Deanship of Graduated Studies

Al- Quds University

Association of OCT1, OCT2 and OCT3 gene polymorphisms with Type 2 Diabetes Mellitus risk in Palestine.

Dua'a Shareef Muhammed Subhi Fakhouri

M.Sc. Thesis

Jerusalem – Palestine

1446/2024

Association of OCT1, OCT2 and OCT3 gene polymorphisms with Type 2 Diabetes Mellitus risk in Palestine

Prepared by:

Dua'a Shareef Muhammed Subhi Fakhouri

B.Sc. in Medical Laboratory Sciences in Hebron University/ Palestine

Supervisor: Dr. Suheir Ereqat

Cosupervisor: Dr. Abdelmajeed Nasereddin

A Thesis Submitted in Partial Fulfillment of Requirements for the Degree of Master of Biochemistry and Molecular Biology/ Faculty of medicine -Al-Quds-University

1446/ 2025

Deanship of Graduated Studies

Al- Quds University

Thesis Approval

Association of OCT1, OCT2 and OCT3 gene polymorphisms with Type 2 Diabetes Mellitus risk in Palestine

Prepared by: Dua'a Shareef Muhammed Subhi Fakhouri

Registration No.: 22212383

Supervisor: Dr. Suheir Ereqat

Cosupervisor: Dr. Abdelmajeed Nassereddin

Master thesis submitted and accepted 8.1.2025

The names and signature of the examining committee member are as follows:

1. Head of committee: Dr. Suheir Ereqat
2. Internal examiner: Dr. Afnan Atallah
3. External examiner: Dr. Amjad Hussein

Jerusalem/ Palestine

1446/ 2025

Dedication:

I dedicate this thesis to my parents, whose believing in my potential

To my son, the sunshine of my life, to my supportive husband.

I also wish to extend my deepest gratitude to my supervisors, especially Dr. Suheir Ereqat, which contributed to the growth my knowledge, and your distinctions motivate me to develop and move forward.

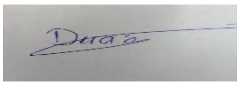
Lastly, to all researchers who work hardly to solve the complications of human health, particularly those striving to combat the global challenging diabetes mellitus type 2, I dedicate this thesis as a little contribution to our shared mission of making power advancements in science and medicine.

With heartfelt gratitude

Dua'a Shareef Muhammed Subhi Fakhouri

Declaration

I certify that this thesis submitted for the degree of master, is the result of my own research, except where otherwise acknowledged, and that this study or any part therefore has not been submitted for a higher to any other university or institution.

Signature: 

Dua'a Shareef Fakhouri

Date: 8.1.2025

Acknowledgments

I am deeply grateful to my supervisor Dr Suheir Ereqat, for skillful help and support. Special thank go to Dr. Abdelamjeed nasereddin, my co-supervisor for his support and help.

My thanks also go to my teachers and all the staff in the department of Biochemistry and Molecular Biology in Al-Quds University, whose resource and facilities made this study possible

Abstract

Type 2 diabetes mellites is a chronic condition when the body fail to balance blood glucose levels due to insulin are not secreted or not doing effectively its job, the insulin is considered as a key anabolic hormone which regulate carbohydrate, protein and fat metabolisms, so any defect in insulin actions or secretion lead to metabolic disorders such as Type 2 Diabetes. In addition, chronic blood glucose elevation can lead to serious complication which cause death. Worldwide, type2 diabetes accounted 95% of all diabetic cases, in average 15.3% of Palestinian individuals are affected according to last published reports. To date, there are many contributing agents including, one or combination of both environmental and genetic factors. A lot of genes may be implicated in onset and progression of type2 diabetes. Solute Carrier transporters22A (1,2 and 3) are belong to these genes, which are responsible of nutrient uptake, regulate ion gradient across membrane, drug metabolism such as metformin.

The study is case control study, the data collection was done in 2024, June- September. The present study cosiest of two individual groups includes type 2 diabetic patients as case and healthy individuals as control. A total of 240 diabetic participants were included in this study 97 healthy participants. All patients aged over 40 years who attending Jericho Health MOH Center and/or AL-Ahili health care center, Palestine. The HbA1c, FBS, lipid profile was performed during the hospital admission examination. Four SNPs, OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) gene polymorphisms in T2DM cases and healthy controls were identified by amplicon based next generation sequencing (NGS), it is the first investigation to be held on Palestinian population.

The frequencies of these polymorphisms were assessed in the studied population (Table 3.5). Moreover, the associations of OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) gene polymorphisms in T2DM cases and healthy controls were investigated. Our results showed that there was no significant association between the genotype in all variants between Case and Control P- value > 0.05 (Table 3.5). There was a significant relationship between gender of T2DM patients compared to those in control group (P=0.000), also significant relationship in the mean age among case and control groups was observed (P = 0.0001) The higher proportion of females in the diabetic group (137 females vs. 23 in controls) suggests a notable gender difference in diabetic prevalence within this specific study populations, show a significant difference in systolic blood pressure P-

value = 0.001, also the results show diastolic blood pressure significant differences between case and control with P- value is 0.002 as it presents in higher amounts in non- diabetes individuals. In addition, significant differences between 2 groups in HbA1c and FBS with P-vale = 0.0001 and the higher value were present in diabetic patents. However, BMI, Cholesterol, TG, LDL and HDL showed no significant differences between diabetic and healthy groups P- value> 0.05.

In conclusion, association between of OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) gene polymorphisms and type 2 diabetes mellitus diseases are varied among different ethnic groups. Our study showed no association between these variants and type 2 diabetic patients in the Palestinian population. Further studies should be done on larger sample to confirm the study results.

Content

List of tables

List of figures

List of appendices

List of abbreviations

Abbreviation	Term
A	Adenine
AMPK	AMP-activated protein kinase
BMI	Body Mass Indices
DBP	Diastolic blood pressure
DM	Diabetes Mellitus
DNA	Deoxyribonucleic acid
EDTA	Ethylene diaminetetraacetic acid
FBS	Fasting Blood Sugar
G	Guanin
GDM	Gestational Diabetes Mellitus
GLUT-4	Glucose transporter type 4
GSIS	Glucose Stimulated Insulin Secretion
GWAS	Genomic wide association studies
HbA1c	Glycosylated hemoglobin
HDL	High density lipoprotein
IR	Insulin Resistant
LDL	Low density lipoprotein
M±SD	Mean±standard deviation
MAF	Minor Allele Frequency
MASLD	Metabolic Associated Steatosis Liver Disease
MDS	Metabolic Dysfunction Syndrome
ND-1000	nanodrop spectrophotometer 1000
NGS	Next Generation Sequencing
NSFA	Non-Stratified Fatty Acids
OCTs	Organic Cation Transporters
OGTT	Oral Glucose Tolerance Test
PCR	Polymerase Chaine Reaction
PI3K	Phosphatidylinositol-3-kinase

RBS	Random Blood Sugar
RTK	Receptor Tyrosine Kinase
SBP	Systolic blood pressure
SLC22A	Solute carrier transporter
SNPs	Single Nucleotide Polymorphisms
SPSS	Statistical product and service solutions
T1DM	Type 1Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TG	Triglyceride level
UV	Ultraviolet light
WHO	Word Health Organizations
X^2	Chi square
β	Beta

Chapter1.

---

1. Introduction:

1.1 Diabetes Mellitus:

Diabetes Mellitus (DM) is a serious and chronic metabolic disorders marked by sustained elevated blood sugar with interruption in carbohydrate, fat and protein metabolism. This arises from either insulin insufficient production by pancreas or the inability of target tissue like (the skeletal muscles, liver, and adipose tissue) to effectively utilize insulin secretion or action or both, this is because the insulin act as anabolic hormone regulation the metabolism of carbohydrate, portions and fat and distortion in its functions lead to metabolic imbalance (Antar et al., 2023). These abnormalities, which represented by hyperglycaemia is accounted for many symptoms significantly reducing the overall quality of life such as polyuria, weight loss, polyphagia, susceptibility to infection and blurred vision beside the acute life-threating complications is ketoacidosis in absence diabetes therapy (Li & Stocum, 2013)

DM is a global health concern combine with highly mortality and morbidity, so that can across all ages, regions, and genders. Type 2 Diabetes which contribute of 90% of all diabetic cases, arise from a combination of hereditary and environmental influences that collectively play important role in its development (The Lancet, 2023), when DM is undiagnosed or poorly managed which may lead to sustained chronic hyperglycaemia, this condition is closely linked to serious complications and this issue can lead to irreversible damage to critical organs such as the kidney glomeruli, nervous system, heart and blood vessels (Baynest, 2015). According to Health Economic Benefits of Diabetes Interventions, keeping blood glucose levels under control by effective management able to reduce risk of diabetic complications by 40%.

1.2 Types of Diabetes Mellitus

Diabetes many-sided syndrome not a single disease distinctive by enduring hyperglycaemia. With time it can lead to serious complications containing damage to the nerves, eyes, and kidneys, in additional of increased risk of cardiovascular problems affecting on medium and large blood vessels. The disease is categorized into three common types: type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM) and, gestational diabetes, in the other hand secondary or other specific types of diabetes may expanded to include known of over fifty distinct subtypes each happen by diverse pathogenic mechanisms or accompanying other syndromes or diseases (Genuth et al., 2021).

1.2.1 Type 1 Diabetes Mellites (T1DM)

Type 1 Diabetes Mellitus is one of common type of diabetes represented by long time hyperglycaemia occurs from the autoimmune illnesses ruin of the beta cells in the pancreas, which restrict the body from producing insulin. The insulin is a necessary anabolic hormone that has an abundance of action of glucose, protein, lipids, and growth in addition to mineral metabolisms. As a result, T1DM demonstrates as a systemic disorder, genetic and environmental factors can participate in its phenotypic illness appearances while also is frequently linked to the presence of other types of autoimmune diseases which anti-islet antibodies typically emerge before to the phenotypic onset of the disease performing as early markers for its development (Popoviciu et al., 2023).

1.2.2 Type 2 Diabetes Mellitus (T2DM)

Type 2 Diabetes Mellitus (T2DM) is one of the most a prevalent metabolic disorder globally characterized by chronic elevated blood sugar, and its appearance and the biological process through which its development is basically driven by one or both of these factors: reduce responsiveness of insulin-sensitive tissue to insulin and impaired insulin secretion from pancreatic β-cells (Galicia-Garcia et al., 2020), this etiological heterogeneity causes of T2DM contribute to significant alteration in patient phenotype, like as the age onset of the illness, the complication presents, also the effectiveness of treatment approaches response. Despite environmental and lifestyle are contributing risk factors, heritability studies the rate to be around 69% between individual 35- 60 years (Suzuki et al., 2024).

1.2.3 Gestational Diabetes Mellitus

Gestational Diabetes Mellitus (GDM) is defined as hyperglycemia that is initial diagnosed in the time of pregnancy, which is the major current short and long-term medical complications of pregnancy including hypertension, pre-eclampsia, macrosomia, undergoing caesarian delivery, in addition, increased likelihood of cardiovascular problems, obesity, and defect in glucose metabolisms resulting in T2DM in infant and mother. GDM affects the rate to be around 15% of pregnancies globally, which represents make up account 18 million birth yearly. The rising rate of GDM not only burden on health outcomes, but also forces economic burdens worth awareness and proactive measures (Choudhury & Devi Rajeswari, 2021; Modzelewski et al., 2022).

1.2.4 Other Specific types of Diabetes

Other specific causes can lead to expression diabetes, such as monogenic diabetes syndromes Including: maturity-onset diabetes of the young, neonatal diabetes, in addition to the condition impacting the exocrine pancreas like pancreatitis and cystic fibrosis, also drugs and chemical induced diabetes including those associated with post-organ transplantation, glucocorticoids and AIDS treatment (Elsayed et al., 2022).

1.3 Prevalence of Diabetes Mellitus

In 2022, about 830 million people were living with diabetes mellitus (14% of whom are adults), more than 95% of them with T2DM, these numbers of diabetes patients are growing rapidly specially in low-income countries verses high income countries, according to Word Health Organizations (WHO); mortality rates resulted from diabetes and its complications is increasing dramatically.

In Palestinian populations, the prevalence of DM is estimated at 15.3 according to International Diabetes Federation published reports, it is a significant high number compared to a worldwide average of 6%. On the other hand, anecdotal evidence from multi-source proposes the reality rate might be greater ranging between 18%- 21%, T2DM constitutes about 95% between Palestinian patients (Palestine National Diabetes Programme, Palestine, 2024). A total of 5466 cases of diabetes were reported in 2023 with a prevalence rate of 186 per 100000, in addition, mortality resulted from diabetes and related complications in Palestine around for 16.3%, ranking third after cardiovascular diseases and cancer (Palestine Health Annual Report 2021 | GHDx, 2021).

1.4 Risk factors of Diabetes

The onset of DM disease is related to many risk factors, foremost genetic predisposition, environmental factors, loss of the initial phase of insulin secretions, smoking, alcoholic consumption, a sedentary lifestyle, physical exercise inactive, obesity, reduced β-cell sensitivity, dyslipidemia, elevated glucagon activity and hyperinsulinemia, these factors together contribute to prediabetes and diabetes by playing a significant role in in insulin non-functionality lead to disease prognosis (Alam et al., 2021).

1.5 Diagnosis of Diabetes Mellitus

Uncontrolled DM can cause significant health complications impact many organs in the body, also can lead to worth outcome if not managed properly. However, early diagnosis of DM can prevent these serious consequences and improve the quality of life (Olisah et al., 2022), many primary symptoms are an indicator of DM, include of persistently raised blood glucose, appetite, increased thirst and excessive urination. The diagnosis of DM or pre-diabetes there are commonly biochemical tests are implemented to screen DM, foremost the glycosylated hemoglobin (HbA1C) test, patient with DM have HbA1C level (≥6.5 DCCT%) , also the Oral Glucose Tolerance Test (OGTT) is another common test can be applied to diagnosed T2DM, the OGTT used to measure how the body cells can deal with 75 gram of glucose who individuals treated orally and after 2 hours of ingestion the plasma glucose levels measured, the individual diagnosed as diabetic if blood glucose levels were found (≥200 mg/dl) (Vijan, 2010), there is another reliable routine test to screen the DM such as Fasting Blood Glucose level and Random blood Glucose Level (Alam et al., 2021). The following table (1.1) summarizes the 2024 WHO cut-off value recommendation diabetes diagnosis.

