c < 7.0% in our study (40.7%) is also similar with two different cross-sectional studies in SSA, from Kenyatta National Hospital, Kenya (36.9%) (Waari et al., 2018) and National Hospital of Abuja, Nigeria (37.9%) (Odume et al., 2015). Thus, the result of our study which is in agreement with several previous studies emanating from the SSA population, confirms the notion that there is generally unsatisfactory level of glycemic control in the region (Kirkman et al., 2018).

The allelic frequency of the two candidate polymorphisms, rs8192675 and rs72552763, assessed in association with metformin treatment outcomes in our study were 66.2% and 9.4 %, respectively. The higher allele frequencies for the rs8192675 (compared to the Caucasian population) (*rs8192675 (SNP) - Population Genetics - Homo_sapiens - Ensembl Genome Browser*) and for rs72552763 (compared to other Africans (Du Plessis et al., 2015) and African descent (Goswami et al., 2014)) shows the potential pharmacologic relevance of both polymorphisms in the Ethiopian population. However, the observed allele frequency differences could be justified by a previous study that reported populations of SSA are the most genetically diverse in the world compared with peoples of non-African ancestry (Campbell & Tishkoff, 2008). Furthermore, the genotype frequency of both polymorphisms in our study is in agreement with the very recent study that identified subjects of African descent were carriers of the SLC22A1 rs72552763 G_G genotype and SLC2A2 rs8192675 C/C genotype, in contrast to the lower percentage of these genotypes among other ethnic groups (Saiz-Rodríguez et al., 2023).

Contrary to the bulk of the evidence in the literature, our study found that carriers of the reduced function *Met420del* variant of *SLC22A1* had a greater likelihood of treatment success from metformin monotherapy. Indeed, T2DM patients with the del_G genotype had more than three times better chance of responding to metformin compared to the wild-type G_G genotype. Furthermore, a significantly lower median treatment HbA1c level was also found in del_G genotypes as compared to the wild-type G_G genotypes despite no significant difference in the baseline HbA1c levels between the two genotypes. In addition, we also found a nearly significant higher rate of good glycemic control with del_G genotypes as compared to the wild-type G_G genotypes after three months of metformin therapy ($p = 0.05$). However, the significant association of del_G genotype with metformin

response was not replicated at the allele level. Thus, the effect of *SLC22A1* rs72552763 polymorphism in metformin response at the genotype and allele levels seems inconsistent in our study. However, we assume the absence of association at the allele level might be related to the limitation in the definition of response and most importantly due to the smaller sample size in our study. Because, the definition was based on an absolute HbA1c reduction over 3 months, which could be influenced by short term life style changes. Our assumption could be further supported by our finding that del allele is significantly associated with good glycemic control. Indeed, patients carrying the del allele had a more than three-fold better chance of good glycemic control than patients not carrying the allele after 3 months of metformin monotherapy. Furthermore, it is of note that glycemic control, which was measured as attainment of HbA1c < 7%, is by far the more valid marker of glycemic response as it shows a long-term cumulative clinical outcome as compared to the surrogate marker of metformin response used in this study.

Thus, although the bulk of the evidence concentrates on the finding that the reduced function allele of *SLC22A1* is associated with reduced cellular metformin uptake but not with metformin treatment response (Dujic et al., 2017), contrary to this widely believed notion, we found a significantly higher metformin response and glycemic control in participants carrying the minor del allele. Thus, although the finding of this study appears to be discordant with the reports in the literature, it seems that the association of Met420del polymorphisms with metformin treatment response in our population might be novel since inconsistent findings on the reduced function alleles of *SLC22A1* across different ethnic populations were reported (Florez, 2017; Sakata et al., 2004; Shu et al., 2007). Our finding also seems in contrast to the generally accepted glucose lowering effect of metformin, which is primarily attributed to its action on the liver (Foretz & Viollet, 2011; Krishan et al., 2015; T. Zhou et al., 2018), as the same variant associated with treatment success in our study is also reported to decrease both intestinal absorption and hepatic distribution of metformin (Shu et al., 2007). However, this effect might be related to the recently recognized mechanism of action of metformin, which is focused on the gastrointestinal tract, as reduced function OCT1 transporter causes the accumulation of metformin in the gut (DeFronzo et al., 2016; Schwartz et al., 2006). Putative gut-based mechanisms of

metformin include direct and indirect enhanced secretion of GLP-1 from intestinal L-cells via various mechanisms (McCreight et al., 2016; Napolitano et al., 2014).

Furthermore, our finding regarding the association of the reduced functional variant *SLC22A1* rs72552763 with metformin response and glycemic control is in accordance with studies done in Denmark, Mexico and China which have reported the association of reduced functional variants of *SLC22A1* with good responses in Danish (rs72552763) (Christensen et al., 2011), Mexican (rs72552763) (Marta et al., 2020) and Chinese (rs628031) population (Y. Zhou et al., 2015). In addition, the GoDART study reported that the R61C (rs12208357) genotype, but not rs72552763, was associated with better metformin outcomes (K. Zhou et al., 2009). In contrast, different studies (Shu et al., 2007; Mahrooz et al., 2015; Becker et al., 2009) reported that *SLC22A1* alleles, including rs72552763, have negative effect on the OGTT, FBG and HbA1c reduction, respectively, following metformin treatment. Furthermore, rs72552763 did not affect HbA1c reduction in Latvian (Tarasova et al., 2012) and Danish population (Christensen et al., 2015b). The reason for such discordant findings might be related to differences in sample size, duration of the study, data analysis, variant considered and definition of response. In addition, the variation in the association between the reduced-function variants and metformin response in different ethnic cohorts might also indicate that the role of *SLC22A1* polymorphisms in modulating metformin treatment response in T2DM is highly dependent on the genetic background of the patients.

On the other hand, it is plausible to assume that the *SLC2A2* rs8192675 polymorphism is not a determinant of metformin treatment response in Ethiopian patients with T2DM. Because in this study, it was observed that the median values for the absolute reduction in HbA1c, absolute reduction in FBG, treatment HbA1c level, and treatment FBG level did not significantly differ among TT, CT, and CC genotypes of rs8192675 polymorphism. Furthermore, no significant intergroup differences in TT, CT, and CC genotypes of rs8192675 were observed between the study participants with poor and good glycemic control. This finding is discordant with a study carried out in the European population, where having a C allele in the T>C substitution SNP of *SLC2A2* gene (rs8192675) was related to a significantly higher on-treatment HbA1c reduction (K. Zhou, Yee, et al., 2016)

and a significantly larger blood glucose reduction (Rathmann et al., 2019). However, it is concordant with several other studies conducted in different populations, including the cohort of ACCORD in USA and Canada (Rotroff et al., 2018), Indigenous Nguni population of South Africa (Abrahams-October et al., 2021) and Russian population (Nasykhova et al., 2022). Furthermore, in our study, there was no significant difference among the three genotypes of rs8192675 in the median baseline HbA1c and FBG. This finding is in agreement with the finding from the GDS, which reported no significant difference in the baseline FBG, baseline HbA1c and fasting glucose at diagnosis between the different genotypes at rs8192675 (Rathmann et al., 2019). Moreover, though studies in Caucasians still suggest that rs8192675 may have an important role in increasing an individual's risk for developing adverse metabolic phenotypes (Le et al., 2013), such as decreased HDL levels and increased TG, no such association were observed in our study. The reason why the effect of this SNP failed to replicate in different studies, including our study, might be related to the difference in the study design and sample size. Furthermore, based on the findings that the association of this variant with the metformin response varies in different ethnic cohorts, genetic heterogeneity for the polymorphic variant in the *SLC2A2* (rs8192675) gene between diverse populations could be assumed. Moreover, given the finding of the very recent GWAS which found no significant variant within the *SLC2A2* (including rs8192675) among participants of African ancestry, in association with metformin treatment response (B. Wu et al., 2024), it is plausible to assume that the variant might not be a good candidate for metformin pharmacogenetic study regarding T2DM in the African population.

Our study also observed no significant intergroup difference in BMI between metformin responder and non-responder groups, which is consistent with studies conducted elsewhere (Ito et al., 2010; Ji et al., 2013; Lund et al., 2007; Ong et al., 2006). This data might suggest that physician's disinclination to prescribing metformin for normal-weight T2DM patients is unfounded. Furthermore, our study showed that in T2DM patients with diabetic dyslipidemia receiving daily statins, a greater absolute reduction in TG was significantly associated with better metformin response. This finding is in agreement with previous study (Kashi et al., 2016), which reported that atorvastatin is more effective in decreasing

the atherogenic lipid parameters in metformin responders compared to non-responders. Thus, as hyperglycemia and dyslipidemia generally co-exist together in T2DM patients manifesting poor glycemic control and increased risk of vascular complications (Afroz et al., 2019), the finding of our study support the notion that, glycemic control should be monitored by assessing both glycemia and dyslipidemia to adjust the intensity of treatment with risk (Sherwani et al., 2016).

In our study, the prevalence of overall dyslipidemia was 92.1%. This is consistent with reports from similar studies in SSA and other developing countries (89.0% to 90.7% in Nigeria (Jisieike-Onuigbo et al., 2011; Okafor et al., 2008), 86.7% in South Africa (Pitso et al., 2021), 88.9% in Thailand (Narindrarangkura et al., 2019) and 88.1% in Nepal (Pokharel et al., 2017)). It is also in line with previous studies in Ethiopia, which have reported a prevalence ranging from 68.1% in southwestern Ethiopia (Haile & Timerga, 2020) to 91% in northern Ethiopia (Gebreyesus et al., 2022). In terms of the pattern of dyslipidemia, our study showed that the most common pattern was the atherogenic mixed dyslipidemia, with gender differences significantly tilted towards women. This finding is also consistent with the recent finding in the northern Ethiopia, which indicated that 87.2% of T2DM patients had high atherogenic risk for coronary heart disease (Gebreyesus et al., 2022). In addition, similar to our study, a recent large epidemiological study in Somalia reported a prevalent atherogenic dyslipidemia among T2DM patients (24.8%) with LDL-C being the most prevalent component dyslipidemia (Alıcı & Genç, 2022). On the other hand, similar studies in Nigerian (Bello-Ovosi et al., 2019), Iranian (Yadegar et al., 2022) and Pakistani (Sarfraz et al., 2016) patients with T2DM reported a relatively smaller prevalence of 3.4%, 13.8% and 17.0%, respectively, for the atherogenic mixed dyslipidemia as compared to our study (29.1 %). Although the difference in cut off points, exercise and dietary pattern, treatment schemes, cultural factors and genetic diversities may affect the pattern of dyslipidemia in patients with T2DM, our study highlighted the high prevalence of the typical atherogenic diabetic dyslipidemia in our study population. Thus, atherogenic dyslipidemia, the commonest pattern in our study, which includes high TG, low HDL-C, and high LDL-C (Hirano, 2018), might predispose to higher risk of

microvascular and/or macrovascular complications, and poor cardiovascular outcomes (Berneis & Krauss, 2002), in our population.