Table 1.1: Diagnostic criteria according to WHO,2024 classification Glucose Tolerance Categories

Measurement	**Diagnostic cut-off value**
HbA1C	≥ 6.5% (48 mmol/mol)
Fasting Blood Sugar	≥7.0 mmol/L (126 mg/dl)
Random Blood Sugar	≥11.1 mmol/L (200mg/dL)
Oral Glucose Tolerance Test	≥11.1 mmol/L (200 mg/dL)

1.6 pathophysiology and signalling pathway

In the past, T2DM was believed as a disease only associated with malfunction in of the pancreas. However, at later time, several evidence have revealed additional factors contributing to the onset of the disease, it's a complex metabolic illness with incompletely understood mechanisms. In the other hand, at the core of T2DM pathophysiology are insulin resistant (IR) and dysfunction of pancreatic β cells, in addition to increasing in hepatic gluconeogenesis (Kesavadev et al., 2023), currently, with the huge progress in scientific research the understanding of the disease was gradually developed from early simplistic model to more holistic frameworks, the ominous octet introduced additional factors may relate to incretin dysfunction, inflammation, oxidative stress, endoplasmic reticulum stress, changed adipocytes metabolism, increased renal glucose reabsorption, elevated glucagon secretion, neurotransmitter abnormalities, in addition to central appetite dysregulation. later, this expanded to the ‘’ dirty dozne” including the role of vitamin D, testosterone, dopamine, and renin-angiotensin system, with the inclusion of the gut, the unlucky 13 further underlines that factors progressing T2DM are still being mysterious (Kesavadev et al., 2023; Lu et al., 2024a).

Factors could lead to insulin resistance and developing T2DM and the mechanism is represented in figure 1.1

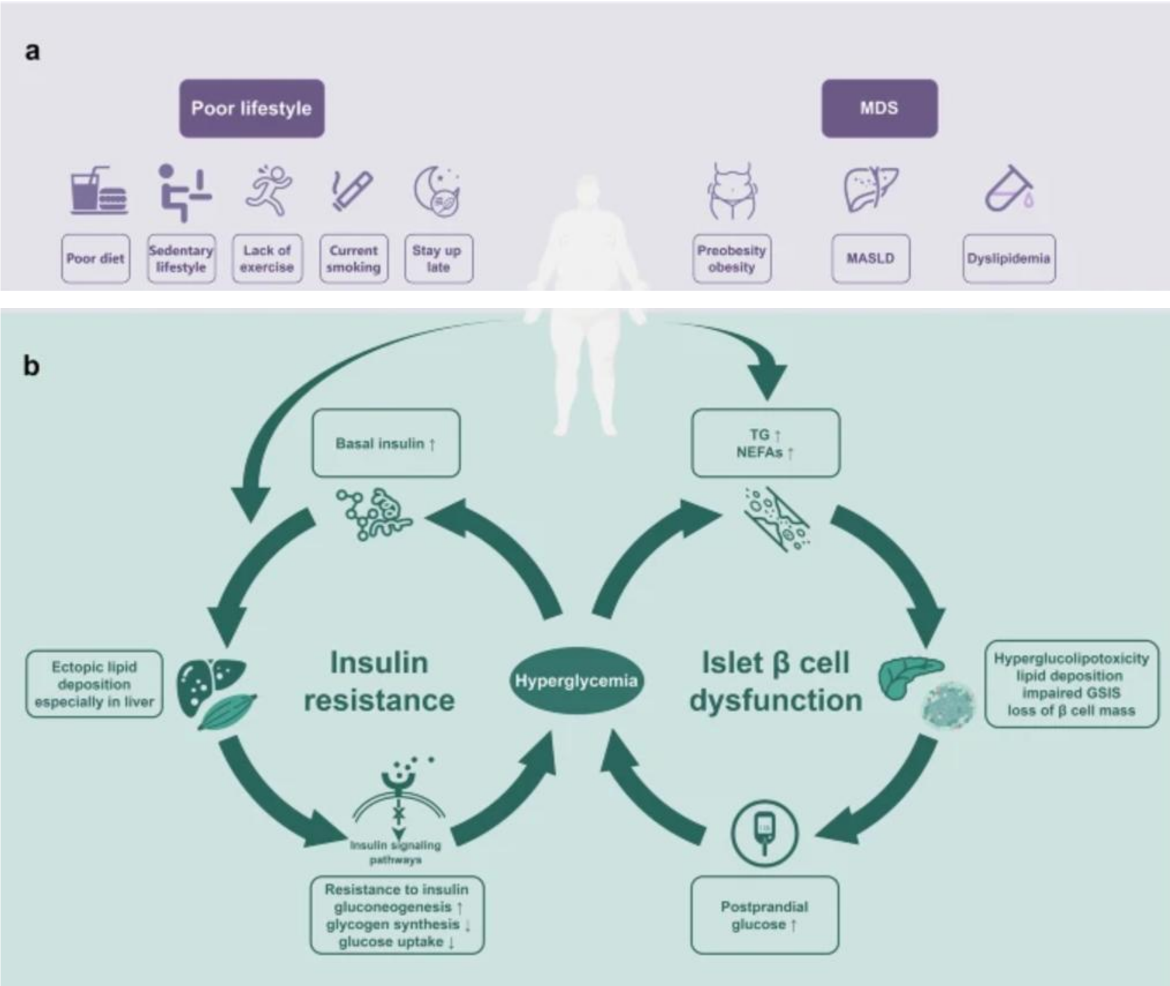


Figure 1.1: T2DM pathophysiology (Lu et al., 2024).

poor lifestyle and metabolic dysfunction syndrome (MDS) are the impulsive force behind the brutal cycle of hyperglycaemia causes increasing triglyceride levels (TG) and non-stratified fatty acids (**a**). Needless lipid is precipitated -abnormally- in non-adipose tissue such as muscles and liver, hinders insulin signalling pathway and participating in insulin resistant. This resistance for example in the liver increases glucose production (Gluconeogenesis) simultaneously decreases the uptake of glucose that way elevating blood glucose level and basal insulin levels, this rise in insulin in turn leads to promotes additional deposition of lipids and worsens insulin resistance and perpetuating this brutal cycle. Meanwhile, elevated in glucose levels and lipid causes hyper glucolipotoxicity a condition that motivates pancreatic β cells damage by lipid deposition within the islets. This affects both the functions and number of β cells, reducing glucose-stimulated insulin secretion (GSIS) in addition to induce hyperglycaemia (**b**). This cycle contributes to MDS, triglycerides (TG), non-stratified fatty acids, GSIS and metabolic associated steatosis liver disease (MASLD) (Lu et al., 2024; Ma et al., 2020; Savage et al., 2007).

1.7 Insulin Signalling Pathway

The action of the insulin hormone started when the insulin binds to its receptors on the surface of the target cells. Insulin receptors is a type II receptor tyrosine kinase (RTK) that plays important roles in metabolism, proliferation, and growth, consist of glycosylated disulfide-linked $\alpha_2\beta_2$ heterodimer also connect to insulin with strong affinity, thereby triggering activation of tyrosine kinase activity, upon activation, the insulin receptors make activation for a numerus intracellular substrate that initiates separate signal transduction pathways. Tyrosine phosphorylation of certain substrates trigger the activation of phosphatidylinositol-3-kinase (PI3K), yielding polyphosphoinositides that interact with protein kinases then activate the kinase Akt, as a result, activated Akt triggers the GLUT4 vesicles trance location from cytoplasm to the membrane then glucose uptake (Jaldin-Fincati et al., 2017). Likewise, Shc (Src homology and collagen) phosphorylation triggers the activation of the Ras/MAP kinase pathway. As well as SH2B2 and of Cbl phosphorylation activate G protein such as TC10. Akt activation by a lot of protein kinases which make phosphorylates of several substrates like GTPase-activating proteins, transcription factors and other kinases that control key metabolic pathway. In addition, insulin acting during vesicle trafficking, degradation insulin, transcription factors and regulating metabolic enzymes, all these mechanisms corresponded together to provide effectively glucose homeostasis (Saltiel, 2021).

1.8 Organic Cation Transporters

Organic cation transporters (OCTs) are mainly part of solute carrier 22 family (SLC22), which itself is considered a part of solute carrier (SLC) superfamily, which represents a large of membrane transporters including 65 different superfamily (SLC1- 65) with approximately 400 genes identified playing important roles in maintaining cellular homeostasis (Samodelov et al., 2020). SLCs are instrumental in orchestrating the regulation, intake and efflux of most of nutrients and molecules necessary for cell life across the membrane. In addition, they are a poly specific Na+ and PH independents facilitated diffusion transporters have a role in uptake and clearance a numerus physiological substance and xenobiotics by achieving vectorial transport in kidney, placenta and liver in mammalian (Hushmandi et al., 2024). Notably, they have been associated with numerus monogenic disorders (César-Razquin et al., 2015). Numerus studies have been published the structure and function of OCTs, confirming at last three main subtypes, including: OCT1, OCT2 and OCT3, the SLC22A1 encoding OCT1 and SLC22A2, SLC22A3 encoding OCT2, OCT3 respectively. SLC22A1 (contain seven exon), SLC22A2 (contain eleven exon) are located together on chromosome 6q26, while SLC22A3(contain eleven exon) is situated on chromosome 6q27 (Zazuli et al., 2020). Genomic location of the solute carrier 22A 1-3 genes and the number are in kilobase (kb) are showed in figure 1.2

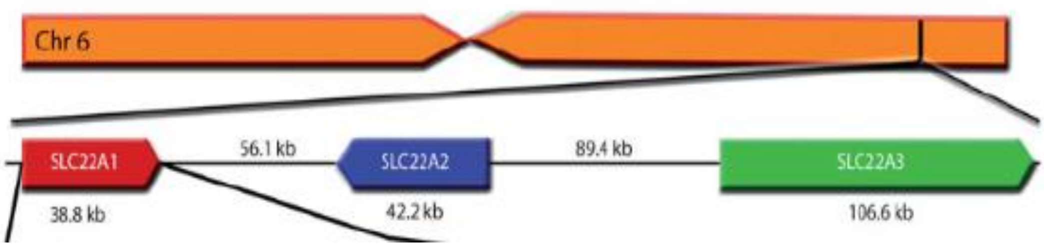


Figure 1.2 Chromosomal location of the solute carrier 22A 1-3

Chromosomal location of the solute carrier 22A 1-3 genes which encode Organic Cation Transporters 1,2 and 3 respectively, and showing numbers in kilobase (kb), that were derived from the GRCh38.p12 sequencing project found in the NCBI database (Brosseau & Ramotar, 2019).

1.8.1 Organic Cation Transporter 1

OCT1 is a key protein mainly expressed on the basolateral membrane of hepatocytes in human, its function mediates the uptake of cation compounds (Na + independent) to the liver for metabolism and excretions(Ciarimboli, 2021), and in rodents, it is present in the kidneys (Holle et al., 2011). OCT1 facilitate the transports both exogenous and endogenous origine substance, exogenous substance like the antidiabetic metformin and the anticholinergic trospium, while endogenous substance includes Acetylcholine and monoamine neurotransmitter (Wenge et al., 2011), OCT1 exhibit high expression in excretory organ and its activity might have profound in effect on both local and systemic level of its substrates (Ciarimboli, 2021). OCT1 is an important determent in the pharmacokinetics of various small molecule drug, like metformin, diltiazem and fenoterol. As a front-line anti-diabetic drug, metformin is well established substrates of OCT1(Kawoosa et al., 2022), so it is responsible for the hepatic uptake of metformin, In hepatocytes, OCT1 is the central way of metformin uptake due to its subsequent effect on the phosphorylation of T172 in the alpha subunit of AMP-activated protein kinase (AMPK), triggering the inhibition of the mitochondrial respiratory chain complex 1 also mG3PDH enzymatic activity, which conduct to occur anti-hyperglycemic effects, a reduction in hepatic gluconeogenesis (Madiraju et al., 2014). The effect of genetic polymorphisms in the OCT1 encoding gene in metformin response has been observed varying among different ethnic groups (Mato et al., 2018).

1.8.1.1 Organic Cation Transporter 1 polymorphisms

Single Nucleotide Polymorphisms (SNP) can modulate the expression, turnover, regulation also the substrate affinity of an uptake transporters can considerably alter the therapeutic efficiency in addition rises the risk of toxic side effects associated with specific drugs (Brosseau & Ramotar, 2019). The OCTs polymorphisms refer to the variation in genetic expression in SLC22A gene which encode the Organic Cation Transporter, abundant variants of OCT1 gene polymorphisms have been discovered, many of these decrease the transporters functions through modifying substrate binding site, decreasing transport rates or altering transport direction. In mammalian, OCT1 is a highly conserved amino acid residues specifically positively charged amino acids and cysteine. Most frequents OCT1 polymorphisms often involve alteration to these conserved amino acids residues (Grinfeld et al., 2013). The OCT1 rs628031 polymorphism (1222A>G) this polymorphism results in the substitution of the amino acid methionine with valine at position 408, it's a one of the most OCT1 polymorphisms have been studied, can influence in OCT1 functions also altering drugs response and side effects. Several studies showing various effects on glycaemic responses to metformin between ethnic groups (Aryastuti et al., 2022; Ghasan Abood Al-Ashoor et al., 2022; Mato, Guewo-Fokeng, Essop, et al., 2018).

1.8.2 Organic Cation Transporter 2

The OCT2 (SLC22A2) is a Na+ independent uniporter-symporter-antiporter family predominantly expression on the basolateral membrane of renal proximal tubule cells. It is having critical function in the initial phase of tubular secretions through moving various substrates from interstitial fluid into the proximal tubular cells. OCT2 transport a range of endogenous substances such as choline and its metabolites trimethylamine N-oxide along with neurotransmitter. It contributes to transport ranging of commonly drugs as metformin, cimetidine, oxaliplatin, cisplatin, gentamicin and fluorinated choline (Hörmann et al., 2020). Organic cation transporter 2 (OCT2) is responsible for 80% metformin clearance (Kashi et al., 2015).

1.8.2.1 Organic Cation Transporter 2 Polymorphism

Many SLC22A2 polymorphisms can changing the function of OCT2 transport and impact the glycaemic response to metformin in different populations have been discovered(Kashi et al., 2015). OCT2 rs145450955 (602 C>T) variant a substitution at 201 first amino acid position, that the threonine (T) is substituted by methionine (M) due to point mutation change from cytosine (C) to thiamin at nucleotide position 602. Various studies suggested that this loss of function variants impact in changes insulin resistant and functions of beta cells by decreasing OCT2 (Vmax) among T2DM patients who treated with metformin (Kashi et al., 2015).

1.8.3 Organic Cation Transporter 3

OCT3 gene symbol (SLC22A3) belong to high- capacity, low-affinity poly-specific transporters that operate as a bidirectional sodium independents carrier, compared to OCT1 and 2 which are found in liver and kidney, the OCT3 is broader expression, which is expressed in various tissue heart, brain, liver and kidney, facilitating cellular uptake cation xenobiotics across different tissue. Furthermore, regulating and the extracellular balance monoamine neurotransmitters including dopamine, serotonin and Norepinephrine that interacts with own specific receptors to promote signaling pathway important in physiological prosses in addition, impacting in various pathophysiology (Khanppnavar et al., 2022; Kuseyri Hübschmann et al., 2021).

1.8.3.1 Organic Cation Transporter3 polymorphism

In OCT3 gene symbol (SLC22A3) many SNPs was discovered, the OCT3 (rs3088442) SNPs is located on 3′-untranslated regions has been related with several clinically outcomes specifically in T2DM, this variant is (G>A) can affects gene expression through interacting with mRNA binding sites, in turn, it affects metformin drug response between ethnic groups (Moeez et al., 2019). The rs2292334(G>A) is another variant of OCT3, where guanin(G) is replaced by adenine (A), many study evidences about potential associations with T2DM, it may influence in uptake and regulation of cation substances like catecholamines and drugs such as metformin (Mahrooz et al., 2017).

1.9 Metformin pharmacokinetics

Metformin, or known as dimethylbiguanide was first synthesized in the 1920s. It keeps the glucose-lowering properties of its origin compound, guanidine, with significantly reduced toxicity. Initially applied in Europe, metformin was later approved by the FDA in 1994 for the treatment of T2DM in the United States. At this time, it is the most widely prescribed oral anti-diabetic medication worldwide, with over 150 million users annually. In addition to its role in managing diabetes, metformin has obtained attention for its potential anti-cancer effects (He & Wondisford, 2015).

Metformin is not metabolized in humans and animals and is eliminated unchanged through renal excretion in urine. The highest approved daily metformin dose for treating DM is 2.5 g (approximately 35 mg/kg of body weight). After oral administration, metformin is absorbed into intestinal enterocytes by the plasma monoamine transporter (PMAT) and organic cation transporter 3 (OCT3) on the apical membrane. It goes out the intestinal enterocytes via OCT1 on the basolateral membrane (Higgins et al., 2012). Once absorbed, metformin is uptake directly to the liver through the portal vein, where its plasma concentrations range between 40 and 70 µM following a therapeutic dose. In the liver, metformin uptake occurs via OCT1 and OCT3 on the hepatocyte membranes. However, it is exit from hepatocytes by the multidrug and toxin extrusion 1 (MATE1) transporter. The rate of metformin uptake in primary hepatocytes follows a curvilinear pattern, with a Km of approximately 220 µM. This Km is 3 to 5 times higher than the concentrations of metformin in the portal vein (Jin et al., 2009). After hepatic uptake, systemic plasma concentrations of metformin decrease to 10–40 µM in both animals and humans (He & Wondisford, 2015).

In the kidney, metformin is absorbed from the bloodstream into renal epithelial cells by OCT2 and excreted into the urine via MATE1 also MATE2k (Gong et al., 2012). Metformin pharmacokinetics present in figure 1.3.

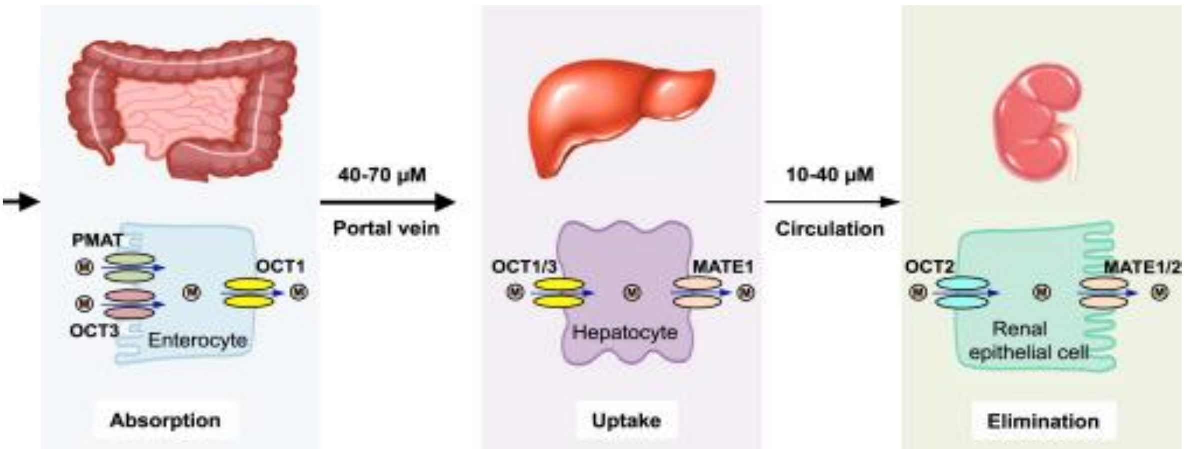


Figure 1.3: Metformin pharmacokinetics (He & Wondisford, 2015)

After oral intake, metformin is absorbed through intestinal enterocytes and transported directly to the liver by the portal vein, where its concentration ranges between 40 to 70 µM.

Metformin is not metabolized in hepatocytes and is excreted unchanged by the kidneys into urine. Also, metformin can be secreted into bile by the MATE1 transporter located on hepatocytes.

1.10 Objectives of the study

The association between genetic variation in Organic Cation Transporters 1, 2 and 3 (OCT1, OCT2 and OCT3) gene in T2DM remains inadequately explored, leaving significant gaps in current understandings. Therefore, the aim of this study is to determine the genotype and allele frequencies of OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) gene polymorphisms and detect their association with T2DM risk in Palestine.

Specific Objectives:

1. To use amplicon based next generation sequencing assay for identifications of the OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) gene polymorphisms.

2. To determine the genotype and allele frequencies of OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) gene polymorphisms in T2DM cases and healthy controls.
3. To assess the association of OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) gene polymorphisms with glycemic control and clinical/biochemical Parameters for T2DM Patients.

1.11 Literature review

The gene family of SLCA22 encoded over the 30 proteins and consist of 11 exons having important roles in the drugs and toxin metabolites eliminations across liver, kidney and other tissue. Accordingly, genetic variation in SLC22A genes could be significant modifiers of individual response to antidiabetic medications (Hakooz et al., 2017), also, the four SNPs most significant among included genes OCT1 *(*rs628031), OCT2 (rs145450955)*,* and OCT3 (rs3088442 and rs2292334) gene polymorphisms (Ghasan Abood Al-Ashoor et al., 2021; Mahrooz et al., 2017). Several studies have explored the association of OCT1, OCT2 and OCT3 with T2DM related to variability in glycemic responses, metformin therapy response additionally, risk and development of T2D between ethnic groups(Mato, Guewo-Fokeng, Faadiel Essop, et al., 2018; Peng et al., 2023). Some studies have been done on OCTs genes polymorphisms in the Arab papulations (Alawadhi, 2013; Al-Eitan et al., 2019; Altall et al., 2019).

A systemic review meta-analysis study from multiple databases aimed to clarify the role of OCTs polymorphisms in metformin glycaemic response and reverse effects across various populations. This study analysed 30 suitable studies containing 2,791 T2DM patients, the OCT1 *(*rs628031) polymorphism correlated with FBS reduction in specific genotypes (GG VS. AA; SMD = -0.60, P = 0.007) also no significant association was observed for other OCTs polymorphism regarding metformin response or intolerance (Peng et al., 2023).

Another study evaluated the effect of specific genetic polymorphism on the effectiveness of metformin therapy in T2DM Iranian patients, this study conducted 140 patients' treatment with metformin, focusing on OCT1 *(*rs628031). The result of this study, this polymorphism showed no significant association with therapeutic response to metformin (Singh et al., 2023). In addition, study conducted in AL-KIWAT showed the same result which is no significant association between this polymorphism and metformin response (P = 0.3) (Alawadhi, 2013).

Two other studies conducted among Chinese population, showed the loss of function variants in OCT1 rs628031 (M480V) reduce the clearance of metformin and imatinib (Qiu et al., 2018; Tarasova et al., 2012).

Furthermore, study among Iranian population, examined the impact OCT2 (rs145450955) (602 C>T) polymorphism on insulin resistant in T2DM patents and who has metformin treatment, showing that the patient with minor allele had higher HbA1c, FBS and HOMA-IR (P = 0.019), (P = 0.023) and (P = 0.03) respectively (Kashi et al., 2015).

A study in Jordan conducted by Al-Eitan et al. (2019), examined the effect of main genetic polymorphisms of organic cation transporters 1,2 and 3 on the pharmacokinetics of metformin, glycaemic control and HbA1c in Jordanians. This study involved 212 patients with T2DM, genotyping was determined for OCT1, OCT2 and OCT3 polymorphisms, there is no statistical differences between OCT3 rs2292334 and glycemic control (P-value = 0.2) (Al-Eitan et al., 2019).

A case control study was performed with 150 T2DM and 152 control in Indian population, this study aimed to investigate the relationship OCT3 variants and T2DM, focusing on (rs3088442 and rs2292334) variants and how these 2 variants interact and affect the development of T2DM. Study result the OCT3 rs3088442 G>A polymorphism was protective against T2DM. However, another variants OCT3 rs2292334 G>A was associated with increased risk of T2DM (Mahrooz et al., 2017).

Genetic analysis investigations have shown many single nucleotide polymorphisms (SNPs) in the three genes that may have an impact on the variation in pharmacokinetics between individuals (Du Plessis et al., 2015), as polymorphism of OCT1-SLC22A1(rs628031), OCT2-SLC22A2 (rs145450955), OCT3-SLC22A3 (rs3088442 and rs2292334) may showed significant association in metformin response (Peng et al., 2023). Moreover, Significant associations between G/G and A/G genotypes of rs628031 and rs461473 variants of SLC22A1 and high levels of HbA1c were detected in T2DM patients from Saudi Arabia (Altall et al., 2019).

Numerous research have also revealed conflicting findings regarding the link between OCT1, OCT2, and OCT3 gene variations and diabetes in different ethnic groups, suggesting that a susceptibility gene may present a varied phenotype across populations or areas (Kawoosa et al., 2022; Mato, Guewo-Fokeng, Faadiel Essop, et al., 2018; Peng et al., 2023b; Rizvi & Tasleem Raza, 2023). In Palestine, there is a lack of information regarding the OCT gene variants linked to type 2 diabetes. In order to determine the genetic variance of the OCT1, OCT2, and OCT3 genes and their correlation with T2DM in Palestine, this study were conducted. This work also advances our knowledge of other potential T2DM-causing factors, such as phenotypic traits, which may have a substantial influence on future individual T2DM medical treatment.

Chapter2.

---

2. Material and Methods

2.1 Study population

Our study included 337 Palestinian participants, comprising 240 case (T2DM patients) and 97 control (non-diabetic individuals). Both groups were aged over 40 years at the time of sampling, who attenuating Jericho Health MOH Center or from AL-Ahili health care center, Palestine.

2.2 Study design and Eligibility criteria

A case control study design was employed to assess the frequency of OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) gene polymorphisms in T2DM patients. Our study participant were unrelated people, recruited within the period from June to September 2024

Inclusion Criteria:

- For cases, Palestinian patients aged 40 years or older with confirmed T2DM, diagnosed according to WHO criteria and under current treatment with metformin and/or other T2DM drugs. For controls, Palestinian individuals aged 40 or older without T2DM and any history of chronic or acute diseases (cardiovascular disease and metabolic syndrome).

Exclusion Criteria:

- Age: Participants under 40 years old were excluded.
- Type 1 Diabetes Mellitus: Individuals with a confirmed diagnosis of Type 1 Diabetes Mellitus were excluded from the study.
- Inadequate Medical Records: Patients with incomplete or unavailable medical records, including missing demographic, clinical, or biochemical data, were excluded.
- Any history of chronic or acute diseases (T1DM, cardiovascular disease and metabolic syndrome).

2.3 Study investigations

2.3.1 Data Collections and biochemical analysis

Demographic and clinical data, including age, gender, BMI, treatment regimens, and diabetic complications, were obtained from medical records. Biochemical measurements (FBG, HbA1c) analyzed using the ARCHITECT C4000 instrument. Genetic analysis of OCT1, OCT2 and OCT3 genes polymorphisms conducted through PCR and sequencing.

2.3.2 Blood sample

Around 5 ml venous blood was drawn from all study participants, under strict quality control and safety procedures. Two milliliters of the collected blood were placed into sterile ethylene diamine tetra acetic acid (EDTA) tubes used for the determination of hemoglobinA1c (HbA1c) and consequent SNPs genotyping. The residual three milliliters were carried in plain tubes and left uncentrifuged to allowing blood to clot. Then these tubes centrifuged at 3000 rpm for 10 minutes and the serum collected into fresh tubes. The obtained serum was used for analyzed FBS by ARCHITECT C4000 Instrument at AL-Ahli Health Laboratory and Jericho Health MOH Center. All these tests were part of routine diagnostic procedures, and the data extracted from the patient's medical record and laboratory tests.

2.4 Molecular analysis

2.4.1 DNA Extraction

Genomic DNAs were extracted from whole blood using Promega Blood kit as manufactures directions.

Representative DNA samples concentration was measured by nanodrop spectrophotometer 1000 (ND-1000) in addition, the 260/280, 260/230 were determined.

2.4.2 Primer's selection

Primer was designed to target the OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334), the process started by searching the NCBI database (https://www.ncbi.nlm.nih.gov/snp) to retrieve the SLC22A1, SLC22A2 AND SLC22A3 genes sequence containing these four SNPs. The flanking region for each four SNPs were recognized, allowing the precise primer design by using online primer 3 software (https://primer3.ut.ee/). The primer was design to amplify DNA fragments of 150 bp and 250 bp in length for four SNPs, this will ensure the accurate amplification of the target region for subsequent analysis. The primer sequence forward and reverse and Expected PCR size/bp are shown in table 2.1

Table 2.1 the sequence of the primers used for PCR amplification of SLC22A1, SLC22A2 and SLC22A3 genes.

Gene	SNP	Forward primer	Reverse prime	Expected PCR size/bp
OCT1	rs628031	GCCTTCATAGCCCTCATCAC	ACACTTTCCCCACACTTCGA	244
OCT2	rs145450955	AGCCTATTAACCAGCCTGCT	GCTATCAGTCTGTGCCTCCT	363
OCT3	rs3088442	TGTTCTCTGCTCACCTTGGT	ATCTGTGGCTCTAGGTGTCC	317
	rs2292334	TTTTCATCTCGGGCGTGGT	CCTAAGAACAAGTGGCTCCC	303

All primers will be modified with over hanged Illumina adapter sequences at the 5′ ends of the forward (5′-CGTCGGCAGCGTCAGATGTGTATAAGAGACA-3′) and reverse primers (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGA-3′), so they will attach the machine flow cell for sequencing.

2.4.3 DNA amplification

The DNA library was done by using two stage PCRs. The 1st stage PCR assay was carried out using 2 μL of DNA sample, 0.3 μL of forward and reverse primers (10 pmol each), 12.5 μL of PCR master mix (PCRBIO HS Taq Mix Red), and 9.4 μL of double distilled water. The PCR amplification condition done as followed: initial denaturation of DNA at 95°C for 5 min, followed by 28 cycles of denaturation at 95°C for 30 s, an annealing at 59°C for 30 s, and extension at 72°C for 30 s and final extension at 72°C for 6 min. All PCR products was cleaned using the Agencourt AMPure XP system (X1, cat. no. A63881; Beckman Coulter Genomics) and subjected to a second round of amplification (2nd stage PCR) for barcoding using Nextera XT Index Kit (Illumina) for samples barcoding. Then they were pooled and cleaned in one tube using the Agencourt AMPure XP system. Prepared samples were sequenced on a NextSeq 500/550 machine using the 150-cycle Mid Output Kit (Illumina, Inc.).