The combined two parameter dyslipidemia was the second common pattern of dyslipidemia in our study with almost equal distribution across both genders. The co-occurrence of low HDL-C and high LDL-C is the predominant combined dyslipidemia while low HDL-C was the most common isolated dyslipidemia. In agreement with the finding of our study, a study in Southern Ethiopia (Ambachew et al., 2015), Nigeria (Chinenye et al., 2012) and the largest study on the quality of diabetes care in six SSA countries (Diabcare Africa study) (Sobngwi et al., 2012) reported low HDL-C concentration to be the most prevalent lipid abnormality among diabetic patients. In addition, a national survey conducted in Ethiopia in 2015 reported low HDL-C as the most prevalent (68%) component lipid abnormality (Gebreyes et al., 2018). Thus, the findings in our study might indicate that increasing HDL-C along with lowering LDL-C comprises an important target for intervention among patients with T2DM in our population, as high LDL-C is also the most prevalent component dyslipidemia.

On the other hand, since metformin induced gastrointestinal intolerance influences treatment response by causing premature termination of therapy (DeFronzo, 1999; Haupt et al., 1991), the present study retrospectively determined metformin induced gastrointestinal intolerance across the study participants and examined the association of *Met420del* variant of *SLC22A1* with metformin induced gastrointestinal intolerance (Paper 2). Furthermore, in the present study in order to assess the association of metformin induced gastrointestinal intolerance with an individual variant of *SLC22A1* (rs72552763), intolerance phenotype was defined to include common and/or severe gastrointestinal intolerance, as both share a common underlying mechanism despite a considerably different duration and intensity of gastrointestinal symptoms (Dujic et al., 2016).

Accordingly, the finding of the present study showed that the *Met420del* variant of *SLC22A1* (*rs72552763*) has no association with metformin induced gastrointestinal intolerance. Indeed, this result is in accordance with earlier finding, which showed that *Met420del* variant has no association with metformin associated gastrointestinal side-

effects, although the same study reported the association of common gastrointestinal adverse effects of metformin therapy with an individual reduced function variants of *SLC22A1* (rs628031 and rs36056065) (Tarasova et al., 2012). Furthermore, the finding from two previous studies showed that individual variants (including rs72552763) were not associated with the occurrence of side effects though the number of *SLC22A1* reduced-function alleles was associated with common gastrointestinal adverse effects to metformin (Dujic et al., 2016) and individuals carrying two deficient *SLC22A1* alleles had higher odds of severe intolerance compared with individuals having one or no deficient alleles (Dujic et al., 2015). Thus, it is plausible to assume that the combined effect of the reduced function variants of *SLC22A1* is valuable to predict metformin -induced gastrointestinal adverse effects rather than the individual variants. However, as our study involved only a single reduced functional variant of *SLC22A1, Met420del* variant, this assumption needs to be confirmed by further studies involving more than one reduced functional variants of the gene.

In the present study, female gender was associated with more than 4 times greater chance of being intolerant to metformin. This finding adds to the previous knowledge that indicated sex differences in metformin intolerance (Dujic et al., 2015, 2016; Tarasova et al., 2012). However, further studies are required to assess the underlying factors of the potential sex differences in metformin intolerance. Furthermore, it is not clear why physical inactivity is related to metformin gastrointestinal intolerance in the present study. Thus, further prospective studies are required to confirm this observation. Age appeared to have influence on the occurrence of side effects depending on the time of T2DM diagnosis. Evidence for this assertion comes from the observation that whilst age had significant effect on the occurrence of side effects in patients with a longer duration of T2DM (Tarasova et al., 2012; Dujic et al., 2015), it was found to have no association in patients with newly diagnosed T2DM (Dujic et al., 2016), which is concordant with our findings. This might have to do with the fact that both metformin tolerant and intolerant patients had similar age in the current study, as all the study participants were recently diagnosed T2DM individuals.

While our study has generated the first metformin pharmacogenetics data in Ethiopian T2DM patients, it has some limitations. The major limitations of this study were its relatively short duration and small sample size. However, long-term data have indicated that the glucose-lowering action of metformin stabilizes after 3 to 4 months of treatment (Ito et al., 2010). In this study the minimum sample size calculated by comparison of two proportion formula was used, although a more reliable results could be achieved if we increased the sample size. Thus, future studies with larger sample size are required to explore the possible associations between the aforementioned polymorphisms and metformin treatment outcomes, both in efficacy and safety. Another limitation is the fact that the study is unable to measure body metformin concentrations and insulin sensitivity. The data on lifestyle interventions such as physical activity and dieting measured using self-reporting questionnaires are subject to recall bias. Nevertheless, it is our belief that the findings of the current study provide an important piece of pharmacogenetic and epidemiological information in Ethiopian patients with T2DM.

6. Conclusions and Recommendations

To the best of our knowledge, this is the first study to investigate the allele frequency distributions of rs8192675 and rs72552763 polymorphisms in the Ethiopian population. In this study, it was demonstrated that metformin response was significantly higher in study participants with heterozygous carriers of the *Met420del* variants of *SLC22A1* as compared to the wild-type G_G genotypes after 3 months of treatment. Furthermore, the results of our study also showed that the minor del_allele was significantly associated with good glycemic control as compared to the wild-type G_allele. In this study, rs8192675 and rs72552763 polymorphisms were not significantly associated with metformin treatment response and metformin induced gastrointestinal intolerance, respectively, in Ethiopian patients with T2DM. In our study we also found that the female gender and physical inactivity were risk factors for metformin induced gastrointestinal intolerance.

Furthermore, a more complete investigation of *SLC22A1* variants would be required to fully assess the effect of the gene on metformin treatment outcome given that several variants with a more severe loss of function have been described. In addition, future studies on the association between variants of *SLC22A1* gene and metformin treatment outcomes should quantify metformin concentrations in the body and insulin level. Similarly, the finding of our study regarding *SLC2A2* gene polymorphism needs to be confirmed by replication study involving larger sample size.

Our study also indicated high prevalence of dyslipidemia among patients with T2DM; with the atherogenic mixed dyslipidemia being the commonest pattern. This implies that most of the T2DM patients in our study are at a higher risk of cardiovascular diseases. Furthermore, the high prevalence of dyslipidemia echoed the need for incorporating serum lipid profile as routine laboratory investigation, in the management of patients with T2DM to minimize the possible complications resulting from terminal dyslipidemia. The data also provided a potentially useful insights on correlating lipid panel data with demographic, clinical, and laboratory variables.

7. References

Abdissa, D., & Hirpa, D. (2022). Poor glycemic control and its associated factors among diabetes patients attending public hospitals in West Shewa Zone, Oromia, Ethiopia: An Institutional based cross-sectional study. *Metabolism Open*, *13*, 100154. https://doi.org/10.1016/j.metop.2021.100154

Abdosh, T., Weldegebreal, F., Teklemariam, Z., & Mitiku, H. (2019). Cardiovascular diseases risk factors among adult diabetic patients in eastern Ethiopia. *JRSM Cardiovascular Disease*, *8*, 2048004019874989. https://doi.org/10.1177/2048004019874989

Abebe, S. M., Berhane, Yemane, Worku, A., Alemu, S., & Mesfin, N. (2015). Level of sustained glycemic control and associated factors among patients with diabetes mellitus in Ethiopia: A hospital-based cross-sectional study. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, 65. https://doi.org/10.2147/DMSO.S75467

Abejew, A. A., Belay, A. Z., & Kerie, M. W. (2015). Diabetic Complications among Adult Diabetic Patients of a Tertiary Hospital in Northeast Ethiopia. *Advances in Public Health*, *2015*, e290920. https://doi.org/10.1155/2015/290920

Abera, R. G., Demesse, E. S., & Boko, W. D. (2022). Evaluation of glycemic control and related factors among outpatients with type 2 diabetes at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia: A cross-sectional study. *BMC Endocrine Disorders*, *22*(1), 54. https://doi.org/10.1186/s12902-022-00974-z

Abraham, R. T. (1998). Mammalian target of rapamycin: Immunosuppressive drugs uncover a novel pathway of cytokine receptor signaling. *Current Opinion in Immunology*, *10*(3), 330–336. https://doi.org/10.1016/s0952-7915(98)80172-6

Abrahams-October, Z., Xhakaza, L., Pearce, B., et al. (2021). Genetic Association of Solute Carrier Transporter Gene Variants with Metformin Response. *Balkan Journal of Medical Genetics : BJMG*, *24*(1), 47–56. https://doi.org/10.2478/bjmg-2021-0004

Adeyemo, A. A., Tekola-Ayele, F., Doumatey, et al. (2015). Evaluation of Genome Wide Association Study Associated Type 2 Diabetes Susceptibility Loci in Sub Saharan Africans. *Frontiers in Genetics*, *6*, 335. https://doi.org/10.3389/fgene.2015.00335

Adeyemo, A. A., Zaghloul, N. A., Chen, G., et al. (2019). ZRANB3 is an African-specific type 2 diabetes locus associated with beta-cell mass and insulin response. *Nature Communications*, *10*(1), 3195. https://doi.org/10.1038/s41467-019-10967-7

Adrian, T. E., Ballantyne, G. H., Longo, et al. (1993). Deoxycholate is an important releaser of peptide YY and enteroglucagon from the human colon. *Gut*, *34*(9), 1219–1224.

Afroz, A., Ali, L., Karim, M. N., et al. (2019). Glycaemic Control for People with Type 2 Diabetes Mellitus in Bangladesh—An urgent need for optimization of management plan. *Scientific Reports*, *9*(1), 10248. https://doi.org/10.1038/s41598-019-46766-9

Agyemang, C., Meeks, K., Beune, E., et al. (2016). Obesity and type 2 diabetes in sub-Saharan Africans – Is the burden in today's Africa similar to African migrants in Europe? The RODAM study. *BMC Medicine*, *14*(1), 166. https://doi.org/10.1186/s12916-016-0709-0

Alebachew Woldu, M., & Diriba Wami, C. (2014). Factors Associated with Poor Glycemic Control among Patients with Type 2 Diabetes Mellitus in Ambo Hospital, Ambo; Ethiopia.