2.4.4 Gel Electrophoresis

The PCR products were subsequently loaded on a 2% agarose gel four bands Crosseponding to DNA fragments for the OCT1, OCT2 and OCT3 genes were observed, indicating successful amplification. For the 2% agarose gel preparation, approximately 2g of agarose was put to 100 ml Tris-acetate-EDTA (TAE) buffer, after that the mixture was boiled for 2 minutes in microwave, after cooling to room temperature, the addition of 2-3 drops (10mg/ml) ethidium bromide. The ready mixture was transferred into an agarose gel casting system (Bio Rad, SUBCELL®GT) for solidification. Posteriorly, 5 μL of every PCR product into the well of the 2% soiled agarose gel. Electrophoresis was conducted for 45 minutes at

120 °C, then the amplified DNA fragments was observed on gel documentation system (GelDoc).

2.5 Bioinformatics Analysis

The DNA sequences was collected via Next Generation Sequencing (NGS) were analyzed and processed by the Galaxy platform (https://usegalaxy.eu/). Four SNPs were successfully identified using single reacted tube within NGS approach, the raw sequence data available as Fast-Q files were striped default parameters of the platform to insure only the reads of highest quality were returned. Thereafter, the filtered sequence was then mapped to their appointed SNPs by using virtual probes designed for their detections. The step-by-step workflow identification and detections is presented in figure 2.1.

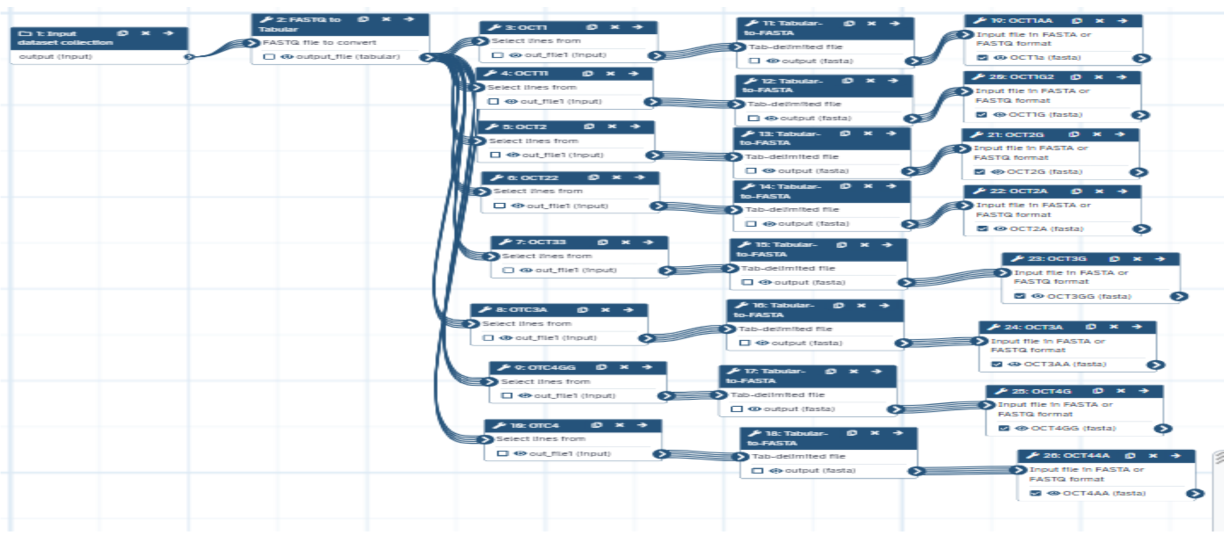


Figure 2.1 NGS Workflow

The workflow pipelines available on galaxy platform ((https://usegalaxy.org.au) tool up for overall approach to analyzing Fast Q files from NGs.

2.6 Statistical Analysis

All of statistical data was analyzed using IBM SPSS software (version 27.0, SPSS Inc., Chicago, USA). Categorical variables were compared by the chi-square test, that the normal continuous variables analyzed through the student's t-test and one-way analysis of variance

(ANOVA). Logistic regression analysis was performed to determine the association between OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) gene polymorphisms with glycemic control in addition.

Chapter3.

---

3. Results

3.1 Biochimical and Demographic characteristics of the study groups

A total of 337 Palestinian participants were recruited between Jun to September 2024 from the Jericho Health MOH Center and/or from AL-Ahili health care center, Palestine. 240 of them are case (T2DM) and 97 control (without T2DM). the female to male ratio in diabetic group (137: 103) VS. (23:74) in control. Also, there was significant relationship between gender of T2DM patients compared to those in control group (P=0.000), also significant relationship in the mean age among case and control groups was observed (P = 0.0001) The higher proportion of females in the diabetic group (137 females vs. 23 in controls) suggests a notable gender difference in diabetic prevalence within this specific study populations. The mean age of diabetic patients (59.76 years) is significantly higher compared to those in controls (53.83 years), as shown in table 3.1.

Table 3.1: the Number and age of the study participants

Variable	Type 2 diabetic Patients n=240	Non-diabetic Controls n=97	Total	*P*-value
Gender				
Female	137	23	160	**.0001**
Male	103	74	177	
Total	240	97	337	
Age (Mean ±SD)	59.76 ± 10.521	53.83 ± 12.63		**.0001**
P-value < 0.05 is considered to be significant, (obtained by $\chi 2 -$ test for categorical variables).				

The table below (3.2) shows a comparison of biochemical and clinical characteristics between case and control groups, the results show that systolic blood pressure was higher in case group compared to control group (P- value = 0.001) whole the diastolic blood pressure was higher in control group compared to cases with P- value is 0.002. In addition, significant differences in the mean values of HbA1c and FBS were higher (P-vale = 0.0001) in diabetic patients. However, BMI, Cholesterol, TG, LDL and HDL showed no significant differences between diabetic and healthy groups P- value> 0.05.

Table 3.2: Biochemical and chemical characteristics mean value comparison between case and control groups

Variable	**Type 2 diabetic Patients n=240**	**Non-diabetic Controls n=97**	***P*-value**
	Mean (± SD)		
BMI	31.3± 6.3	30.5 ± 16.2	0.516
Systolic blood pressure (SBP)	136.421 ± 22.297	128.402 ± 14.8448	**0.001**
Diastolic blood pressure (DBP)	75.171 ± 15.388	80.722± 13.451	**0.002**
HbA1C	7.995± 1.971	5.437 ± 0.4398	**0.0001**
Cholesterol	169.271 ± 38.73	172.70± 43.806	0.479
TG	148.01 ± 109.66	173.737 ± 134.217	0.069
LDL	100.563 ± 33.239	112.503 ± 40.5016	0.005
HDL	42.21 ± 11.316	40.46 ± 17.773	0.282
FBS	174.137+ 79.310	83.631 ± 9.527	**0.0001**
P < 0.05 was considered significant (from t − tests), data are shown as mean ± standard deviation.			

3.1.1 Purity and concentration amusement of DNA samples

DNA concentration and purity were assessed by using the Nanodrop 1000 spectrophotometer. The Concentration values were given as ng/µl. However, purity was assessed by the 260/280 and 260/230 absorbance ratios. The samples analyzed given concentrations between 50 to 200 ng/µl, which reflecting valid DNA extraction. The 260/230 ratio considered an indicator of DNA sample purity, indicating ideal range between 2.0 and 2.2. Deviations from this range due to contaminants such as phenol that absorb at a wavelength of 230 nm, propose that the DNA extraction protocol couldn't have been properly done.

DNA purity can be evaluated by using 260/280 ratio because proteins commonly absorb at 280 nm. A ratio of around 1.8 is a good indication of pure DNA. Any variation from the approximate value of 1.8 suggests contamination with protein and can be corrected by the addition of proteinase K, which digests and thus removes the proteins. Table 3.3 shows the concentration and purity for 5 samples.

Table 3.3: Representative DNA sample concentration and purity for five random samples measured using nanodrop spectrophotometer 1000 (ND-1000).

DNA sample	ng/µl	260/280	260/230
1	162.28	1.98	1.23
2	56.95	2.50	1.71
3	44.67	1.84	1.92
4	35.95	1.99	1.07
5	107.14	1.03	1.66

3.1.2 Amplification of OCT1, OCT2 gene polymorphisms and OCT3 and Gel Electrophoresis

The PCR products appeared four distinct bands, approximately 240 - 370 bp in size, identical to the targeted fragments of the OCT1, OCT2, and OCT3 gene. Figure 3. shows representative samples visualized through agarose gel electrophoresis.

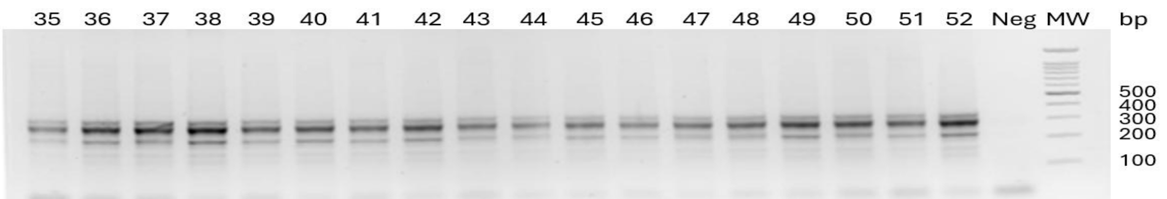


Figure 3.1 Agarose gel electrophoresis

Agarose gel electrophoresis results exhibiting Multiplex-PCR products of the OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) gene polymorphisms. Lane L represents the 100 bp DNA ladder, whilst –ve arrow the negative control. Visualized PCR products using the Tapestation machine and the High Sensitivity D100 kit (Agilent) provided by Macrogen.

3.1.3 Genotyping of OCT1, OCT2 and OCT3 variants by next generation sequencing Galaxy program

Genotypes were specified by accounting the input ratio of read counts for every allele in individual samples. as mentioned in table 3.4 and 3.4b

Table 3.4-A Genotyping of OCT1, OCT2 and OCT3 variants along with count reads analyzed Galaxy program.

Sample Numbers	Variants	G Allele	A Allele	ratio	Variant Genotype
1	**OCT1 (rs628031)**	383286	5044	76.0	GG
	OCT2 (rs145450955)	199474	236	0.001183	GG
	OCT3 (rs3088442)	126928	122158	1.0	GA
	OCT3 (rs2292334)	153725	152460	0.991771	GA
2	**OCT1 (rs628031)**	236445	240796	1.9	GA
	OCT2 (rs145450955)	164238	169	0.001029	GG

Table 3.4-B Genotyping of OCT1, OCT2 and OCT3 variants along with count reads analyzed Galaxy program.

	OCT3 (rs3088442)	227931	4516	0.0	GG
	OCT3 (rs2292334)	151511	152460	0.990951	GA
100	**OCT1 (rs628031)**	13936	13185	1.1	GA
	OCT2 (rs145450955)	11598	23	0.001983	GG
	OCT3 (rs3088442)	9919	8948	0.0	GA
	OCT3 (rs2292334)	13340	11210	0.84033	GA
150	**OCT1 (rs628031)**	9548	9842	1.0	GA
	OCT2 (rs145450955)	9490	16	0.001686	GG
	OCT3 (rs3088442)	6807	6285	0.9	GA
	OCT3 (rs2292334)	1451	15826	10.90696	AA

3.1.4 Multiple Sequence Alignment

The genotype findings were validated by applying multiple alignment for the reference genes using the MultAlin platform available online at http://multalin.toulouse.inra.fr/multalin/.

Sample Number	G	A	Ratio	Genotype
3	430595	6667	64.6	GG

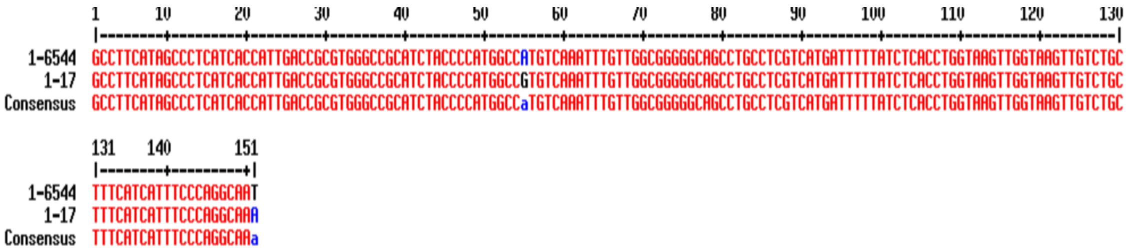


Figure 3.2 the Multiple Sequence Alignment for represented homozygous sample (3)

Sample number	G	A	Ratio	Genotype
37	9499	9701	1.0	GA

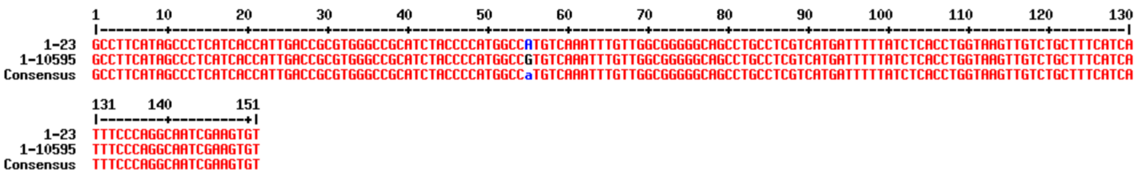


Figure 3.3 the Multiple Sequence Alignment for represented heterozygous sample (37)

Sample number	G	A	Ratio	Genotype
84	826	74536	0.0	AA

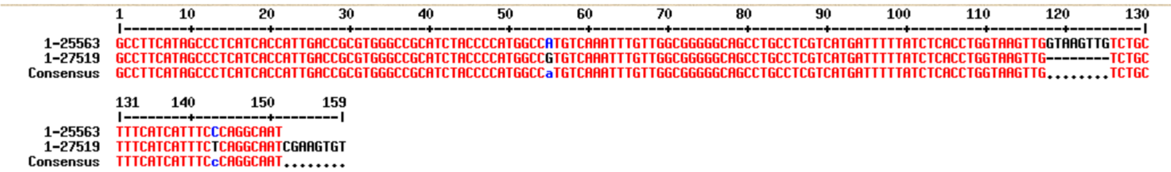


Figure 3.4 the Multiple Sequence Alignment for represented homozygous sample (84)

3.2.2.1 Distribution of OCT1, OCT2 and OCT3 genotype between T2DM and Control

All genotype distributions and Minor Allele Frequences (MAF) for all variants between cases and controls are shown in table 3.5, the genotype in four SNPs show no significant difference between the two groups case and control P- value > 0.05. In OCT1 rs628031 SNPs case shows a MAF of 0.204, which is slightly lower than controls of 0.235. While the MAF in OCT3 rs3088442 SNPs case higher than control (0.168 vs 0.113) respectively. In addition, the MAF of OCT3 rs2292334 SNPs in case of 0.295 higher than MAF in control at 0.202.