Endocrinology & Metabolic Syndrome, *03*(04). https://doi.org/10.4172/2161-1017.1000143

Alıcı, G., & Genç, Ö. (2022). The pattern of dyslipidemia among Somali type 2 diabetic patients: A cross-sectional study. *European Journal of Medical Research*, *27*(1), 253. https://doi.org/10.1186/s40001-022-00882-x

Al-Ma'aitah, O. H., Demant, D., Jakimowicz, S., & Perry, L. (2022). Glycaemic control and its associated factors in patients with type 2 diabetes in the Middle East and North Africa: An updated systematic review and meta-analysis. *Journal of Advanced Nursing*, *78*(8), 2257–2276. https://doi.org/10.1111/jan.15255

Alshawi, A., & Agius, L. (2019). Low metformin causes a more oxidized mitochondrial NADH/NAD redox state in hepatocytes and inhibits gluconeogenesis by a redox-independent mechanism. *The Journal of Biological Chemistry*, *294*(8), 2839–2853. https://doi.org/10.1074/jbc.RA118.006670

Altall, R. M., Qusti, S. Y., Filimban, N., et al. (2019). SLC22A1 And ATM Genes Polymorphisms Are Associated With The Risk Of Type 2 Diabetes Mellitus In Western Saudi Arabia: A Case-Control Study. *The Application of Clinical Genetics*, *12*, 213–219. https://doi.org/10.2147/TACG.S229952

Ambachew, H., Shimelis, T., & Lemma, K. (2015). Dyslipidemia among diabetic patients in Southern Ethiopia: Cross-sectional study. *Journal of Diabetes and Endocrinology*, *6*, 19–24. https://doi.org/10.5897/JDE2015.0086

American Diabetes Association. (2009). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, *32 Suppl 1*(Suppl 1), S62-67. https://doi.org/10.2337/dc09-S062

American Diabetes Association. (2018). 6. Glycemic Targets: Standards of Medical Care in Diabetes-2018. *Diabetes Care*, *41*(Suppl 1), S55–S64. https://doi.org/10.2337/dc18-S006

American Diabetes Association. (2022). Standards of Medical Care in Diabetes—2022 Abridged for Primary Care Providers. *Clinical Diabetes*, *40*(1), 10–38. https://doi.org/10.2337/cd22-as01

Bachtiar, M., & Lee, C. G. L. (2013). Genetics of Population Differences in Drug Response. *Current Genetic Medicine Reports*, *1*(3), 162–170. https://doi.org/10.1007/s40142-013-0017-3

Bauer, P. V., Duca, F. A., Waise, T. M. Z., et al. (2018). Metformin Alters Upper Small Intestinal Microbiota that Impact a Glucose-SGLT1-Sensing Glucoregulatory Pathway. *Cell Metabolism*, *27*(1), 101-117.e5. https://doi.org/10.1016/j.cmet.2017.09.019

Becker, M. L., Visser, L. E., van Schaik, et al. (2009). Genetic variation in the organic cation transporter 1 is associated with metformin response in patients with diabetes mellitus. *The Pharmacogenomics Journal*, *9*(4), 242–247. https://doi.org/10.1038/tpj.2009.15

Bekele, S., Yohannes, T., & Mohammed, A. E. (2017). Dyslipidemia and associated factors among diabetic patients attending Durame General Hospital in Southern Nations, Nationalities, and People's Region. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *10*, 265–271. https://doi.org/10.2147/DMSO.S135064

Bello-Ovosi, B. O., Ovosi, J. O., Ogunsina, M. A., Asuke, S., & Ibrahim, M. S. (2019). Prevalence and pattern of dyslipidemia in patients with type 2 diabetes mellitus in Zaria, Northwestern Nigeria. *The Pan African Medical Journal*, *34*, 123. https://doi.org/10.11604/pamj.2019.34.123.18717

Bennett, P. H. (1999). Impact of the new WHO classification and diagnostic criteria. *Diabetes, Obesity & Metabolism*, *1 Suppl 2*, S1-6. https://doi.org/10.1046/j.1463-1326.1999.0010s2001.x

Berneis, K. K., & Krauss, R. M. (2002). Metabolic origins and clinical significance of LDL heterogeneity. *Journal of Lipid Research*, *43*(9), 1363–1379. https://doi.org/10.1194/jlr.r200004-jlr200

Brunetti, A., Chiefari, E., & Foti, D. P. (2017). Pharmacogenetics in type 2 diabetes: Still a conundrum in clinical practice. *Expert Review of Endocrinology & Metabolism*, *12*(3), 155–158. https://doi.org/10.1080/17446651.2017.1316192

Burcelin, R., Dolci, W., & Thorens, B. (2000). Glucose sensing by the hepatoportal sensor is GLUT2-dependent: In vivo analysis in GLUT2-null mice. *Diabetes*, *49*(10), 1643–1648. https://doi.org/10.2337/diabetes.49.10.1643

Buse, J. B., Wexler, D. J., Tsapas, A., et al. (2020). 2019 Update to: Management of Hyperglycemia in Type 2 Diabetes, 2018. A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care*, *43*(2), 487–493. https://doi.org/10.2337/dci19-0066

Cynthia Mohandas, Riccardo Bonadonna, Fariba Shojee-Moradie , et al. (2018). Ethnic differences in insulin secretory function between black African and white European men with early type 2 diabetes. *Diabetes, Obesity & Metabolism*, *20*(7):1678-1687. https://doi.org/10.1111/dom.13283

Campbell, M. C., & Tishkoff, S. A. (2008). African Genetic Diversity: Implications for Human Demographic History, Modern Human Origins, and Complex Disease Mapping. *Annual Review of Genomics and Human Genetics*, *9*(1), 403–433. https://doi.org/10.1146/annurev.genom.9.081307.164258

Cannon, C. P., Braunwald, E., McCabe, C. H., et al. (2004). Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *The New England Journal of Medicine*, *350*(15), 1495–1504. https://doi.org/10.1056/NEJMoa040583

Cao, J., Meng, S., Chang, E., et al. (2014). Low Concentrations of Metformin Suppress Glucose Production in Hepatocytes through AMP-activated Protein Kinase (AMPK). *The Journal of Biological Chemistry*, *289*(30), 20435–20446. https://doi.org/10.1074/jbc.M114.567271

Carls, G., Huynh, J., Tuttle, E., Yee, J., & Edelman, S. V. (2017). Achievement of Glycated Hemoglobin Goals in the US Remains Unchanged Through 2014. *Diabetes Therapy: Research, Treatment and Education of Diabetes and Related Disorders*, *8*(4), 863–873. https://doi.org/10.1007/s13300-017-0280-5

Chen, L., Takizawa, M., Chen, E., et al. (2010). Genetic polymorphisms in organic cation transporter 1 (OCT1) in Chinese and Japanese populations exhibit altered function. *The Journal of Pharmacology and Experimental Therapeutics*, *335*(1), 42–50. https://doi.org/10.1124/jpet.110.170159

Chen, Z., Chen, G., Qin, H., et al. (2020). Higher triglyceride to high-density lipoprotein cholesterol ratio increases cardiovascular risk: 10-year prospective study in a cohort of Chinese adults. *Journal of Diabetes Investigation*, *11*(2), 475–481. https://doi.org/10.1111/jdi.13118

Chinenye, S., Uloko, A. E., Ogbera, A. O., et al. (2012). Profile of Nigerians with diabetes mellitus – Diabcare Nigeria study group (2008): Results of a multicenter study. *Indian Journal of Endocrinology and Metabolism*, *16*(4), 558–564. https://doi.org/10.4103/2230-8210.98011

Christensen, M. M. H., Brasch-Andersen, C., Green, H., et al. (2011). The pharmacogenetics of metformin and its impact on plasma metformin steady-state levels and glycosylated hemoglobin A1c. *Pharmacogenetics and Genomics*, *21*(12), 837–850. https://doi.org/10.1097/FPC.0b013e32834c0010

Christensen, M. M. H., Højlund, K., Hother-Nielsen, O., et al. (2015a). Endogenous glucose production increases in response to metformin treatment in the glycogen-depleted state in humans: A randomised trial. *Diabetologia*, *58*(11), 2494–2502. https://doi.org/10.1007/s00125-015-3733-2

Christensen, M. M. H., Højlund, K., Hother-Nielsen, O., et al. (2015b). Steady-state pharmacokinetics of metformin is independent of the OCT1 genotype in healthy volunteers. *European Journal of Clinical Pharmacology*, *71*(6), 691–697. https://doi.org/10.1007/s00228-015-1853-8

Cook, M. N., Girman, C. J., Stein, P. P., & Alexander, C. M. (2007). Initial monotherapy with either metformin or sulphonylureas often fails to achieve or maintain current glycaemic goals in patients with Type 2 diabetes in UK primary care. *Diabetic Medicine: A Journal of the British Diabetic Association*, *24*(4), 350–358. https://doi.org/10.1111/j.1464-5491.2007.02078.x

Dagnew, B., Debalkie Demissie, G., & Abebaw Angaw, D. (2021). Systematic Review and Meta-Analysis of Good Self-Care Practice among People Living with Type 2 Diabetes Mellitus in Ethiopia: A National Call to Bolster Lifestyle Changes. *Evidence-Based Complementary and Alternative Medicine: eCAM*, *2021*, 8896896. https://doi.org/10.1155/2021/8896896

Davies, M. J., D'Alessio, D. A., Fradkin, J., et al. (2018). Management of hyperglycaemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetologia*, *61*(12), 2461–2498. https://doi.org/10.1007/s00125-018-4729-5

Dawed, A. Y., Zhou, K., van Leeuwen, N., et al. (2019). Variation in the Plasma Membrane Monoamine Transporter (PMAT) (Encoded by SLC29A4) and Organic Cation Transporter 1 (OCT1) (Encoded by SLC22A1) and Gastrointestinal Intolerance to Metformin in Type 2 Diabetes: An IMI DIRECT Study. *Diabetes Care*, *42*(6), 1027–1033. https://doi.org/10.2337/dc18-2182

Daya, R., Bayat, Z., & Raal, F. (2017). Prevalence and pattern of dyslipidaemia in type 2 diabetes mellitus patients at a tertiary care hospital. *Journal of Endocrinology, Metabolism and Diabetes of South Africa*, *22*(3), 31–35. https://doi.org/10.1080/16089677.2017.1360064

De Vadder, F., Kovatcheva-Datchary, P., Goncalves, D., et al. (2014). Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell*, *156*(1–2), 84–96. https://doi.org/10.1016/j.cell.2013.12.016

DeFronzo, R. A. (1999). Pharmacologic therapy for type 2 diabetes mellitus. *Annals of Internal Medicine*, *131*(4), 281–303. https://doi.org/10.7326/0003-4819-131-4-199908170-00008

DeFronzo, R. A., & Goodman, A. M. (1995). Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. The Multicenter Metformin Study Group. *The New England Journal of Medicine*, *333*(9), 541–549. https://doi.org/10.1056/NEJM199508313330902

Dixit, A. K., Dey, R., Suresh, A., et al. (2014). The prevalence of dyslipidemia in patients with diabetes mellitus of ayurveda Hospital. *Journal of Diabetes and Metabolic Disorders*, *13*, 58. https://doi.org/10.1186/2251-6581-13-58

Dodds, S. (2017). The How-To for Type 2: An Overview of Diagnosis and Management of Type 2 Diabetes Mellitus. *The Nursing Clinics of North America*, *52*(4), 513–522. https://doi.org/10.1016/j.cnur.2017.07.002