Table 3.5-A: genotype frequency for rs628031, rs145450955, rs3088442 and rs2292334 variants between case and control.

rs628031 SNPs		Case	Control	All Subjects	P-value
Genotype	**GG**	151 (74.9%)	52 (73.3%)	203 (73.3%)	.242
	GA	80 (39.6%)	39 (44.0%)	119 (44.0%)	
	AA	9 (4.5%)	6 (5.6%)	15 (5.6%)	
MAF		0.204	0.235		0.990

Table 3.5-B: genotype frequency for rs628031, rs145450955, rs3088442 and rs2292334 variants between case and control.

rs145450955 SNPs		**Case**	**Control**	**All Subjects**	**P-value**
Genotype	**GG**	240 (100%)	97 (100%)	337 (100%)	
	GA	0	0	0	
	AA	0	0	0	
rs3088442 SNPs		**Case**	**Control**	**All Subjects**	**P-value**
Genotype	**GG**	176 (87.6%)	79 (84.9 %)	255 (85.3%)	.279
	GA	59 (28.9%)	17 (23.0%)	76 (27.5%)	
	AA	5 (2.5%)	1 (1.4%)	6 (2.2%)	
MAF		0.168	0.113		0.990
rs2292334 SNPs		**Case**	**Control**	**All Subjects**	**P-value**
Genotype	**GG**	133 (65.6%)	62 (61.2%)	195 (62.5%)	.213
	GA	94 (46.3%)	33 (44.6%)	127 (47.0%)	
	AA	13 (6.4%)	2 (2.7%)	15 (5.6%)	
MAF		0.295	0.202		0.982
P < 0.05 was considered significant					

Additional analysis was applied to determine if there is an association between rs628031, rs145450955, rs3088442 and rs2292334Variants and clinical/ biochemical parameters in patients with T2DM groups. The study data was stratified according to genotype within T2DM group. The finding for rs628031 variant, rs3088442 variant and rs2292334 variant are presented in table 3.6, 3.7 and 3.8, respectively. As 100% of cases and controls had the GG genotype of the rs145450955 variant, this SNP was excluded from any further analysis. However, our findings revealed no significant differences between rs628031, rs3088442 and rs2292334 variants and the mean values HbA1c, FBS, BMI, age, lipid profile, SBP and DBP.

Table 3.6: Differences between rs628031 Variant and age, BMI, blood pressure, FBS, lipid profile and HbA1C in T2DM.

Parameters	rs628031 SNPs			P-value
	GG	GA	AA	
Age	59.1	60.2	66.4	0.117
BMI	31.0	31.8	31.1	0.704
SBP	136.5	137.5	126.3	0.364
DBP	75.4	75.4	70.3	0.632
FBS	176.4	170.8	166.6	0.844
HbA1c	8.1	7.9	7.5	0.681
Cholesterol	167.9	171.5	172.0	0.787
TG	140.7	163.4	133.0	0.301
HDL	43.5	40.1	39.7	0.084
LDL	100.6	100.6	99.0	0.990

Table3.7: Differences between rs3088442 Variant and age, BMI, blood pressure, FBS, lipid profile and HbA1C in T2DM.

Parameters	rs3088442 SNPs			P-value
	GG	GA	AA	
Age	59.3	60.7	66.8	0.665
BMI	31.1	31.6	33.4	0.211
SBP	135.9	137.5	142.0	0.763
DBP	75.3	74.9	73.0	0.932
FBS	177.5	164.9	164.6	0.559
HbA1c	8.0	7.9	7.4	0.758
Cholesterol	171.1	165.5	150.4	0.349
TG	152.8	138.0	97.4	0.390
HDL	42.3	42.2	40.7	0.953
LDL	101.6	98.5	86.8	0.532

Table3.8 : Differences between rs2292334 Variant and age, BMI, blood pressure, FBS, lipid profile and HbA1C in T2DM.

Parameters	rs2292334 SNPs			P-value
	GG	GA	AA	
Age	59.9	59.1	36.3	0.385
BMI	31.2	31.3	31.3	0.994
SBP	136.2	136.5	137.8	0.971
DBP	75.7	74.1	77.2	0.668
FBS	173.7	175.1	171.5	0.985
HbA1c	8.0	8.0	7.4	0.490
Cholesterol	173.9	164.6	154.8	0.077
TG	161.1	130.1	143.2	0.109
HDL	41.7	43.5	37.6	0.162
LDL	104.4	96.6	90.2	0.110

3.2.2.2 The genotype distribution frequency for rs628031, rs145450955, rs3088442 and rs2292334 variants according to HbA1c level.

The diabetic group was stratified based on glycaemic control as presented by HbA1c levels (>6.5 vs. <6.5). The genotype distribution of the rs628031, rs3088442, and rs2292334 variants is presented in Table 3.11-a, 3.11-b, which showed no significant association between the genotypes of all variants and HbA1c categories (>6.5 and <6.5). Among the 240 patients (case) included in the study, only 10 were receiving a combination therapy of insulin and metformin (0.04). Therefore, subgroup analysis for this specific treatment group could not be conducted due to the limited sample size.

Table 3.9 : The genotype distribution for rs628031, rs3088442 and rs2292334 Variants between HbA1c > 6.5 vs HbA1c < 6.5

rs628031 SNPs		HbA1c > 6.5	HbA1c < 6.5	P-value
Genotype	GG	114	37	.639
	GA	56	24	
	AA	7	2	
rs3088442 SNPs		HbA1c > 6.5	HbA1c < 6.5	P-value
Genotype	GG	45	131	.758
	GA	16	43	
	AA	2	3	
rs2292334 SNPs		HbA1c > 6.5	HbA1c < 6.5	P-value
Genotype	GG	98	35	.561
	GA	71	23	
	AA	8	5	
P < 0.05 was considered significant				

Chapter4.

4.1 Discussion

Our study adds to the increasing amounts of research investigating the genetic determinants of Type 2 Diabetes Mellitus (T2DM) by studied the polymorphisms in OCT1, OCT2, and OCT3 genes. Although significant progress in understanding the complex pathophysiology of T2DM has been made, it essentially involves a multifactorial interplay of genetic, environmental, and lifestyle factors, the exact role of OCTs in modifying disease susceptibility remains incompletely understood (Koepsell, 2020).

Over the years, genome-wide association studies (GWAS) have advanced achievements to explore genetic variants joined to raised risk of type 2 diabetes (T2D). These results have paved the way for the progression of polygenic risk scores (PRSs), that quantify the genetic contribution to an individual's disease risk. PRSs are measured as a weighted total of the risk alleles an individual carries, with each allele's weight Cross ponding its effect size as governed by GWAS data. PRSs exhibit valuable insights into both absolute and relative disease risk parallel to a wider population. Substantially, PRSs applications spread three key domains: enhancing the accuracy and timeliness of predicting disease onset and progression, optimizing therapeutic planning to manage, or treat the disease, and given critical directions for genetic counseling and family planning. This application hold promises for more personalized and proactive approaches in healthcare (Boecker & Lai, 2020; Torkamani et al., 2018). In this context, we investigated the relationship between OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) gene polymorphisms and their associations with T2DM risk in Palestine. A total of 337 subjects were recruited for this study, including 250 subjects with T2DM and 97 control subjects. To our knowledge, this is the first study reporting the frequency of OCT1, OCT2 and OCT3 gene polymorphisms among Palestinian population.

In this study, the number of females who's affected in T2DM higher showed higher proportion than those in control (P=0.000), the mean age of diabetic patients (59.76 years) is higher compared to those in controls (53.83 years), this may suggest the influence of gender and age in risk and management for diabetes disease. This result agree with Health Annual Report Palestine 2023, also There is studies agree with the and with present study (Ciarambino et al., 2022; Manicardi et al., 2016). However, another study showed the higher proportion in male of T2DM than women(Kumar et al., 2023), emphasizing that gender-related differences impact psychosocial and cultural consideration like distinct behaviors, lifestyles, also their style toward prevention and treatment, contribute to differences in both gender tendency to the progression of type 2 diabetes (Ciarambino et al., 2022). Moreover, the age increases are the important risk factors in the onset and progression T2DM (Yan et al., 2023).

The recognized elevation in HbA1c, FBS, and systolic blood pressure in T2DM patients compared to controls coordinates with the clearly established in pathophysiological interplay between hyperglycemia, vascular dysfunction, and hypertension studies (Ambelu & Teferi, 2023; Rabizadeh et al., 2021; Shaphe et al., 2024). Chronic hyperglycemia aggravates endothelial dysfunction, increased oxidative stress, in addition to elevate systemic inflammation, resulted to increased arterial hardness and systolic pressure. Moreover, insulin resistance a marker of T2DM, exacerbate the regulation of glucose and blood pressure, promoting their chronic elevation in a half patient with T2DM (Tatsumi & Ohkubo, 2017). However, our present study showed higher DBP value in non-diabetic group (P- value = 0.002), this result aligns with previous study finding that diabetic patients tend to have high systolic blood pressure (SBP) and somewhat reduced DBP versus non-diabetic counterparts(Atmaca et al., 2022). Another finding by Ocher et al demonstrated in a related study (Osher & Stern, 2008) that the diabetic patient have a tendency to higher systolic blood pressure (SBP) and slightly lower diastolic blood pressure (DBP).

This can be partially explained by that healthy individuals mostly have more elastic arterial walls, letting better diastolic function. Although, diabetes related to vascular damage frequently leads to raised arterial stiffness and reducing the elasticity in ordered to maintain higher DBP levels. Also, arterial stiffness primarily effects systolic blood pressure, leaving diastolic pressure relatively lower in diabetic patients. In addition to this reason, lifestyle factors such as physical activity, diet, and hypertension management drugs could contribute to these differences (Azimi et al., 2016; Huang et al., 2017; Osher & Stern, 2008).

In our case control study, the Minor Allele Frequency of OCT1 (rs628031) in case vs control (0.204) Vs. (0.235). Whereas, the OCT2 (rs145450955) frequency are 100% GG in both case and control. However, OCT3 (rs3088442) variant MAF between case and control respectively (0.168) VS (0.113). In addition, OCT3 (rs2292334) MAF in case Vs control (0.295) vs (0.202). The OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) variants were studied among various populations. A study reported in Sudi Arabian populations, the G allele frequency in OCT1 (rs628031) was (0.69) in T2DM VS (0.71) in control. While, MAF of the same variant (0.31) in T2DM and (0.29) in control group, in this study, the allele frequency is similar to our allele frequency findings (Altall et al., 2019). However, a study among Iranian populations conflict with our results of OCT1 (rs628031) showed, The G allele frequency was 0.32 in T2DM and 0.33 in control. The A allele was 0.68 among diabetic patients. While, 0.66 (A) in control (Shokri et al., 2016).

The OCT3 rs3088442 variant was studied among Japanese populations showed equal allele frequency for G and A allele, G= 0.51 and A= 0.49 (Ghaffari-Cherati et al., 2016). While, the OCT3 rs2292334 variant was studied among Jordanian population reported that the G allele frequency was 0.86 and A allele was 0.14 (Hakooz et al., 2017b) .

When we investigated the potential role of the OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) gene polymorphisms with risk of T2DM, we found no significant difference in the genotype distribution of all these variants between the T2DM patients and control group. These results suggest that these variants could not play important role in the T2DM pathogenesis among Palestinian populations and may be the relatively small sample size was unable to reveal such association.

Moreover, this study included additional analysis to explore if the OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) gene polymorphisms can explain some differences in HbA1c level which may reflect the response for Metformin treatment, the diabetic subject was stratified into 2 groups according to HbA1c level (more than 6.5 vs less than 6.5). Our results show no significant differences in all these variants and the level of glycated hemoglobin. This result has implicit meaning, which is these variants no affect metformin responses. In this regard, a lot of researches have also revealed conflicting findings regarding the link between OCT1, OCT2, and OCT3 gene variations and diabetes in different ethnic groups.

A lot of research have also revealed conflicting findings regarding the link between OCT1, OCT2, and OCT3 gene variations and diabetes in different ethnic groups (Aladhab et al., 2023; Peng et al., 2023d; Rizvi et al., 2024).

Several studies investigated OCT1 (rs628031) polymorphism with diabetes and having variation in their results. Study in al Iraq agree with presents study, showed no significant associations between rs628031 genotypes and the metformin efficiency (Aladhab et al., 2023), another study in Iranian population showed no significant in metformin responses (P= 0.47) (Shokri et al., 2016b) , In addition, study conducted in AL-KIWAT showed the same result which is no significant association between this polymorphism and metformin response (P = 0.3) (Alawadhi, 2013). However, study in Saudi Arabia showed The G allele in OCT1 rs628031 polymorphisms identified as a possible risk factor, showing evidence suggesting that patients carrying the G/G genotype in OCT1 (rs628031) tend to demonstrate higher levels of HbA1c and fasting blood sugar (FBS) versus individual carrying the A/G or A/A genotypes in the same variant (Altall et al., 2019).

In OCT2 (rs145450955) polymorphism, among Iranian population, there is a study showed the patients carry A minor allele has higher glycated hemoglobin and fasting blood sugar (Kashi et al., 2015b), although (Ghasan Abood Al-Ashoor et al., 2021) showed no significant association between rs145450955 in genotype and allele frequency in rs145450955 polymorphisms between T2DM and control groups ($P > 0.05$).

In our presented study, when we stratified the T2DM data according to genotypes to determine the association between all variants (OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) and the mean value of HbA1c, FBS, BMI, age, lipid profile, SBP and DBP, we found no significant differences in the all-mean value of clinicals and biochemical parameters.

OCT3 (rs3088442) is another variant was investigated between diabetic patients, A study conducted by Hosseyni-Talei and colleagues showed that individuals who is carrying GG genotype had lower level of HbA1C than those carrion A allele after three month of metformin therapy (Hosseyni-Talei et al., 2017). Also, another study found this variant has a protective effect against T2DM progression which can decrease the diabetes susceptibility and increased the clinical efficacy of metformin responses in patients(Mahrooz et al., 2017), also in Pakistan population, a study found the A allele in OCT3 (rs3088442) may considered as a protective allele for metformin responses (Moeez et al., 2019).

OCT3 (rs2292334) variant have shown variability in result between different ethnic groups. In north India, a study showed a significant association betweenOCT3 (rs2292334) variant and metformin response, considered the AA genotype of rs2292334 enhanced efficiency of metformin clearance. While G allele in the same variant was associated with decreased metformin respond in T2DM (Rizvi et al., 2024). Although, a systemic review and meta-analysis study showed no statistical association was found between the OCT3 (rs2292334) variants and metformin response and intolerance (Peng et al., 2023). The differences in results regarding OCT1 (rs628031) and OCT2 (rs145450955) polymorphisms and their association with glycemic control or metformin response across various studies can be attributed to factors such as ethnic variations, genotyping accuracy, and methodological differences. Ethnic diversity significantly influences the distribution of genetic polymorphisms and their phenotypic effects. These inconsistencies may also stem from differences in genotyping methods, as variations in techniques (e.g., PCR-based assays versus sequencing) can influence accuracy, while small sample sizes and limited statistical power may obscure significant findings. Additionally, variations in study design, inclusion criteria, and adjustment for confounding factors such as BMI, age, or lipid profile further complicate comparisons. In the current study, we utilized a newly introduced assay based on next-generation sequencing (NGS), which effectively ruled out any genotyping errors. The NGS assay successfully detected all four targeted SNPs across three fragments in a single-tube reaction, with FAST-Q files ensuring high detection accuracy through thousands of sequencings reads per SNP. This method is not only highly specific but also less labor-intensive, cost-effective, and suitable for individual-level SNP quantification, making it superior to traditional genotyping techniques. In our analysis, stratification of T2DM data based on OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) genotypes revealed no significant differences in HbA1c, FBS, BMI, age, lipid profile, SBP, or DBP, aligning with several previous findings. These results emphasize the need for future large-scale studies, leveraging accurate and advanced methods like NGS, to clarify the role of these polymorphisms in glycemic control and metformin response across diverse populations.

4.2 Study limitations

The main limitation of the current study was the relatively small sample size, which may have affected the ability to detect significant associations. Future research with larger cohorts could provide a deeper understanding of the role of OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) gene polymorphisms in T2DM and their interactions with other clinical parameters. This is particularly important for understanding variability in drug pharmacokinetics, pharmacodynamics, efficacy, and toxicity profiles, including potential risks such as nephrotoxicity. Another limitation is that the study focused on specific genetic variants within the OCT1, OCT2, and OCT3 genes, while other potential polymorphisms within these genes, which could also have significant functional roles, were not explored.

4.2.1 Study recommendations

Given the lack of association between the OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) gene polymorphisms and T2DM observed in Palestinian patients, future research should focus on exploring additional factors such as other medications, detailed clinical information that contribute to T2DM in this population to enhance the comprehensiveness of future analyses. Long-term studies are needed to assess the effects of OCT1, OCT2, and OCT3 polymorphisms on metformin response and the development of T2DM over time. Furthermore, implementing personalized medicine strategies tailored to patients' medical histories and lifestyles, including smoking cessation support, regular screenings, and periodic check-ups, is essential to prevent complications and improve patient outcomes. Functional validation studies would also be highly beneficial in providing a comprehensive understanding of interindividual variability in drug pharmacokinetics, pharmacodynamics, efficacy, and toxicity profiles, particularly with regard to risks such as nephrotoxicity.

4.3 Conclusion

In conclusion, the current study is the first in Palestine that examines the effect of the OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) gene polymorphisms and T2DM. The study results showed no association the OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) gene polymorphisms and T2DM. In addition, there is a variation was observed in age, gender, systolic and diastolic blood pressure between T2DM and control.

References

Aladhab, R. A., Abd, A. H., Alidrisi, H. A., & Alabbood, M. H. (2023). Genetic Polymorphism in the Organic Cation Transporters 1 (OCT1) Gene and its Effect on Therapeutic Efficacy and Gastrointestinal Side Effects of Metformin in Patients with Type 2 Diabetes Mellitus in Basrah/ Southern Iraq. *Biomedical and Pharmacology Journal*, *16*(2), 1189–1199. https://doi.org/10.13005/BPJ/2699

Alam, S., Hasan, M. K., Neaz, S., Hussain, N., Hossain, M. F., & Rahman, T. (2021). Diabetes Mellitus: Insights from Epidemiology, Biochemistry, Risk Factors, Diagnosis, Complications and Comprehensive Management. *Diabetology 2021, Vol. 2, Pages 36-50*, *2*(2), 36–50. https://doi.org/10.3390/DIABETOLOGY2020004

Alawadhi, S. (2013). *Factors affecting metformin plasma concentrations*. https://espace.curtin.edu.au/bitstream/20.500.11937/1383/2/193209_Alawadhi2010.pdf

Al-Eitan, L. N., Almomani, B. A., Nassar, A. M., Elsaqa, B. Z., & Saadeh, N. A. (2019). Metformin Pharmacogenetics: Effects of SLC22A1, SLC22A2, and SLC22A3 Polymorphisms on Glycemic Control and HbA1c Levels. *Journal of Personalized Medicine 2019, Vol. 9, Page 17*, *9*(1), 17. https://doi.org/10.3390/JPM9010017

Altall, R. M., Qusti, S. Y., Filimban, N., Alhozali, A. M., Alotaibi, N. A., Dallol, A., Chaudhary, A. G., & Bakhashab, S. (2019a). 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

Altall, R. M., Qusti, S. Y., Filimban, N., Alhozali, A. M., Alotaibi, N. A., Dallol, A., Chaudhary, A. G., & Bakhashab, S. (2019b). 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. https://doi.org/10.2147/TACG.S229952

Altall, R. M., Qusti, S. Y., Filimban, N., Alhozali, A. M., Alotaibi, N. A., Dallol, A., Chaudhary, A. G., & Bakhashab, S. (2019c). SLC22A1 and ATM genes polymorphisms are associated with the risk of type 2 diabetes mellitus in western Saudi Arabia: A case-control study. *Application of Clinical Genetics*, *12*, 213–219. https://doi.org/10.2147/TACG.S229952

Ambelu, T., & Teferi, G. (2023). The impact of exercise modalities on blood glucose, blood pressure and body composition in patients with type 2 diabetes mellitus. *BMC Sports Science, Medicine and Rehabilitation*, *15*(1), 1–11. https://doi.org/10.1186/S13102-023-00762-9/TABLES/3

Antar, S. A., Ashour, N. A., Sharaky, M., Khattab, M., Ashour, N. A., Zaid, R. T., Roh, E. J., Elkamhawy, A., & Al-Karmalawy, A. A. (2023). Diabetes mellitus: Classification, mediators, and complications; A gate to identify potential targets for the development of new effective treatments. *Biomedicine & Pharmacotherapy*, *168*, 115734. https://doi.org/10.1016/J.BIOPHA.2023.115734

Aryastuti, S. A., Sintya, E., Lestarini, A., & Witari, N. P. D. (2022). Allele Frequency of a Common Variants and Two Common Loss-of-Function Variants in Organic Cation Transporter 1 (OCT1) among Balinese Diabetic Patients. *Biomedical and Pharmacology Journal*, *15*(1), 269–275. https://doi.org/10.13005/BPJ/2363

Atmaca, H., Cuglan, B., Yalta, K., & Yetkin, E. (2022). Systolic Blood Pressure to Diastolic Blood Pressure Ratios in Diabetic and Non-diabetic Patients: Deviation from Golden Ratio. *High Blood Pressure & Cardiovascular Prevention : The Official Journal of the Italian Society of Hypertension*, *29*(4), 401–404. https://doi.org/10.1007/S40292-022-00518-5

Azimi, H., Masroor, D., Haghani, H., & Rafii, F. (2016). Effect of Aerobic Exercise on Blood Pressure of Patients With Type 2 Diabetes: A Randomized Controlled Trial. *Journal of Client-Centered Nursing Care*, 169–176. https://doi.org/10.32598/JCCNC.2.3.169

Baynest, H. W. (2015). Classification, Pathophysiology, Diagnosis and Management of Diabetes Mellitus. *Journal of Diabetes & Metabolism*, *6*(5), 1–9. https://doi.org/10.4172/2155-6156.1000541

Boecker, M., & Lai, A. G. (2020). Could personalised risk prediction for type 2 diabetes using polygenic risk scores direct prevention, enhance diagnostics, or improve treatment? [version 1; peer review: awaiting peer review]. *Wellcome Open Research ,* *5* *p.* *206.* *(2020)* , 5, 206. https://doi.org/10.12688/WELLCOMEOPENRES.16251.1

Brosseau, N., & Ramotar, D. (2019). The human organic cation transporter OCT1 and its role as a target for drug responses. *Drug Metabolism Reviews*, *51*(4), 389–407. https://doi.org/10.1080/03602532.2019.1670204

César-Razquin, A., Snijder, B., Frappier-Brinton, T., Isserlin, R., Gyimesi, G., Bai, X., Reithmeier, R. A., Hepworth, D., Hediger, M. A., Edwards, A. M., & Superti-Furga, G. (2015). A Call for Systematic Research on Solute Carriers. *Cell*, *162*(3), 478–487. https://doi.org/10.1016/J.CELL.2015.07.022/ATTACHMENT/1A575FF5-9531-4F8D-B2A9-A32807843D17/MMC4.PDF

Choudhury, A. A., & Devi Rajeswari, V. (2021). Gestational diabetes mellitus - A metabolic and reproductive disorder. *Biomedicine & Pharmacotherapy*, *143*, 112183. https://doi.org/10.1016/J.BIOPHA.2021.112183

Ciarambino, T., Crispino, P., Leto, G., Mastrolorenzo, E., Para, O., & Giordano, M. (2022). Influence of Gender in Diabetes Mellitus and Its Complication. *International Journal of Molecular Sciences*, *23*(16), 8850. https://doi.org/10.3390/IJMS23168850

Ciarimboli, G. (2021). Regulation Mechanisms of Expression and Function of Organic Cation Transporter 1. *Frontiers in Pharmacology*, *11*, 607613. https://doi.org/10.3389/FPHAR.2020.607613

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

Elsayed, N. A., Aleppo, G., Aroda, V. R., Bannuru, R. R., Brown, F. M., Bruemmer, D., Collins, B. S., Hilliard, M. E., Isaacs, D., Johnson, E. L., Kahan, S., Khunti, K., Kosiborod, M., Leon, J., Lyons, S. K., Murdock, L., Perry, M. Lou, Prahalad, P., Pratley, R. E., … Gabbay, R. A. (2022). 2. Classification and Diagnosis of Diabetes: Standards of Care in Diabetes—2023. *Diabetes Care*, *46*(Suppl 1), S19. https://doi.org/10.2337/DC23-S002

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., Ostolaza, H., & Martín, C. (2020). Pathophysiology of Type 2 Diabetes Mellitus.

International Journal of Molecular Sciences 2020, Vol. 21, Page 6275, *21*(17), 6275. https://doi.org/10.3390/IJMS21176275

Genuth, S. M., Palmer, J. P., & Nathan, D. M. (2021). Classification and Diagnosis of Diabetes. *Diabetes in America, 3rd Edition*, *2*(4), 1–39. http://europepmc.org/books/NBK568014

Ghaffari-Cherati, M., Mahrooz, A., Hashemi-Soteh, M. B., Hosseyni-Talei, S. R., Alizadeh, A., & Nakhaei, S. M. (2016). Allele frequency and genotype distribution of a common variant in the 3´-untranslated region of the SLC22A3 gene in patients with type 2 diabetes: Association with response to metformin. *Journal of Research in Medical Sciences : The Official Journal of Isfahan University of Medical Sciences*, *21*(6), 92. https://doi.org/10.4103/1735-1995.192508

Ghasan Abood Al-Ashoor, S., Ramachandran, V., Inche Mat, L. N., Mohamad, N. A., Mohamed, M. H., & Wan Sulaiman, W. A. (2021). Analysis of OCT1, OCT2 and OCT3 gene polymorphisms among Type 2 diabetes mellitus subjects in Indian ethnicity, Malaysia. *Saudi Journal of Biological Sciences*, *29*(1), 453. https://doi.org/10.1016/J.SJBS.2021.09.008

Ghasan Abood Al-Ashoor, S., Ramachandran, V., Inche Mat, L. N., Mohamad, N. A., Mohamed, M. H., & Wan Sulaiman, W. A. (2022). Analysis of OCT1, OCT2 and OCT3 gene polymorphisms among Type 2 diabetes mellitus subjects in Indian ethnicity, Malaysia. *Saudi Journal of Biological Sciences*, *29*(1), 453–459. https://doi.org/10.1016/J.SJBS.2021.09.008

Gong, L., Goswami, S., Giacomini, K. M., Altman, R. B., & Klein, T. E. (2012). Metformin pathways: pharmacokinetics and pharmacodynamics. *Pharmacogenetics and Genomics*, *22*(11), 820. https://doi.org/10.1097/FPC.0B013E3283559B22

Grinfeld, J., Gerrard, G., Alikian, M., Alonso-Dominguez, J., Ale, S., Valgañon, M., Nteliopoulos, G., White, D., Marin, D., Hedgley, C., O'Brien, S., Clark, R., Goldman, J. M., Milojkovic, D., Apperley, J. F., & Foroni, L. (2013). A common novel splice variant of SLC22A1 (OCT1) is associated with impaired responses to imatinib in patients with chronic myeloid leukaemia. *British Journal of Haematology*, *163*(5), 631–639. https://doi.org/10.1111/BJH.12591

Hakooz, N., Jarrar, Y. B., Zihlif, M., Imraish, A., Hamed, S., & Arafat, T. (2017a). Effects of the genetic variants of organic cation transporters 1 and 3 on the pharmacokinetics of metformin in Jordanians. *Drug Metabolism and Personalized Therapy*, *32*(3), 157–162. https://doi.org/10.1515/DMPT-2017-0019

Hakooz, N., Jarrar, Y. B., Zihlif, M., Imraish, A., Hamed, S., & Arafat, T. (2017b). Effects of the genetic variants of organic cation transporters 1 and 3 on the pharmacokinetics of metformin in Jordanians. *Drug Metabolism and Personalized Therapy*, *32*(3), 157–162. https://doi.org/10.1515/DMPT-2017-0019/MACHINEREADABLECITATION/RIS

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

Higgins, J. W., Bedwell, D. W., & Zamek-Gliszczynski, M. J. (2012). Ablation of both organic cation transporter (OCT)1 and OCT2 alters metformin pharmacokinetics but has no effect on tissue drug exposure and pharmacodynamics. *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, *40*(6), 1170–1177. https://doi.org/10.1124/DMD.112.044875

Holle, S. K., Ciarimboli, G., Edemir, B., Neugebauer, U., Pavenstädt, H., & Schlatter, E. (2011). Properties and regulation of organic cation transport in freshly isolated mouse proximal tubules analyzed with a fluorescence reader-based method. *Pflugers Archiv : European Journal of Physiology*, *462*(2), 359–369. https://doi.org/10.1007/S00424-011-0969-7

Hörmann, S., Gai, Z., Kullak-Ublick, G. A., & Visentin, M. (2020). Plasma Membrane Cholesterol Regulates the Allosteric Binding of 1-Methyl-4-Phenylpyridinium to Organic Cation Transporter 2 (SLC22A2). *Journal of Pharmacology and Experimental Therapeutics*, *372*(1), 46–53. https://doi.org/10.1124/JPET.119.260877

Hosseyni-Talei, S. R., Mahrooz, A., Hashemi-Soteh, M. B., Ghaffari-Cherati, M., & Alizadeh, A. (2017). Association between the synonymous variant organic cation transporter 3 (OCT3)-1233G>A and the glycemic response following metformin therapy in patients with type 2 diabetes. *Iranian Journal of Basic Medical Sciences*, *20*(3), 250–255. https://doi.org/10.22038/IJBMS.2017.8351

Huang, C., Dhruva, S. S., Coppi, A. C., Warner, F., Li, S. X., Lin, H., Nasir, K., & Krumholz, H. M. (2017). Systolic blood pressure response in SPRINT (Systolic Blood Pressure Intervention Trial) and ACCORD (Action to Control Cardiovascular Risk in Diabetes): A possible explanation for discordant trial results. *Journal of the American Heart Association*, *6*(11). https://doi.org/10.1161/JAHA.117.007509/SUPPL_FILE/JAH32778-SUP-0001-TABSS1-S5-FIGS1-S3.PDF