Donnelly, L. A., Doney, A. S. F., Hattersley, A. T., Morris, A. D., & Pearson, E. R. (2006). The effect of obesity on glycaemic response to metformin or sulphonylureas in Type 2 diabetes. *Diabetic Medicine: A Journal of the British Diabetic Association*, *23*(2), 128–133. https://doi.org/10.1111/j.1464-5491.2005.01755.x

Du Plessis, M., Pearce, B., Jacobs, C., Hoosain, N., & Benjeddou, M. (2015). Genetic polymorphisms of the organic cation transporter 1 gene (SLC22A1) within the Cape Admixed population of South Africa. *Molecular Biology Reports*, *42*(3), 665–672. https://doi.org/10.1007/s11033-014-3813-2

Duca, F. A., Côté, C. D., Rasmussen, B. A., et al. (2015). Metformin activates a duodenal Ampk-dependent pathway to lower hepatic glucose production in rats. *Nature Medicine*, *21*(5), 506–511. https://doi.org/10.1038/nm.3787

Dujic, T., Causevic, A., Bego, T., et al. (2016). Organic cation transporter 1 variants and gastrointestinal side effects of metformin in patients with Type 2 diabetes. *Diabetic Medicine: A Journal of the British Diabetic Association*, *33*(4), 511–514. https://doi.org/10.1111/dme.13040

Dujic, T., Zhou, K., Donnelly, L. A., Tavendale, R., Palmer, C. N. A., & Pearson, E. R. (2015). Association of Organic Cation Transporter 1 With Intolerance to Metformin in Type 2 Diabetes: A GoDARTS Study. *Diabetes*, *64*(5), 1786–1793. https://doi.org/10.2337/db14-1388

Dujic, T., Zhou, K., Yee, S. W., et al. (2017). Variants in Pharmacokinetic Transporters and Glycemic Response to Metformin: A Metgen Meta-Analysis. *Clinical Pharmacology and Therapeutics*, *101*(6), 763–772. https://doi.org/10.1002/cpt.567

Dupuis, J., Langenberg, C., Prokopenko, I., et al. (2010). New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nature Genetics*, *42*(2), 105–116. https://doi.org/10.1038/ng.520

Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. (2001). Executive Summary of The Third Report of The National Cholesterol Education

Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA*, *285*(19), 2486–2497. https://doi.org/10.1001/jama.285.19.2486

Feher, M. D. (2004). Diabetes: Preventing coronary heart disease in a high risk group. *Heart (British Cardiac Society)*, *90 Suppl 4*(Suppl 4), iv18-21. https://doi.org/10.1136/hrt.2004.037572

Fitchett, D. H., Leiter, L. A., Goodman, S. G., & Langer, A. (2006). Lower is better: Implications of the Treating to New Targets (TNT) study for Canadian patients. *The Canadian Journal of Cardiology*, *22*(10), 835–839.

Florez, J. C. (2011). Does metformin work for everyone? A genome-wide association study for metformin response. *Current Diabetes Reports*, *11*(6), 467–469. https://doi.org/10.1007/s11892-011-0220-0

Florez, J. C. (2017). The pharmacogenetics of metformin. *Diabetologia*, *60*(9), 1648–1655. https://doi.org/10.1007/s00125-017-4335-y

Foretz, M., Guigas, B., & Viollet, B. (2019). Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus. *Nature Reviews. Endocrinology*, *15*(10), 569–589. https://doi.org/10.1038/s41574-019-0242-2

Foretz, M., Hébrard, S., Leclerc, J., et al. (2010). Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. *The Journal of Clinical Investigation*, *120*(7), 2355–2369. https://doi.org/10.1172/JCI40671

Foretz, M., & Viollet, B. (2011). Regulation of hepatic metabolism by AMPK. *Journal of Hepatology*, *54*(4), 827–829. https://doi.org/10.1016/j.jhep.2010.09.014

Forslund, K., Hildebrand, F., Nielsen, T., et al. (2015). Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature*, *528*(7581), 262–266. https://doi.org/10.1038/nature15766

Fullerton, M. D., Galic, S., Marcinko, K., et al. (2013). Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin. *Nature Medicine*, *19*(12), 1649–1654. https://doi.org/10.1038/nm.3372

Galderisi, A., Polidori, D., Weiss, R., et al. (2019). Lower Insulin Clearance Parallels a Reduced Insulin Sensitivity in Obese Youths and Is Associated With a Decline in β-Cell Function Over Time. *Diabetes*, *68*(11), 2074–2084. https://doi.org/10.2337/db19-0120

Gatti, R. A., Berkel, I., Boder, E., et al. (1988). Localization of an ataxia-telangiectasia gene to chromosome 11q22-23. *Nature*, *336*(6199), 577–580. https://doi.org/10.1038/336577a0

Gebreyes, Y. F., Goshu, D. Y., Geletew, T. K., et al. (2018). Prevalence of high bloodpressure, hyperglycemia, dyslipidemia, metabolic syndrome and their determinants in Ethiopia: Evidences from the National NCDs STEPS Survey, 2015. *PLOS ONE*, *13*(5), e0194819. https://doi.org/10.1371/journal.pone.0194819

Gebreyesus, H. A., Abreha, G. F., Besherae, S. D., et al. (2022). High atherogenic risk concomitant with elevated HbA1c among persons with type 2 diabetes mellitus in North Ethiopia. *PloS One*, *17*(2), e0262610. https://doi.org/10.1371/journal.pone.0262610

Gebreyohannes, E. A., Netere, A. K., & Belachew, S. A. (2019). Glycemic control among diabetic patients in Ethiopia: A systematic review and meta-analysis. *PLOS ONE*, *14*(8), e0221790. https://doi.org/10.1371/journal.pone.0221790

Gill, G., English, P., Dedicoat, M., & Tesfaye, S. (2010). *The variable African diabetic phenotype: Tales from the north and the south*. https://www.semanticscholar.org/paper/The-variable-African-diabetic-phenotype%3A-tales-from-Gill-English/3bb9beeae97e1a11595ac1d94c2fa50b323590fe

Gill, G. V., Tekle, A., Reja, A., et al. (2011). Immunological and C-peptide studies of patients with diabetes in northern Ethiopia: Existence of an unusual subgroup possibly related to malnutrition. *Diabetologia*, *54*(1), 51–57. https://doi.org/10.1007/s00125-010-1921-7

Goedecke, J. H., & Olsson, T. (2020). Pathogenesis of type 2 diabetes risk in black Africans: A South African perspective. *Journal of Internal Medicine*, *288*(3), 284–294. https://doi.org/10.1111/joim.13083

Goldberg, I. J. (2001). Diabetic Dyslipidemia: Causes and Consequences. *The Journal of Clinical Endocrinology & Metabolism*, *86*(3), 965–971. https://doi.org/10.1210/jcem.86.3.7304

Goswami, S., Gong, L., Giacomini, K., Altman, R. B., & Klein, T. E. (2014). PharmGKB summary: Very important pharmacogene information for SLC22A1. *Pharmacogenetics and Genomics*, *24*(6), 324–328. https://doi.org/10.1097/FPC.0000000000000048

Griffin, S. J., Leaver, J. K., & Irving, G. J. (2017). Impact of metformin on cardiovascular disease: A meta-analysis of randomised trials among people with type 2 diabetes. *Diabetologia*, *60*(9), 1620–1629. https://doi.org/10.1007/s00125-017-4337-9

Gründemann, D., Gorboulev, V., Gambaryan, S., Veyhl, M., & Koepsell, H. (1994). Drug excretion mediated by a new prototype of polyspecific transporter. *Nature*, *372*(6506), Article 6506. https://doi.org/10.1038/372549a0

Grundy, S. M. (1997). Small LDL, atherogenic dyslipidemia, and the metabolic syndrome. *Circulation*, *95*(1), 1–4. https://doi.org/10.1161/01.cir.95.1.1

Grundy, S. M., Stone, N. J., Bailey, A. L., et al. (2019). 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation*, *139*(25), e1082–e1143. https://doi.org/10.1161/CIR.0000000000000625

Guariguata, L., Whiting, D. R., Hambleton, I., et al. (2014). Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Research and Clinical Practice*, *103*(2), 137–149. https://doi.org/10.1016/j.diabres.2013.11.002

Hiroshi Takane, Eriko Shikata, Kenji Otsubo, Shun Higuchi & Ichiro Ieiri (2008).Polymorphism in human organic cation transporters and metformin action. *Pharmacogenomics*, *9*(4).https://doi.org/10.2217/14622416.9.4.415

Haile, K., & Timerga, A. (2020). Dyslipidemia and Its Associated Risk Factors Among Adult Type-2 Diabetic Patients at Jimma University Medical Center, Jimma, Southwest Ethiopia. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *13*, 4589–4597. https://doi.org/10.2147/DMSO.S283171

Hansen, M., Scheltema, M. J., Sonne, D. P., et al. (2016). Effect of chenodeoxycholic acid and the bile acid sequestrant colesevelam on glucagon-like peptide-1 secretion. *Diabetes, Obesity & Metabolism*, *18*(6), 571–580. https://doi.org/10.1111/dom.12648

Hardie, D. G. (2014). AMPK--sensing energy while talking to other signaling pathways. *Cell Metabolism*, *20*(6), 939–952. https://doi.org/10.1016/j.cmet.2014.09.013

Haupt, E., Knick, B., Koschinsky, T., et al. (1991). Oral antidiabetic combination therapy with sulphonylureas and metformin. *Diabete & Metabolisme*, *17*(1 Pt 2), 224–231.

Hawley, S. A., Ross, F. A., Chevtzoff, C., et al. (2010). Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. *Cell Metabolism*, *11*(6), 554–565. https://doi.org/10.1016/j.cmet.2010.04.001

Hayfron-Benjamin, C., van den Born, B.J., Maitland-van der Zee, A. H., et al. (2019). Microvascular and macrovascular complications in type 2 diabetes Ghanaian residents in Ghana and Europe: The RODAM study. *Journal of Diabetes and Its Complications*, *33*(8), 572–578. https://doi.org/10.1016/j.jdiacomp.2019.04.016

He, L., & Wondisford, F. E. (2015). Metformin action: Concentrations matter. *Cell Metabolism*, *21*(2), 159–162. https://doi.org/10.1016/j.cmet.2015.01.003

Hermann, L. S., Scherstén, B., & Melander, A. (1994). Antihyperglycaemic efficacy, response prediction and dose-response relations of treatment with metformin and sulphonylurea, alone and in primary combination. *Diabetic Medicine: A Journal of the British Diabetic Association*, *11*(10), 953–960. https://doi.org/10.1111/j.1464-5491.1994.tb00253.x

Hirano, T. (2018). Pathophysiology of Diabetic Dyslipidemia. *Journal of Atherosclerosis and Thrombosis*, *25*(9), 771–782. https://doi.org/10.5551/jat.RV17023

Holst, J. J., Gasbjerg, L. S., & Rosenkilde, M. M. (2021). The Role of Incretins on Insulin Function and Glucose Homeostasis. *Endocrinology*, *162*(7), bqab065. https://doi.org/10.1210/endocr/bqab065

Holt, R. (2016). Personalized medicine for diabetes: A special issue. *Diabetic Medicine*, *33*, 711–711. https://doi.org/10.1111/dme.13144

Howlett, D. H. C. S., & Bailey, C. J. (1999). A Risk-Benefit Assessment of Metformin in Type 2 Diabetes Mellitus. *Drug Safety*, *20*(6), 489–503. https://doi.org/10.2165/00002018-199920060-00003

Huang, P. L. (2009). A comprehensive definition for metabolic syndrome. *Disease Models & Mechanisms*, *2*(5–6), 231–237. https://doi.org/10.1242/dmm.001180

Hunter, R. W., Hughey, C. C., Lantier, L., et al. (2018). Metformin reduces liver glucose production by inhibition of fructose-1-6-bisphosphatase. *Nature Medicine*, *24*(9), 1395–1406. https://doi.org/10.1038/s41591-018-0159-7

Ieiri, I., Takane, H., Hirota, T., Otsubo, K., & Higuchi, S. (2006). Genetic polymorphisms of drug transporters: Pharmacokinetic and pharmacodynamic consequences in pharmacotherapy. *Expert Opinion on Drug Metabolism & Toxicology*, *2*(5), 651–674. https://doi.org/10.1517/17425255.2.5.651

Ito, H., Ishida, H., Takeuchi, Y., et al. (2010). Long-term effect of metformin on blood glucose control in non-obese patients with type 2 diabetes mellitus. *Nutrition & Metabolism*, *7*(1), 83. https://doi.org/10.1186/1743-7075-7-83

Ji, L., Li, H., Guo, X., Li, Y., Hu, R., & Zhu, Z. (2013). Impact of Baseline BMI on Glycemic Control and Weight Change with Metformin Monotherapy in Chinese Type 2 Diabetes Patients: Phase IV Open-Label Trial. *PLOS ONE*, *8*(2), e57222. https://doi.org/10.1371/journal.pone.0057222

Jisieike-Onuigbo, N. N., Unuigbe, E. I., & Oguejiofor, C. O. (2011). Dyslipidemias in type 2 diabetes mellitus patients in Nnewi South-East Nigeria. *Annals of African Medicine*, *10*(4), 285–289. https://doi.org/10.4103/1596-3519.87045

Karita, E., Ketter, N., Price, M. A., et al. (2009). CLSI-Derived Hematology and Biochemistry Reference Intervals for Healthy Adults in Eastern and Southern Africa. *PLoS ONE*, *4*(2), e4401. https://doi.org/10.1371/journal.pone.0004401

Kashi, Z., Mahrooz, A., Kianmehr, A., & Alizadeh, A. (2016). The Role of Metformin Response in Lipid Metabolism in Patients with Recent-Onset Type 2 Diabetes: HbA1c Level as a Criterion for Designating Patients as Responders or Nonresponders to Metformin. *PloS One*, *11*(3), e0151543. https://doi.org/10.1371/journal.pone.0151543

Kassahun, T., Eshetie, T., & Gesesew, H. (2016). Factors associated with glycemic control among adult patients with type 2 diabetes mellitus: A cross-sectional survey in Ethiopia. *BMC Research Notes*, *9*, 78. https://doi.org/10.1186/s13104-016-1896-7

Khatami, F., Mohajeri-Tehrani, M. R., & Tavangar, S. M. (2019). The Importance of Precision Medicine in Type 2 Diabetes Mellitus (T2DM): From Pharmacogenetic and Pharmacoepigenetic Aspects. *Endocrine, Metabolic & Immune Disorders Drug Targets*, *19*(6), 719–731. https://doi.org/10.2174/1871530319666190228102212

Kibirige, D., Lumu, W., Jones, A. G., et al. (2019). Understanding the manifestation of diabetes in sub Saharan Africa to inform therapeutic approaches and preventive strategies: A narrative review. *Clinical Diabetes and Endocrinology*, *5*(1), 2. https://doi.org/10.1186/s40842-019-0077-8

Kiplagat, S. V., Lydia, K., Jemimah, K., & Drusilla, M. (2017). Prevalence of Dyslipidemia and The Associated Factors Among Type 2 Diabetes Patients in Turbo Sub-County kenya. *Journal of Endocrinology and Diabetes*, *4*(5). https://symbiosisonlinepublishing.com/endocrinology-diabetes/

Kirkman, M. S., Mahmud, H., & Korytkowski, M. T. (2018). Intensive Blood Glucose Control and Vascular Outcomes in Patients with Type 2 Diabetes Mellitus. *Endocrinology and Metabolism Clinics of North America*, *47*(1), 81–96. https://doi.org/10.1016/j.ecl.2017.10.002

Kirpichnikov, D., McFarlane, S. I., & Sowers, J. R. (2002). Metformin: An update. *Annals of Internal Medicine*, *137*(1), 25–33. https://doi.org/10.7326/0003-4819-137-1-200207020-00009

Knight, M. G., Goedecke, J. H., Ricks, M., et al. (2011). The TG/HDL-C ratio does not predict insulin resistance in overweight women of African descent: A study of South African, African American and West African women. *Ethnicity & Disease*, *21*(4), 490–494.

Koepsell, H., & Endou, H. (2004). The SLC22 drug transporter family. *Pflugers Archiv: European Journal of Physiology*, *447*(5), 666–676. https://doi.org/10.1007/s00424-003-1089-9

Koepsell, H., Lips, K., & Volk, C. (2007). Polyspecific Organic Cation Transporters: Structure, Function, Physiological Roles, and Biopharmaceutical Implications. *Pharmaceutical Research*, *24*, 1227–1251. https://doi.org/10.1007/s11095-007-9254-z

Krishan, S., Richardson, D. R., & Sahni, S. (2015). Adenosine monophosphate-activated kinase and its key role in catabolism: Structure, regulation, biological activity, and pharmacological activation. *Molecular Pharmacology*, *87*(3), 363–377. https://doi.org/10.1124/mol.114.095810

Le, M. T., Lobmeyer, M. T., Campbell, M., et al. (2013). Impact of Genetic Polymorphisms of SLC2A2, SLC2A5, and KHK on Metabolic Phenotypes in Hypertensive Individuals. *PLoS ONE*, *8*(1), e52062. https://doi.org/10.1371/journal.pone.0052062

Li, H., Liu, T., Lim, J., et al. (2015). Increased biogenesis of glucagon-containing secretory granules and glucagon secretion in BIG3-knockout mice. *Molecular Metabolism*, *4*(3), 246–252. https://doi.org/10.1016/j.molmet.2015.01.001

Li, H., Wei, S., Cheng, K., et al. (2014). BIG3 inhibits insulin granule biogenesis and insulin secretion. *EMBO Reports*, *15*(6), 714–722. https://doi.org/10.1002/embr.201338181

Li, J. H., Brenner, L. N., Kaur, V., et al. (2023). Genome-wide association analysis identifies ancestry-specific genetic variation associated with acute response to metformin and glipizide in SUGAR-MGH. *Diabetologia*, *66*(7), 1260–1272. https://doi.org/10.1007/s00125-023-05922-7

Li, J. H., Perry, J. A., Jablonski, K. A., et al. (2023). Identification of Genetic Variation Influencing Metformin Response in a Multiancestry Genome-Wide Association Study in the Diabetes Prevention Program (DPP). *Diabetes*, *72*(8), 1161–1172. https://doi.org/10.2337/db22-0702

Liang, X., & Giacomini, K. M. (2017). Transporters Involved in Metformin Pharmacokinetics and Treatment Response. *Journal of Pharmaceutical Sciences*, *106*(9), 2245–2250. https://doi.org/10.1016/j.xphs.2017.04.078

Lim, M. T., Ab Rahman, N., Teh, X. R., et al. (2021). Optimal cut-off points for adherence measure among patients with type 2 diabetes in primary care clinics: A retrospective analysis. *Therapeutic Advances in Chronic Disease*, *12*, 2040622321990264. https://doi.org/10.1177/2040622321990264

Lin, H. V., & Accili, D. (2011). Hormonal regulation of hepatic glucose production in health and disease. *Cell Metabolism*, *14*(1), 9–19. https://doi.org/10.1016/j.cmet.2011.06.003

Liu, C., Feng, X., Li, Q., Wang, Y., Li, Q., & Hua, M. (2016). Adiponectin, TNF-α and inflammatory cytokines and risk of type 2 diabetes: A systematic review and meta-analysis. *Cytokine*, *86*, 100–109. https://doi.org/10.1016/j.cyto.2016.06.028

Lonsdale, J., Thomas, J., Salvatore, M., et al. (2013). The Genotype-Tissue Expression (GTEx) project. *Nature Genetics*, *45*(6), 580–585. https://doi.org/10.1038/ng.2653

L S Hermann, B Scherstén, P O Bitzén, et al. (1994). Therapeutic comparison of metformin and sulfonylurea, alone and in various combinations. A double-blind controlled study. *Diabetes Care, 17*(10) :1100-9. https://doi.org/10.2337/diacare.17.10.1100

Lund, S. S., Tarnow, L., Stehouwer, C. D. A., et al. (2007). Targeting hyperglycaemia with either metformin or repaglinide in non-obese patients with type 2 diabetes: Results from a randomized crossover trial. *Diabetes, Obesity & Metabolism*, *9*(3), 394–407. https://doi.org/10.1111/j.1463-1326.2007.00713.x

Ma, T., Tian, X., Zhang, B., et al. (2022). Low-dose metformin targets the lysosomal AMPK pathway through PEN2. *Nature*, *603*(7899), 159–165. https://doi.org/10.1038/s41586-022-04431-8

MacDonald, M. R., Petrie, M. C., Varyani, F., et al. (2008). Impact of diabetes on outcomes in patients with low and preserved ejection fraction heart failure: An analysis of the Candesartan in Heart failure: Assessment of Reduction in Mortality and morbidity (CHARM) programme. *European Heart Journal*, *29*(11), 1377–1385. https://doi.org/10.1093/eurheartj/ehn153

Macho-González, A., Garcimartín, A., López-Oliva, M. E., et al. (2019). Can Carob-Fruit-Extract-Enriched Meat Improve the Lipoprotein Profile, VLDL-Oxidation, and LDL Receptor Levels Induced by an Atherogenic Diet in STZ-NAD-Diabetic Rats? *Nutrients*, *11*(2), 332. https://doi.org/10.3390/nu11020332

Madiraju, A. K., Erion, D. M., Rahimi, Y., et al. (2014). Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. *Nature*, *510*(7506), 542–546. https://doi.org/10.1038/nature13270

Mahrooz, A., Parsanasab, H., Hashemi-Soteh, M. B., et al. (2015). The role of clinical response to metformin in patients newly diagnosed with type 2 diabetes: A monotherapy study. *Clinical and Experimental Medicine*, *15*(2), 159–165. https://doi.org/10.1007/s10238-014-0283-8

Maruthur, N. M., Tseng, E., Hutfless, S., et al. (2016). Diabetes Medications as Monotherapy or Metformin-Based Combination Therapy for Type 2 Diabetes: A Systematic Review and Meta-analysis. *Annals of Internal Medicine*, *164*(11), 740–751. https://doi.org/10.7326/M15-2650

Mbanya, J. C. N., Motala, A. A., Sobngwi, E., Assah, F. K., & Enoru, S. T. (2010). Diabetes in sub-Saharan Africa. *Lancet (London, England)*, *375*(9733), 2254–2266. https://doi.org/10.1016/S0140-6736(10)60550-8

McCreight, L. J., Bailey, C. J., & Pearson, E. R. (2016). Metformin and the gastrointestinal tract. *Diabetologia*, *59*(3), 426–435. https://doi.org/10.1007/s00125-015-3844-9

Menjivar M., Katy S.P., Joel J.S. et al. (2020). Pharmacogenetic Evaluation of Metformin and Sulphonylurea Response in Mexican Mestizos with Type 2 Diabetes. Curr Drug Metab, 21(4):291-300. doi: 10.2174/1389200221666200514125443.