Hushmandi, K., Einollahi, B., Saadat, S. H., Lee, E. H. C., Farani, M. R., Okina, E., Huh, Y. S., Nabavi, N., Salimimoghadam, S., & Kumar, A. P. (2024). Amino acid transporters within the solute carrier superfamily: Underappreciated proteins and novel opportunities for cancer therapy. *Molecular Metabolism*, *84*, 101952. https://doi.org/10.1016/J.MOLMET.2024.101952

Jaldin-Fincati, J. R., Pavarotti, M., Frendo-Cumbo, S., Bilan, P. J., & Klip, A. (2017). Update on GLUT4 Vesicle Traffic: A Cornerstone of Insulin Action. *Trends in Endocrinology and Metabolism: TEM*, *28*(8), 597–611. https://doi.org/10.1016/J.TEM.2017.05.002

Jin, H. E., Hong, S. S., Choi, M. K., Maeng, H. J., Kim, D. D., Chung, S. J., & Shim, C. K. (2009). Reduced antidiabetic effect of metformin and down-regulation of hepatic oct1 in rats with ethynylestradiol-induced cholestasis. *Pharmaceutical Research*, *26*(3), 549–559. https://doi.org/10.1007/S11095-008-9770-5/METRICS

Kashi, Z., Masoumi, P., Mahrooz, A., Hashemi-Soteh, M. B., Bahar, A., & Alizadeh, A. (2015a). The variant organic cation transporter 2 (OCT2)-T201M contribute to changes in insulin resistance in patients with type 2 diabetes treated with metformin. *Diabetes Research and Clinical Practice*, *108*(1), 78–83. https://doi.org/10.1016/J.DIABRES.2015.01.024

Kashi, Z., Masoumi, P., Mahrooz, A., Hashemi-Soteh, M. B., Bahar, A., & Alizadeh, A. (2015b). The variant organic cation transporter 2 (OCT2)-T201M contribute to changes in insulin resistance in patients with type 2 diabetes treated with metformin. *Diabetes Research and Clinical Practice*, *108*(1), 78–83. https://doi.org/10.1016/J.DIABRES.2015.01.024

Kawoosa, F., Shah, Z. A., Masoodi, S. R., Amin, A., Rasool, R., Fazili, K. M., Dar, A. H., Lone, A., & ul Bashir, S. (2022). Role of human organic cation transporter-1 (OCT-1/SLC22A1) in modulating the response to metformin in patients with type 2 diabetes. *BMC Endocrine Disorders*, *22*(1). https://doi.org/10.1186/S12902-022-01033-3

Kesavadev, J., Jawad, F., Deeb, A., Coetzee, A., Ansari, M. A. J., Shrestha, D., Somasundaram, N., & Kalra, S. (2023). Pathophysiology of Type 2 Diabetes. *The Diabetes Textbook: Clinical Principles, Patient Management and Public Health Issues, Second Edition*, 127–142. https://doi.org/10.1007/978-3-031-25519-9_9

Khanppnavar, B., Maier, J., Herborg, F., Gradisch, R., Lazzarin, E., Luethi, D., Yang, J.-W., Qi, C., Holy, M., Jäntsch, K., Kudlacek, O., Schicker, K., Werge, T., Gether, U., Stockner, T., Korkhov, V. M., & Sitte, H. H. (2022). Structural basis of organic cation transporter-3 inhibition. *BioRxiv*, 2022.07.14.499921. https://doi.org/10.1101/2022.07.14.499921

Koepsell, H. (2020). Glucose transporters in the small intestine in health and disease. *Pflugers Archiv : European Journal of Physiology*, *472*(9), 1207–1248. https://doi.org/10.1007/S00424-020-02439-5

Kumar, A., Gangwar, R., Ahmad Zargar, A., Kumar, R., & Sharma, A. (2023). Prevalence of Diabetes in India: A Review of IDF Diabetes Atlas 10th Edition. *Current Diabetes Reviews*, *20*(1). https://doi.org/10.2174/1573399819666230413094200

Kuseyri Hübschmann, O., Horvath, G., Cortès-Saladelafont, E., Yıldız, Y., Mastrangelo, M., Pons, R., Friedman, J., Mercimek-Andrews, S., Wong, S. N., Pearson, T. S., Zafeiriou, D. I., Kulhánek, J., Kurian, M. A., López-Laso, E., Oppebøen, M., Kılavuz, S., Wassenberg, T., Goez, H., Scholl-Bürgi, S., … Opladen, T. (2021). Insights into the expanding phenotypic spectrum of inherited disorders of biogenic amines. *Nature Communications 2021 12:1*, *12*(1), 1–15. https://doi.org/10.1038/s41467-021-25515-5

Li, J., & Stocum, D. L. (2013). Fracture Healing. *Basic and Applied Bone Biology*, 205–223. https://doi.org/10.1016/B978-0-12-416015-6.00010-1

Lu, X., Xie, Q., Pan, X., Zhang, R., Zhang, X., Peng, G., Zhang, Y., Shen, S., & Tong, N. (2024a). Type 2 diabetes mellitus in adults: pathogenesis, prevention and therapy. *Signal Transduction and Targeted Therapy 2024 9:1*, *9*(1), 1–25. https://doi.org/10.1038/s41392-024-01951-9

Lu, X., Xie, Q., Pan, X., Zhang, R., Zhang, X., Peng, G., Zhang, Y., Shen, S., & Tong, N. (2024b). Type 2 diabetes mellitus in adults: pathogenesis, prevention and therapy. *Signal Transduction and Targeted Therapy 2024 9:1*, *9*(1), 1–25. https://doi.org/10.1038/s41392-024-01951-9

Ma, M., Liu, H., Yu, J., He, S., Li, P., Ma, C., Zhang, H., Xu, L., Ping, F., Li, W., Sun, Q., & Li, Y. (2020). Triglyceride is independently correlated with insulin resistance and islet beta cell function: A study in population with different glucose and lipid metabolism states. *Lipids in Health and Disease*, *19*(1), 1–12. https://doi.org/10.1186/S12944-020-01303-W/TABLES/3

Madiraju, A. K., Erion, D. M., Rahimi, Y., Zhang, X. M., Braddock, D. T., Albright, R. A., Prigaro, B. J., Wood, J. L., Bhanot, S., MacDonald, M. J., Jurczak, M. J., Camporez, J. P., Lee, H. Y., Cline, G. W., Samuel, V. T., Kibbey, R. G., & Shulman, G. I. (2014). Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. *Nature*, *510*(7506), 542. https://doi.org/10.1038/NATURE13270

Mahrooz, A., Alizadeh, A., Hashemi-Soteh, M. B., Ghaffari-Cherati, M., & Hosseyni-Talei, S. R. (2017a). Polymorphic Variants rs3088442 and rs2292334 in the Organic Cation Transporter 3 (OCT3) Gene and Susceptibility Against Type 2 Diabetes: Role of their Interaction. *Archives of Medical Research*, *48*(2), 162–168. https://doi.org/10.1016/J.ARCMED.2017.03.010

Mahrooz, A., Alizadeh, A., Hashemi-Soteh, M. B., Ghaffari-Cherati, M., & Hosseyni-Talei, S. R. (2017b). Polymorphic Variants rs3088442 and rs2292334 in the Organic Cation Transporter 3 (OCT3) Gene and Susceptibility Against Type 2 Diabetes: Role of their Interaction. *Archives of Medical Research*, *48*(2), 162–168. https://doi.org/10.1016/J.ARCMED.2017.03.010

Mahrooz, A., Alizadeh, A., Hashemi-Soteh, M. B., Ghaffari-Cherati, M., & Hosseyni-Talei, S. R. (2017c). Polymorphic Variants rs3088442 and rs2292334 in the Organic Cation Transporter 3 (OCT3) Gene and Susceptibility Against Type 2 Diabetes: Role of their Interaction. *Archives of Medical Research*, *48*(2), 162–168. https://doi.org/10.1016/J.ARCMED.2017.03.010

Mahrooz, A., Alizadeh, A., Hashemi-Soteh, M. B., Ghaffari-Cherati, M., & Hosseyni-Talei, S. R. (2017d). The Polymorphic Variants rs3088442 and rs2292334 in the

Organic Cation Transporter 3 (OCT3) Gene and Susceptibility Against Type 2 Diabetes: Role of their Interaction. *Archives of Medical Research*, *48*(2), 162–168. https://doi.org/10.1016/J.ARCMED.2017.03.010

Manicardi, V., Russo, G., Napoli, A., Torlone, E., Volsi, P. L., Giorda, C. B., Musacchio, N., Nicolucci, A., Suraci, C., Lucisano, G., & Rossi, M. C. (2016). Gender-Disparities in Adults with Type 1 Diabetes: More Than a Quality of Care Issue. A Cross-Sectional Observational Study from the AMD Annals Initiative. *PLoS ONE*, *11*(10), e0162960. https://doi.org/10.1371/JOURNAL.PONE.0162960

Mato, E. P. M., Guewo-Fokeng, M., Essop, M. F., & Owira, P. M. O. (2018a). 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

Mato, E. P. M., Guewo-Fokeng, M., Essop, M. F., & Owira, P. M. O. (2018b). 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). https://doi.org/10.1097/MD.0000000000011349

Mato, E. P. M., Guewo-Fokeng, M., Faadiel Essop, M., & Owira, P. M. O. (2018). Genetic polymorphisms of organic cation transporters 1 (OCT1) and responses to metformin therapy in individuals with type 2 diabetes mellitus: A systematic review protocol. *Systematic Reviews*, *7*(1), 1–6. https://doi.org/10.1186/S13643-018-0773-Y/TABLES/1

Modzelewski, R., Stefanowicz-Rutkowska, M. M., Matuszewski, W., & Bandurska-Stankiewicz, E. M. (2022). Gestational Diabetes Mellitus—Recent Literature Review. *Journal of Clinical Medicine 2022, Vol. 11, Page 5736*, *11*(19), 5736. https://doi.org/10.3390/JCM11195736

Moeez, S., Riaz, S. K., Masood, N., Kanwal, N., Arif, M. A., Niazi, R., & Khalid, S. (2019a). Evaluation of the rs3088442 G>A SLC22A3 Gene Polymorphism and the Role of microRNA 147 in Groups of Adult Pakistani Populations With Type 2 Diabetes in Response to Metformin. *Canadian Journal of Diabetes*, *43*(2), 128-135.e3. https://doi.org/10.1016/J.JCJD.2018.07.001

Moeez, S., Riaz, S. K., Masood, N., Kanwal, N., Arif, M. A., Niazi, R., & Khalid, S. (2019b). Evaluation of the rs3088442 G>A SLC22A3 Gene Polymorphism and the Role of microRNA 147 in Groups of Adult Pakistani Populations With Type 2 Diabetes in Response to Metformin. *Canadian Journal of Diabetes*, *43*(2), 128-135.e3. https://doi.org/10.1016/J.JCJD.2018.07.001

Olisah, C. C., Smith, L., & Smith, M. (2022). Diabetes mellitus prediction and diagnosis from a data preprocessing and machine learning perspective. *Computer Methods and Programs in Biomedicine*, *220*, 106773. https://doi.org/10.1016/J.CMPB.2022.106773

Osher, E., & Stern, N. (2008a). Diastolic pressure in type 2 diabetes: can target systolic pressure be reached without "diastolic hypotension"? *Diabetes Care*, *31 Suppl 2*. https://doi.org/10.2337/DC08-S262

Osher, E., & Stern, N. (2008b). Diastolic pressure in type 2 diabetes: can target systolic pressure be reached without "diastolic hypotension"? *Diabetes Care*, *31 Suppl 2*. https://doi.org/10.2337/DC08-S262

Palestine Health Annual Report 2021 | GHDx. (n.d.). Retrieved November 24, 2024, from https://ghdx.healthdata.org/record/palestine-health-annual-report-2021

Palestine national diabetes programme, Palestine. (n.d.). Retrieved November 24, 2024, from https://www.worlddiabetesfoundation.org/what-we-do/projects/wdf15-1304/

Peng, A., Gong, C., Xu, Y., Liang, X., Chen, X., Hong, W., & Yan, J. (2023a). 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*, 1183879. https://doi.org/10.3389/FPUBH.2023.1183879/BIBTEX

Peng, A., Gong, C., Xu, Y., Liang, X., Chen, X., Hong, W., & Yan, J. (2023b). 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*, 1183879. https://doi.org/10.3389/FPUBH.2023.1183879/BIBTEX

Peng, A., Gong, C., Xu, Y., Liang, X., Chen, X., Hong, W., & Yan, J. (2023c). 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://doi.org/10.3389/FPUBH.2023.1183879/FULL

Peng, A., Gong, C., Xu, Y., Liang, X., Chen, X., Hong, W., & Yan, J. (2023d). 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*, 1183879. https://doi.org/10.3389/FPUBH.2023.1183879/BIBTEX

Popoviciu, M. S., Kaka, N., Sethi, Y., Patel, N., Chopra, H., & Cavalu, S. (2023). Type 1 Diabetes Mellitus and Autoimmune Diseases: A Critical Review of the Association and the Application of Personalized Medicine. *Journal of Personalized Medicine 2023, Vol. 13, Page 422*, *13*(3), 422. https://doi.org/10.3390/JPM13030422

Qiu, H. B., Zhuang, W., Wu, T., Xin, S., Lin, C. Z., Ruan, H. L., Zhu, X., Huang, M., Li, J. L., Hou, X. Y., Zhou, Z. W., & Wang, X. D. (2018). Imatinib-induced ophthalmological side-effects in GIST patients are associated with the variations of EGFR, SLC22A1, SLC22A5 and ABCB1. *The Pharmacogenomics Journal*, *18*(3), 460–466. https://doi.org/10.1038/TPJ.2017.40

Rabizadeh, S., Gholami, B., Mahmoudzadeh Kani, S., Rajab, A., Farrokhpour, H., Esteghamati, A., & Nakhjavani, M. (2021). Uncontrolled hypertension in patients with type 2 diabetes: What are the correlates? *The Journal of Clinical Hypertension*, *23*(9), 1776. https://doi.org/10.1111/JCH.14352

Rizvi, S., Raza, S. T., & Mahdi, F. (2024). Association of Single Nucleotide Polymorphism in OCT1 and OCT3 Genes with the Efficacy of Metformin Response in North Indian Type 2 Diabetes Mellitus Patients. *Current Pharmacogenomics and Personalized Medicine (Formerly Current Pharmacogenomics)*, *21*(2), 88–99. https://doi.org/10.2174/0118756921316739240816095335

Rizvi, S., & Tasleem Raza, S. (2023). *Association of Single Nucleotide Polymorphism in OCT1 and OCT3 Genes with the Ecacy of Metformin Response in North Indian Type 2 Diabetes Mellitus Patients*. https://doi.org/10.21203/rs.3.rs-3227562/v1

Saltiel, A. R. (2021). Insulin signaling in health and disease. *The Journal of Clinical Investigation*, *131*(1). https://doi.org/10.1172/JCI142241