Miks S., B. J. (2018, September). *Gene-Calc [Computer software]*. Www.Gene-Calc.Pl. https://gene-calc.pl/contact

Miller, R. A., Chu, Q., Xie, J., Foretz, M., Viollet, B., & Birnbaum, M. J. (2013). Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. *Nature*, *494*(7436), 256–260. https://doi.org/10.1038/nature11808

Milo, R. B., & Connelly, C. D. (2019). Predictors of glycemic management among patients with type 2 diabetes. *Journal of Clinical Nursing*, *28*(9–10), 1737–1744. https://doi.org/10.1111/jocn.14779

Mithal, A., Majhi, D., Shunmugavelu, M., Talwarkar, P. G., Vasnawala, H., & Raza, A. S. (2014). Prevalence of dyslipidemia in adult Indian diabetic patients: A cross sectional study (SOLID). *Indian Journal of Endocrinology and Metabolism*, *18*(5), 642–647. https://doi.org/10.4103/2230-8210.139220

Mittelman, S. D., Van Citters, G. W., Kim, S. P., et al. (2000). Longitudinal compensation for fat-induced insulin resistance includes reduced insulin clearance and enhanced beta-cell response. *Diabetes*, *49*(12), 2116–2125. https://doi.org/10.2337/diabetes.49.12.2116

Mofo Mato, E. P., Guewo-Fokeng, M., Essop, M. F., & Owira, P. M. O. (2018). Genetic polymorphisms of organic cation transporter 1 (OCT1) and responses to metformin therapy in individuals with type 2 diabetes: A systematic review. *Medicine*, *97*(27), e11349. https://doi.org/10.1097/MD.0000000000011349

Morrice, N., Vainio, S., Mikkola, K., et al. (2023). Metformin increases the uptake of glucose into the gut from the circulation in high-fat diet-fed male mice, which is enhanced by a reduction in whole-body *Slc2a2* expression. *Molecular Metabolism*, *77*, 101807. https://doi.org/10.1016/j.molmet.2023.101807

Motala, A. A., Mbanya, J. C., Ramaiya, K., Pirie, F. J., & Ekoru, K. (2022). Type 2 diabetes mellitus in sub-Saharan Africa: Challenges and opportunities. *Nature Reviews Endocrinology*, *18*(4), Article 4. https://doi.org/10.1038/s41574-021-00613-y

Motuma, A., Shiferaw, K., Gobena, T., Roba, K. T., Berhane, Y., & Worku, A. (2023). Dyslipidemia and its predictors among adult workers in eastern Ethiopia: An institution-based cross-sectional study. *PLOS ONE*, *18*(10), e0291665. https://doi.org/10.1371/journal.pone.0291665

Mueller, N. T., Differding, M. K., Zhang, M., et al. (2021). Metformin Affects Gut Microbiome Composition and Function and Circulating Short-Chain Fatty Acids: A Randomized Trial. *Diabetes Care*, *44*(7), 1462–1471. https://doi.org/10.2337/dc20-2257

Napolitano, A., Miller, S., Nicholls, A. W., et al. (2014). Novel Gut-Based Pharmacology of Metformin in Patients with Type 2 Diabetes Mellitus. *PLOS ONE*, *9*(7), e100778. https://doi.org/10.1371/journal.pone.0100778

Narindrarangkura, P., Bosl, W., Rangsin, R., & Hatthachote, P. (2019). Prevalence of dyslipidemia associated with complications in diabetic patients: A nationwide study in Thailand. *Lipids in Health and Disease*, *18*(1), 90. https://doi.org/10.1186/s12944-019-1034-3

Nasykhova, Y. A., Barbitoff, Y. A., Tonyan, Z. N., et al. (2022). Genetic and Phenotypic Factors Affecting Glycemic Response to Metformin Therapy in Patients with Type 2 Diabetes Mellitus. *Genes*, *13*(8), Article 8. https://doi.org/10.3390/genes13081310

Nies, A. T., Koepsell, H., Winter, S., et al. (2009). Expression of organic cation transporters OCT1 (SLC22A1) and OCT3 (SLC22A3) is affected by genetic factors and cholestasis in human liver. *Hepatology (Baltimore, Md.)*, *50*(4), 1227–1240. https://doi.org/10.1002/hep.23103

Nolan, C. J., Ruderman, N. B., Kahn, S. E., Pedersen, O., & Prentki, M. (2015). Insulin resistance as a physiological defense against metabolic stress: Implications for the management of subsets of type 2 diabetes. *Diabetes*, *64*(3), 673–686. https://doi.org/10.2337/db14-0694

Obesity: Preventing and managing the global epidemic. Report of a WHO consultation. (2000). *World Health Organization Technical Report Series*, *894*, i–xii, 1–253.

Odume, B. B., Ofoegbu, O. S., Aniwada, E. C., & Okechukwu, E. F. (2015). The influence of family characteristics on glycaemic control among adult patients with type 2 diabetes mellitus attending the general outpatient clinic, National Hospital, Abuja, Nigeria. *South African Family Practice*, *57*(6), 347–352. https://doi.org/10.1080/20786190.2015.1090688

Okafor, C. I., Fasanmade, O. A., & Oke, D. A. (2008). Pattern of dyslipidaemia among Nigerians with type 2 diabetes mellitus. *Nigerian Journal of Clinical Practice*, *11*(1), 25–31.

Ong, C. R., Molyneaux, L. M., Constantino, M. I., Twigg, S. M., & Yue, D. K. (2006). Long-Term Efficacy of Metformin Therapy in Nonobese Individuals With Type 2 Diabetes. *Diabetes Care*, *29*(11), 2361–2364. https://doi.org/10.2337/dc06-0827

Osei, K., Schuster, D. P., Owusu, S. K., & Amoah, A. G. B. (1997). Race and ethnicity determine serum insulin and C-peptide concentrations and hepatic insulin extraction and insulin clearance: Comparative studies of three populations of West African Ancestry and White Americans. *Metabolism*, *46*(1), 53–58. https://doi.org/10.1016/S0026-0495(97)90167-0

Overbeek, J. A., Heintjes, E. M., Prieto-Alhambra, D., et al. (2017). Type 2 Diabetes Mellitus Treatment Patterns Across Europe: A Population-based Multi-database Study. *Clinical Therapeutics*, *39*(4), 759–770. https://doi.org/10.1016/j.clinthera.2017.02.008

Owen, M. R., Doran, E., & Halestrap, A. P. (2000). Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *The Biochemical Journal*, *348 Pt 3*(Pt 3), 607–614.

Palmer, S. C., Mavridis, D., Nicolucci, A., et al. (2016). Comparison of Clinical Outcomes and Adverse Events Associated With Glucose-Lowering Drugs in Patients With Type 2 Diabetes: A Meta-analysis. *JAMA*, *316*(3), 313–324. https://doi.org/10.1001/jama.2016.9400

Park, J. E., Jeong, G. H., Lee, I. K., et al. (2018). A Pharmacometabolomic Approach to Predict Response to Metformin in Early-Phase Type 2 Diabetes Mellitus Patients. *Molecules (Basel, Switzerland)*, *23*(7), 1579. https://doi.org/10.3390/molecules23071579

Pastakia, S. D., Pekny, C. R., Manyara, S. M., & Fischer, L. (2017). Diabetes in sub-Saharan Africa – from policy to practice to progress: Targeting the existing gaps for future care for diabetes. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *10*, 247–263. https://doi.org/10.2147/DMSO.S126314

Pawlyk, A. C., Giacomini, K. M., McKeon, C., Shuldiner, A. R., & Florez, J. C. (2014). Metformin pharmacogenomics: Current status and future directions. *Diabetes*, *63*(8), 2590–2599. https://doi.org/10.2337/db13-1367

Pearson, E. R. (2016). Personalized medicine in diabetes: The role of "omics" and biomarkers. *Diabetic Medicine: A Journal of the British Diabetic Association*, *33*(6), 712–717. https://doi.org/10.1111/dme.13075

Pearson, E. R. (2019). Type 2 diabetes: A multifaceted disease. *Diabetologia*, *62*(7), 1107–1112. https://doi.org/10.1007/s00125-019-4909-y

Peng, A., Gong, C., Xu, Y., et al. (2023). Association between organic cation transporter genetic polymorphisms and metformin response and intolerance in T2DM individuals: A systematic review and meta-analysis. *Frontiers in Public Health*, *11*. https://www.frontiersin.org/articles/10.3389/fpubh.2023.1183879

Petersen, M. C., & Shulman, G. I. (2018). Mechanisms of Insulin Action and Insulin Resistance. *Physiological Reviews*, *98*(4), 2133–2223. https://doi.org/10.1152/physrev.00063.2017

Petersen, M. C., Vatner, D. F., & Shulman, G. I. (2017). Regulation of hepatic glucose metabolism in health and disease. *Nature Reviews. Endocrinology*, *13*(10), 572–587. https://doi.org/10.1038/nrendo.2017.80

Phate, S. D., Daswani, B. R., Mishra, D. N., & Joshi, K. S. (2020). Genetic analysis of SLC47A1, SLC22A1, SLC22A2, ATM gene polymorphisms among diabetics in an Indian population. *International Journal of Basic & Clinical Pharmacology*, *9*(6), 891–896. https://doi.org/10.18203/2319-2003.ijbcp20202189

Pitso, L., Mofokeng, T. R. P., & Nel, R. (2021). Dyslipidaemia pattern and prevalence among type 2 diabetes mellitus patients on lipid-lowering therapy at a tertiary hospital in central South Africa. *BMC Endocrine Disorders*, *21*(1), 159. https://doi.org/10.1186/s12902-021-00813-7

Pokharel, D. R., Khadka, D., Sigdel, M., et al. (2017). Prevalence and pattern of dyslipidemia in Nepalese individuals with type 2 diabetes. *BMC Research Notes*, *10*(1), 146. https://doi.org/10.1186/s13104-017-2465-4

Polianskyte-Prause, Z., Tolvanen, T. A., Lindfors, S., et al. (2019). Metformin increases glucose uptake and acts renoprotectively by reducing SHIP2 activity. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *33*(2), 2858–2869. https://doi.org/10.1096/fj.201800529RR

Ralph A DeFronzo, John B Buse , Terri Kim, et al. (2016). Once-daily delayed-release metformin lowers plasma glucose and enhances fasting and postprandial GLP-1 and PYY: Results from two randomised trials. *Diabetologia*, *59*(8):1645-54 https://doi.org/10.1007/s00125-016-3992-6

Rathmann, W., Strassburger, K., Bongaerts, B., et al. (2019). A variant of the glucose transporter gene SLC2A2 modifies the glycaemic response to metformin therapy in recently diagnosed type 2 diabetes. *Diabetologia*, *62*(2), 286–291. https://doi.org/10.1007/s00125-018-4759-z

Reaven, P. D., Emanuele, N. V., Wiitala, W. L., et al. (2019). Intensive Glucose Control in Patients with Type 2 Diabetes—15-Year Follow-up. *The New England Journal of Medicine*, *380*(23), 2215–2224. https://doi.org/10.1056/NEJMoa1806802

Rena, G., Hardie, D. G., & Pearson, E. R. (2017). The mechanisms of action of metformin. *Diabetologia*, *60*(9), 1577–1585. https://doi.org/10.1007/s00125-017-4342-z

Rena, G., & Sakamoto, E. R. P. K. (2012). Molecular action and pharmacogenetics of metformin: Current understanding of an old drug. *Diabetes Management*, *2*(5), 439.

Rotroff, D. M., Yee, S. W., Zhou, K., et al. (2018). Genetic Variants in CPA6 and PRPF31 Are Associated With Variation in Response to Metformin in Individuals With Type 2 Diabetes. *Diabetes*, *67*(7), 1428–1440. https://doi.org/10.2337/db17-1164

rs8192675 (SNP)—Population genetics—Homo_sapiens—Ensembl genome browser. Retrieved January 21, 2023, from https://www.ensembl.org/Homo_sapiens/Variation/Population

Saeedi, P., Petersohn, I., Salpea, P., et al. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*, *157*, 107843. https://doi.org/10.1016/j.diabres.2019.107843

Saiz-Rodríguez, M., Ochoa, D., Zubiaur, P., et al. (2023). Identification of Transporter Polymorphisms Influencing Metformin Pharmacokinetics in Healthy Volunteers. *Journal of Personalized Medicine*, *13*(3), 489. https://doi.org/10.3390/jpm13030489

Sakata, T., Anzai, N., Shin, H. J., et al. (2004). Novel single nucleotide polymorphisms of organic cation transporter 1 (SLC22A1) affecting transport functions. *Biochemical and Biophysical Research Communications*, *313*(3), 789–793. https://doi.org/10.1016/j.bbrc.2003.11.175

Sanchez-Rangel, E., & Inzucchi, S. E. (2017). Metformin: Clinical use in type 2 diabetes. *Diabetologia*, *60*(9), 1586–1593. https://doi.org/10.1007/s00125-017-4336-x

Sansome, D. J., Xie, C., Veedfald, S., Horowitz, M., Rayner, C. K., & Wu, T. (2020). Mechanism of glucose-lowering by metformin in type 2 diabetes: Role of bile acids. *Diabetes, Obesity & Metabolism*, *22*(2), 141–148. https://doi.org/10.1111/dom.13869

Sarfraz, M., Sajid, S., & Ashraf, M. A. (2016). Prevalence and pattern of dyslipidemia in hyperglycemic patients and its associated factors among Pakistani population. *Saudi Journal of Biological Sciences*, *23*(6), 761–766. https://doi.org/10.1016/j.sjbs.2016.03.001

Savage, D. B., Choi, C. S., Samuel, V. T., et al. (2006). Reversal of diet-induced hepatic steatosis and hepatic insulin resistance by antisense oligonucleotide inhibitors of acetyl-CoA carboxylases 1 and 2. *The Journal of Clinical Investigation*, *116*(3), 817–824. https://doi.org/10.1172/JCI27300

Scarpello, J. H., Hodgson, E., & Howlett, H. C. (1998). Effect of metformin on bile salt circulation and intestinal motility in type 2 diabetes mellitus. *Diabetic Medicine: A Journal of the British Diabetic Association*, *15*(8), 651–656. https://doi.org/10.1002/(SICI)1096-9136(199808)15:8<651::AID-DIA628>3.0.CO;2-A

Schaller, L., & Lauschke, V. M. (2019). The genetic landscape of the human solute carrier (SLC) transporter superfamily. *Human Genetics*, *138*(11–12), 1359–1377. https://doi.org/10.1007/s00439-019-02081-x

Schofield, J. D., Liu, Y., Rao-Balakrishna, P., Malik, R. A., & Soran, H. (2016). Diabetes Dyslipidemia. *Diabetes Therapy: Research, Treatment and Education of Diabetes and Related Disorders*, *7*(2), 203–219. https://doi.org/10.1007/s13300-016-0167-x

Schwartz, S., Fonseca, V., Berner, B., Cramer, M., Chiang, Y. K., & Lewin, A. (2006). Efficacy, tolerability, and safety of a novel once-daily extended-release metformin in patients with type 2 diabetes. *Diabetes Care*, *29*(4), 759–764. https://doi.org/10.2337/diacare.29.04.06.dc05-1967

Steven E Kahn, Steven M Haffner, Mark A Heise, et al. (2006). Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *The New England Journal of Medicine*, *355*(23):2427-43. https://doi.org/10.1056/NEJMoa066224

Seid, M. A., Akalu, Y., Gela, Y. Y., et al. (2021). Microvascular complications and its predictors among type 2 diabetes mellitus patients at Dessie town hospitals, Ethiopia. *Diabetology & Metabolic Syndrome*, *13*, 86. https://doi.org/10.1186/s13098-021-00704-w

Seitz, T., Stalmann, R., Dalila, N., et al. (2015). Global genetic analyses reveal strong inter-ethnic variability in the loss of activity of the organic cation transporter OCT1. *Genome Medicine*, *7*(1), 56. https://doi.org/10.1186/s13073-015-0172-0

Semiz, S., Dujic, T., & Causevic, A. (2013). Pharmacogenetics and personalized treatment of type 2 diabetes. *Biochemia Medica*, *23*(2), 154–171. https://doi.org/10.11613/bm.2013.020

Sert, M., Morgul, G., & Tetiker, B. T. (2019). Diabetic dyslipidemia is a well-known issue, but what about lipoprotein a levels in Type 2 diabetics? *International Journal of Diabetes and Metabolism*, *18*(2), 81–87. https://doi.org/10.1159/000497697

Seyer, P., Vallois, D., Poitry-Yamate, C., et al. (2013). Hepatic glucose sensing is required to preserve β cell glucose competence. *The Journal of Clinical Investigation*, *123*(4), 1662–1676. https://doi.org/10.1172/JCI65538

Shan, S., Gu, L., Lou, Q., et al. (2017). Evaluation of glycemic control in patients with type 2 diabetes mellitus in Chinese communities: A cross-sectional study. *Clinical and Experimental Medicine*, *17*(1), 79–84. https://doi.org/10.1007/s10238-015-0406-x

Shaw, J. E., Sicree, R. A., & Zimmet, P. Z. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice*, *87*(1), 4–14. https://doi.org/10.1016/j.diabres.2009.10.007

Shaw, R. J., Lamia, K. A., Vasquez, D., et al. (2005). The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. *Science (New York, N.Y.)*, *310*(5754), 1642–1646. https://doi.org/10.1126/science.1120781

Sherwani, S. I., Khan, H. A., Ekhzaimy, A., Masood, A., & Sakharkar, M. K. (2016). Significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients. *Biomarker Insights*, *11*, 95–104. https://doi.org/10.4137/BMI.S38440

Shikata, E., Yamamoto, R., Takane, H., et al. (2007). Human organic cation transporter (OCT1 and OCT2) gene polymorphisms and therapeutic effects of metformin. *Journal of Human Genetics*, *52*(2), 117–122. https://doi.org/10.1007/s10038-006-0087-0

Shokri, F., Ghaedi, H., Ghafouri Fard, S., et al. (2016). Impact of ATM and SLC22A1 Polymorphisms on Therapeutic Response to Metformin in Iranian Diabetic Patients. *International Journal of Molecular and Cellular Medicine*, *5*(1), 1–7.

Shu, Y., Sheardown, S. A., Brown, C., et al. (2007). Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *The Journal of Clinical Investigation*, *117*(5), 1422–1431. https://doi.org/10.1172/JCI30558

SNP Genotyping Analysis Using TaqMan Assays. Retrieved July 29, 2023, from https://www.thermofisher.com/ng/en/home/life-science/pcr/real-time-pcr/real-time-pcr-assays/snp-genotyping-taqman-assays.html

Sobngwi, E., Ndour-Mbaye, M., Boateng, K. A., et al. (2012). Type 2 diabetes control and complications in specialised diabetes care centres of six sub-Saharan African countries: The Diabcare Africa study. *Diabetes Research and Clinical Practice*, *95*(1), 30–36. https://doi.org/10.1016/j.diabres.2011.10.018

Song, I. S., Shin, H. J., Shim, E. J., et al. (2008). Genetic variants of the organic cation transporter 2 influence the disposition of metformin. *Clinical Pharmacology and Therapeutics*, *84*(5), 559–562. https://doi.org/10.1038/clpt.2008.61

Song, R. (2016). Mechanism of Metformin: A Tale of Two Sites. *Diabetes Care*, *39*(2), 187–189. https://doi.org/10.2337/dci15-0013

Sun, L., Xie, C., Wang, G., et al. (2018). Gut microbiota and intestinal FXR mediate the clinical benefits of metformin. *Nature Medicine*, *24*(12), 1919–1929. https://doi.org/10.1038/s41591-018-0222-4

Tanabe, M., Motonaga, R., Terawaki, Y., Nomiyama, T., & Yanase, T. (2017). Prescription of oral hypoglycemic agents for patients with type 2 diabetes mellitus: A retrospective cohort study using a Japanese hospital database. *Journal of Diabetes Investigation*, *8*(2), 227–234. https://doi.org/10.1111/jdi.12567

Tarasova, L., Kalnina, I., Geldnere, K., et al. (2012). Association of genetic variation in the organic cation transporters OCT1, OCT2 and multidrug and toxin extrusion 1 transporter protein genes with the gastrointestinal side effects and lower BMI in metformin-treated type 2 diabetes patients. *Pharmacogenetics and Genomics*, *22*(9), 659–666. https://doi.org/10.1097/FPC.0b013e3283561666