Samodelov, S. L., Kullak-Ublick, G. A., Gai, Z., & Visentin, M. (2020). Organic Cation Transporters in Human Physiology, Pharmacology, and Toxicology. *International Journal of Molecular Sciences 2020, Vol. 21, Page 7890*, *21*(21), 7890. https://doi.org/10.3390/IJMS21217890

Savage, D. B., Petersen, K. F., & Shulman, G. I. (2007). Disordered Lipid Metabolism and the Pathogenesis of Insulin Resistance. *Physiological Reviews*, *87*(2), 507. https://doi.org/10.1152/PHYSREV.00024.2006

Shaphe, M. A., Alshehri, M. M., Alajam, R. A., Alfaifi, B., Hakamy, A., Aldhahi, M. I., Ahmad, A., Khan, A., Aafreen, & Khan, A. R. (2024). Evaluating Blood Pressure Variability in Type 2 Diabetic Patients: An Insight into Non-Dipping Patterns and Their Clinical Implications. *International Journal of General Medicine*, *17*, 4445. https://doi.org/10.2147/IJGM.S484183

Shokri, F., Ghaedi, H., Fard, S. G., Movafagh, A., Abediankenari, S., Mahrooz, A., Kashi, Z., & Omrani, M. D. (2016a). 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. https://pmc.ncbi.nlm.nih.gov/articles/PMC4916778/

Shokri, F., Ghaedi, H., Fard, S. G., Movafagh, A., Abediankenari, S., Mahrooz, A., Kashi, Z., & Omrani, M. D. (2016b). 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. /pmc/articles/PMC4916778/

Singh, S., Shukla, A. K., Usman, K., & Banerjee, M. (2023). Pharmacogenetic impact of SLC22A1 gene variant rs628031 (G/A) in newly diagnosed Indian type 2 diabetes patients undergoing metformin monotherapy. *Pharmacogenetics and Genomics*, *33*(3), 51–58. https://doi.org/10.1097/FPC.0000000000000493

Suzuki, K., Hatzikotoulas, K., Southam, L., Taylor, H. J., Yin, X., Lorenz, K. M., Mandla, R., Huerta-Chagoya, A., Melloni, G. E. M., Kanoni, S., Rayner, N. W., Bocher, O., Arruda, A. L., Sonehara, K., Namba, S., Lee, S. S. K., Preuss, M. H., Petty, L. E., Schroeder, P., … Zeggini, E. (2024). Genetic drivers of heterogeneity in type 2 diabetes pathophysiology. *Nature 2024 627:8003*, *627*(8003), 347–357. https://doi.org/10.1038/s41586-024-07019-6

Tarasova, L., Kalnina, I., Geldnere, K., Bumbure, A., Ritenberga, R., Nikitina-Zake, L., Fridmanis, D., Vaivade, I., Pirags, V., & Klovins, J. (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

Tatsumi, Y., & Ohkubo, T. (2017). Hypertension with diabetes mellitus: significance from an epidemiological perspective for Japanese. *Hypertension Research 2017 40:9*, *40*(9), 795–806. https://doi.org/10.1038/hr.2017.67

The Lancet. (2023). Diabetes: a defining disease of the 21st century. *The Lancet*, *401*(10394), 2087. https://doi.org/10.1016/S0140-6736(23)01296-5

Torkamani, A., Wineinger, N. E., & Topol, E. J. (2018). The personal and clinical utility of polygenic risk scores. *Nature Reviews. Genetics*, *19*(9), 581–590. https://doi.org/10.1038/S41576-018-0018-X

Vijan, S. (2010). In the clinic. Type 2 diabetes. *Annals of Internal Medicine*, *152*(5), ITC31-15; quiz ITC316. https://doi.org/10.7326/0003-4819-152-5-201003020-01003

Wenge, B., Geyer, J., & Bönisch, H. (2011). Oxybutynin and trospium are substrates of the human organic cation transporters. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *383*(2), 203–208. https://doi.org/10.1007/S00210-010-0590-X

Yan, Z., Cai, M., Han, X., Chen, Q., & Lu, H. (2023). The Interaction Between Age and Risk Factors for Diabetes and Prediabetes: A Community-Based Cross-Sectional Study. *Diabetes, Metabolic Syndrome and Obesity*, *16*, 85. https://doi.org/10.2147/DMSO.S390857

Zazuli, Z., Duin, N. J. C. B., Jansen, K., Vijverberg, S. J. H., Maitland-Van der Zee, A. H., & Masereeuw, R. (2020). The Impact of Genetic Polymorphisms in Organic Cation Transporters on Renal Drug Disposition. *International Journal of Molecular Sciences 2020, Vol. 21, Page 6627*, *21*(18), 6627. https://doi.org/10.3390/IJMS21186627

Appendices

Appendix 1:Research approval by research ethics committee at Al-Quds University.

Al-Quds University
Faculty of Medicine
Abu-Dies, Jerusalem

جامعة القدس
كلية الطب
أبوديس – القدس

Date:28/4/2024

Research Ethics subcommittee of Faculty of medicine

Letter of Ethical approval

Date:28/4/2024
Ref#: Re2-28-24

Dear Applicants: Dr. Abdelmajeed Naseeddin and Miss. Duaa Fakhouri

Biochemistry and Molecular Biology master program
The Research Ethics subcommittee of faculty of medicine has recently reviewed your proposal entitled “Association of OTC1, OTC2 and OTC3 gene polymorphisms with the risk of Type 2 Diabetes Mellitus in Palestine”
Your proposal is deemed to meet the requirements of research ethics subcommittee at Al-Quds University. This approval does not substitute for any administrative or other approvals that may be necessary.

Note: This letter can be used to apply for the central Al-Quds University research ethics committee if needed

Best of luck,

Dr. Suheir Ereqat
Head of research ethics subcommittee
Biochemistry and Molecular Biology master program
Faculty of Medicine-Al-Quds University

Appendix 2: Consent form

2024

Al-Quds University

Faculty of Graduate Studies

Consent Form

Dear Participant,

You are invited to participate in a research study conducted by student of graduate studies from Al-Quds University in order to fulfill master's degree of Biochemistry and Molecular biology. This study aims to determine the genotype and allele frequencies of OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) gene polymorphisms and detect their association with T2DM risk in Palestine at Jericho health center and Al-Ahli health center.

The purpose of this research is to enhance our understanding of genetic factors that may contribute in individuals with type 2 diabetes mellitus. By participating in this study, you can help us advance knowledge in this area and potentially contribute to improved diabetes management strategies in the future. Taking permission will be part of the diagnosis and follow-up which could lead to improved treatments.

Participation in this study is entirely voluntary. You have the right to withdraw your consent and discontinue participation at any time without penalty. Your decision to participate or not will not affect your medical care or treatment in any way. All data will be recorded and kept confidential. Data will be anonymized by a coding system and may be used in other research studies.

The procedures will be including:

1. Your blood sample will be taken after completing routine tests for DNA extraction and genetic analysis.

2. We will collect relevant clinical and medical data from your medical records.

3. Your participation will involve no cost to you.

Al-Quds University

Faculty of Graduate Studies

• Filling out this form means accepting to participate in this research

I have read and understood the information provided in this Informed Consent Form. I have had the opportunity to ask questions and have received satisfactory answers. I voluntarily agree to participate in this research study.

[Participant's Signature]: ______________________________ Date: _______________

Researcher: Doaa Fakhoury / Al-Quds University - College of Graduate Studies

[The researcher]: ______________________________ Date _______________

Appendix 3: DNA Extraction Method Protocol

1. A 900 µL cell lysis were pipetted into a sterile 1.5ml microcentrifuge tube.
2. After gently rock the blood tube until mixed, 300 µL of blood transferred into the tube congaing cell lysis.
3. Mixture incubated for 10 minutes at room temperature, then centrifugated at 15,00 x g for 20 second.
4. The supernatant was removed as much as possible without disturbing the visible white pellet.
5. The tube was vortexed vigorously until the white blood cells were resuspended (10 – 15 seconds)
6. 300 µL nuclei lysis was added to the tube containing the resuspended cells, then pipetted the solution 5-6 times to lyse white blood cells and the solution became very viscous.
7. 300 µL RNase solution was added and mixed 2 – 5 times, then allowed the mixture incubated at 37 °C for 15 minutes.
8. 100 µL protein precipitation solution was added to the nuclear lysate, then vortexed vigorously fo 10 – 20 seconds until small protein clumps were visible
9. Centrifuged at 13,000 – 16,000 x g for 3 minutes at room temperature.
10. The supernatant was transferred to a clean 1.5 ml microcentrifuge tube containing 300 µL of room temperature isopropanol.
11. The solution was gently mixed by inversion until the white thread-like strands of DNA formed a visible mass
12. The solution was centrifugated at 13,000 – 16,000 x g for 1 minute at room temperature.
13. Decanted the supernatant and one sample volume of 70% ethanol was added to the DNA, then the tube was gently inverted several times to wash the DNA after that the solution centrifugated 13,000 – 16,000 x g for 1 minute.
14. The ethanol was carefully aspirated, and the tube was inverted on clean absorbent paper and air-dried the pallet for 10 – 15 minutes.

15. 100 μL DNA rehydration solution was added then incubated the tube at 65°C for 1 hour
16. DNA was stored at 2 – 8 °C

العلاقة بين تعدد أشكال في الجينات OCT1 وOCT2 و OCT3 وخطر الإصابة بالسكري من النوع الثاني في فلسطين

إعداد: دعاء شريف "محمد صبحي" فاخوري

إشراف: د. سهير عريقات

المشرف الثاني: د. عبد المجيد ناصر الدين

الملخص

مرض السكري من النوع الثاني هو حالة مزمنة تنتج عندما يعاني الجسم من عدم تحقيق توازن في مستويات السكر في الدم بسبب عد القدرة على افراز الانسولين أو عدم قدرة الانسولين على القيام بوظائفه بشكل فعّال، حيث يعتبر الأنسولين من أحد أهم الهرمونات الأيضية البنائية الذي يلعب دوراً مهماً في تنظيم التمثيل الغذائي لكلٍ من الكربوهيدرات، البروتين والدهون، لذلك حدوث أي خلل في عمل هذا الهرمون يؤدي الى اضطرابات التمثيل الغذائي ومرض السكري من النوع الثاني هو أحد أهم هذه الاضطرابات. أيضاً، ارتفاع السكر في الدم لفترات طويلة مزمنة تؤدي الى العديد من المضاعفات التي بدورها تؤدي الى الوفاة. يحتل مرض السكري من النوع الثاني نسبة 95% من إجمالي حالات مرض السكري، وينتشر بنسبة 15.3% بين المواطنين الفلسطينيين بحسب آخر تقرير نشرته وزارة الصحة الفلسطينية. هناك العديد من العوامل التي تساهم في ظهور المرض أو تطوره، وكل هذه العوامل تصنف إما جينية أو بيئية أو تشارك بين هذين العاملين يؤدي الى ظهور مرض السكري من النوع الثاني. قد يكون هناك الكثير من الجينات المتسببة في الإصابة بمرض السكري من النوع الثاني وتطور مضاعفاته، ومن بين هذه الجينات: (SLC22A1, SLC22A2و SLC22A3) التي تقوم بعدة وظائف من أهمها: امتصاص العناصر الغذائية، تنظيم التدرج الأيوني عبر الغشاء، واستقلاب الأدوية التي من بينها الميتفورمين.

تعد هذه الدراسة هي دراسة حالة–شواهد، تم جمع بيانات هذه الدراسة في بين شهر حزيران الى شهر أيلول من العام 2024، تضمنت الدراسة الحلية مجموعتين، المجموعة الأولى كانت مجموعه الحالة

للأشخاص المصابين بمرض السكري من النوع الثاني، والمجموعة الثانية– الشواهد– المشاركين الأصحاء، كان عدد الأشخاص المصابين بالسكري من النوع الثاني عددهم 240 بينما كان عدد المشاركين الأصحاء 97 مشاركاً. جميع المرضى في المجموعتين كانت أعمارهم 40 فما فوق وكانوا من المراجعين في مركز صحة أريحا والمستشفى الأهلي في الخليل– فلسطين. تم إجراء مجموعة من التحاليل لجميع المشاركين في المستشفى الأهلي وصحة أريحا تضمنت فحص السكر التراكمي، وفحص السكر الصائم وتحاليل الدهون. تم إجراء تحليل التسلسل الجيني للجين القائم على الأمبليكون لأربعة أشكال جينية وهي OCT1 (rs628031), OCT2 (rs145450955), and OCT3 (rs3088442 and rs2292334) وتم تحديدها بين الحالات والشواهد، وتعتبر هذه الدراسة هي أول دراسة أجريت على السكان الفلسطينيين

تم تقييم ترددات جميع الأشكال الجينية في مجتمع الدراسة، علاوة على ذلك تم التحقيق في ارتباطات الأشكال الجينية (OCT1 (rs628031، (OCT2 (rs145450955، وOCT3 (rs3088442 وrs2292334) في الحالات– مرضى السكري، والشواهد–الأصحاء. أظهرت نتائج الدراسة أنه لا يوجد ارتباط كبير بين النمط الوراثي في جميع المتغيرات بين الحالة والشواهد P–> 0.05. كانت هناك علاقة بين الجنس والمرضى مقارنة بمجموعة الأصحاء (P=0.000)، كما لوحظت علاقة في متوسط العمر بين مجموعات الحالة ومجموعة الشواهد(P=0.0001) وكانت نسبة الإناث أعلى في مجموعة مرضى السكري 137 أنثى مقابل 23 في مجموعة الأصحاء)، يمكن أن تشير إلى اختلاف ملحوظ بين الجنسين. وأظهرت نتائج الدراسة أيضا فرقاً احصائياً في ضغط الدم الانقباضي P=0.001، وهي أقل من 0.05، كما أظهرت النتائج اختلافات إحصائية في ضغط الدم الانبساطي بين الحالة والشواهد حيث بلغت قيمة p =0.0002 وأن ضغط الدم الانبساطي كان أعلى لدى الأفراد غير المصابين بالسكري. بالإضافة إلى ذلك، كانت هناك فروق ذات دلالة إحصائية بين مجموعتين في نسبة HbA1c وFBS مع قيمة P = 0.0001 والقيمة الأعلى كانت مرضى السكري. ومع ذلك، لم يظهر مؤشر كتلة الجسم والكوليسترول وTG وLDL وHDL أي فروق ذات دلالة إحصائية بين المجموعات المصابة بالسكري والأصحاء بقيمة P> 0.05.

في الختام، تتنوع العلاقة بين تعدد الأشكال الجينية (OCT1 (rs628031، OCT2 (rs145450955)، وOCT3 (rs3088442 وrs2292334) وأمراض داء السكري من النوع 2 بين المجموعات العرقية المختلفة. أظهرت دراستنا عدم وجود ارتباط بين هذه المتغيرات ومرضى السكري من النوع الثاني في السكان الفلسطينيين. وينبغي إجراء المزيد من الدراسات على عينة أكبر لتأكيد نتائج الدراسة.