Taskinen, M. R. (2002). Diabetic dyslipidemia. *Atherosclerosis Supplements*, *3*(1), 47–51. https://doi.org/10.1016/s1567-5688(01)00006-x

Thomas, C., Gioiello, A., Noriega, L., et al. (2009). TGR5-mediated bile acid sensing controls glucose homeostasis. *Cell Metabolism*, *10*(3), 167–177. https://doi.org/10.1016/j.cmet.2009.08.001

Thorens, B., Charron, M. J., & Lodish, H. F. (1990). Molecular physiology of glucose transporters. *Diabetes Care*, *13*(3), 209–218. https://doi.org/10.2337/diacare.13.3.209

Todd, J. N., & Florez, J. C. (2014). An update on the pharmacogenomics of metformin: Progress, problems and potential. *Pharmacogenomics*, *15*(4), 529–539. https://doi.org/10.2217/pgs.14.21

Umamaheswaran, G., Praveen, R. G., Damodaran, S. E., Das, A. K., & Adithan, C. (2015). Influence of SLC22A1 rs622342 genetic polymorphism on metformin response in South Indian type 2 diabetes mellitus patients. *Clinical and Experimental Medicine*, *15*(4), 511–517. https://doi.org/10.1007/s10238-014-0322-5

United Kingdom Prospective Diabetes Study 24: A 6-year, randomized, controlled trial comparing sulfonylurea, insulin, and metformin therapy in patients with newly diagnosed type 2 diabetes that could not be controlled with diet therapy. (UKPDS 24). (1998). *Annals of Internal Medicine*, *128*(3), 165–175. https://doi.org/10.7326/0003-4819-128-3-199802010-00001

United Kingdom Prospective Diabetes Study 33: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). (1998). *The Lancet*, *352*(9131), 837–853. https://doi.org/10.1016/S0140-6736(98)07019-6

United Kingdom Prospective Diabetes Study 34: Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). (1998). *The Lancet*, *352*(9131), 854–865. https://doi.org/10.1016/S0140-6736(98)07037-8

van Leeuwen, N., Nijpels, G., Becker, M. L., et al. (2012). A gene variant near ATM is significantly associated with metformin treatment response in type 2 diabetes: A replication and meta-analysis of five cohorts. *Diabetologia*, *55*(7), 1971–1977. https://doi.org/10.1007/s00125-012-2537-x

van Leeuwen, N., Swen, J. J., Guchelaar, H. J., & 't Hart, L. M. (2013). The role of pharmacogenetics in drug disposition and response of oral glucose-lowering drugs. *Clinical Pharmacokinetics*, *52*(10), 833–854. https://doi.org/10.1007/s40262-013-0076-3

Vilvanathan, S., Gurusamy, U., Mukta, V., Das, A. K., & Chandrasekaran, A. (2014). Allele and genotype frequency of a genetic variant in ataxia telangiectasia mutated gene affecting glycemic response to metformin in South Indian population. *Indian Journal of Endocrinology and Metabolism*, *18*(6), 850–854. https://doi.org/10.4103/2230-8210.119944

Waari, G., Mutai, J., & Gikunju, J. (2018). Medication adherence and factors associated with poor adherence among type 2 diabetes mellitus patients on follow-up at Kenyatta National Hospital, Kenya. *The Pan African Medical Journal*, *29*, 82. https://doi.org/10.11604/pamj.2018.29.82.12639

Wagner, D. J., Hu, T., & Wang, J. (2016). Polyspecific organic cation transporters and their impact on drug intracellular levels and pharmacodynamics. *Pharmacological Research*, *111*, 237–246. https://doi.org/10.1016/j.phrs.2016.06.002

Wang, H., & Chow, S. C. (2007). Sample Size Calculation for Comparing Proportions. In *Wiley Encyclopedia of Clinical Trials*. John Wiley & Sons, Ltd. https://doi.org/10.1002/9780471462422.eoct005

Wilcock, C., & Bailey, C. J. (1994). Accumulation of metformin by tissues of the normal and diabetic mouse. *Xenobiotica; the Fate of Foreign Compounds in Biological Systems*, *24*(1), 49–57. https://doi.org/10.3109/00498259409043220

Wilson, P. W. F., D'Agostino, R. B., Parise, H., Sullivan, L., & Meigs, J. B. (2005). Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. *Circulation*, *112*(20), 3066–3072. https://doi.org/10.1161/CIRCULATIONAHA.105.539528

Wu, B., Yee, S. W., Xiao, S., et al. (2024). Genome-Wide Association Study Identifies Pharmacogenomic Variants Associated With Metformin Glycemic Response in African American Patients With Type 2 Diabetes. *Diabetes Care*, *47*(2), 208–215. https://doi.org/10.2337/dc22-2494

Wu, H., Esteve, E., Tremaroli, V., et al. (2017). Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nature Medicine*, *23*(7), 850–858. https://doi.org/10.1038/nm.4345

Wu, K., Li, X., Xu, Y., et al. (2020). SLC22A1 rs622342 Polymorphism Predicts Insulin Resistance Improvement in Patients with Type 2 Diabetes Mellitus Treated with Metformin: A Cross-Sectional Study. *International Journal of Endocrinology*, *2020*, 2975898. https://doi.org/10.1155/2020/2975898

Wu, L., & Parhofer, K. G. (2014). Diabetic dyslipidemia. *Metabolism: Clinical and Experimental*, *63*(12), 1469–1479. https://doi.org/10.1016/j.metabol.2014.08.010

Wu, T., Bound, M. J., Standfield, S. D., et al. (2013). Effects of rectal administration of taurocholic acid on glucagon-like peptide-1 and peptide YY secretion in healthy humans. *Diabetes, Obesity & Metabolism*, *15*(5), 474–477. https://doi.org/10.1111/dom.12043

Xie, D., Chen, F., Zhang, Y., et al. (2022). Let-7 underlies metformin-induced inhibition of hepatic glucose production. *Proceedings of the National Academy of Sciences*, *119*(14), e2122217119. https://doi.org/10.1073/pnas.2122217119

Yadegar, A., Mohammadi, F., Rabizadeh, S., Qahremani, R., Esteghamati, A., & Nakhjavani, M. (2022). Prevalence of different patterns of dyslipidemia in patients with type 2 diabetes in an Iranian population. *Translational Medicine Communications*, *7*(1), 23. https://doi.org/10.1186/s41231-022-00129-1

Yee, S. W., Chen, L., & Giacomini, K. M. (2012). The role of ATM in response to metformin treatment and activation of AMPK. *Nature Genetics*, *44*(4), 359–360. https://doi.org/10.1038/ng.2236

Yoval-Sánchez, B., Ansari, F., Lange, D., & Galkin, A. (2022). Effect of metformin on intact mitochondria from liver and brain: Concept revisited. *European Journal of Pharmacology*, *931*, 175177. https://doi.org/10.1016/j.ejphar.2022.175177

Zeller, T., Wild, P., Szymczak, S., et al. (2010). Genetics and beyond—The transcriptome of human monocytes and disease susceptibility. *PloS One*, *5*(5), e10693. https://doi.org/10.1371/journal.pone.0010693

Zeru, M. A., Tesfa, E., Mitiku, A. A., Seyoum, A., & Bokoro, T. A. (2021). Prevalence and risk factors of type-2 diabetes mellitus in Ethiopia: Systematic review and meta-analysis. *Scientific Reports*, *11*(1), 21733. https://doi.org/10.1038/s41598-021-01256-9

Zhang, J., Yang, Z., Xiao, J., et al. (2015). Association between Family History Risk Categories and Prevalence of Diabetes in Chinese Population. *PLOS ONE*, *10*(2), e0117044. https://doi.org/10.1371/journal.pone.0117044

Zhou, G., Myers, R., Li, Y., et al. (2001). Role of AMP-activated protein kinase in mechanism of metformin action. *The Journal of Clinical Investigation*, *108*(8), 1167–1174. https://doi.org/10.1172/JCI13505

Zhou, K., Bellenguez, C., Spencer, C. C., et al. (2011). Common variants near ATM are associated with glycemic response to metformin in type 2 diabetes. *Nature Genetics*, *43*(2), 117–120. https://doi.org/10.1038/ng.735

Zhou, K., Donnelly, L. A., Kimber, C. H., et al. (2009). Reduced-function SLC22A1 polymorphisms encoding organic cation transporter 1 and glycemic response to metformin: A GoDARTS study. *Diabetes*, *58*(6), 1434–1439. https://doi.org/10.2337/db08-0896

Zhou, K., Donnelly, L., Yang, J., et al. (2014). Heritability of variation in glycaemic response to metformin: A genome-wide complex trait analysis. *The Lancet. Diabetes & Endocrinology*, *2*(6), 481–487. https://doi.org/10.1016/S2213-8587(14)70050-6

Zhou, K., Pedersen, H. K., Dawed, A. Y., & Pearson, E. R. (2016). Pharmacogenomics in diabetes mellitus: Insights into drug action and drug discovery. *Nature Reviews. Endocrinology*, *12*(6), 337–346. https://doi.org/10.1038/nrendo.2016.51

Zhou, K., Yee, S. W., Seiser, E. L., et al. (2016). Variation in the glucose transporter gene SLC2A2 is associated with glycemic response to metformin. *Nature Genetics*, *48*(9), 1055–1059. https://doi.org/10.1038/ng.3632

Zhou, T., Xu, X., Du, M., Zhao, T., & Wang, J. (2018). A preclinical overview of metformin for the treatment of type 2 diabetes. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *106*, 1227–1235. https://doi.org/10.1016/j.biopha.2018.07.085

Zhou, Y., Guo, Y., Ye, W., et al. (2014). RS11212617 is associated with metformin treatment response in type 2 diabetes in Shanghai local Chinese population. *International Journal of Clinical Practice*, *68*(12), 1462–1466. https://doi.org/10.1111/ijcp.12534

Zhou, Y., Ye, W., Wang, Y., et al. (2015). Genetic variants of OCT1 influence glycemic response to metformin in Han Chinese patients with type-2 diabetes mellitus in Shanghai. *International Journal of Clinical and Experimental Pathology*, *8*(8), 9533–9542.

Zhu, H., Jia, Z., Li, Y. R., & Danelisen, I. (2023). Molecular mechanisms of action of metformin: Latest advances and therapeutic implications. *Clinical and Experimental Medicine*, 1–11. https://doi.org/10.1007/s10238-023-01051-y

Appendix

Annex 1: PUBLICATIONS

Annex 2: ETHICAL APPROVAL DOCUMENTS

Annex 3: MATERIAL TRANSFER AGREEMENT

Annex 4: PARTICIPANT INFORMATION SHEET AND INFORMED VOLUNTARY CONSENT FORMS

Annex 5: QUESTIONNAIRES

Annex 6: CHECKLIST FOR ABSTRACTION OF CLINICAL AND LABORATORY DATA