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Association of GLP-1R Gene Polymorphisms with the risk of Dyslipidemia in Palestinian Patients with Type 2 Diabetes Mellitus

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Thesis approval

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Dedication

To my family, especially my husband, who has supported me greatly throughout this academic journey. Thank you for standing by me through the challenges and triumphs, for your love, patience, and understanding.

I dedicate this thesis to my supervisors, whose guidance, expertise, and wisdom have shaped my academic growth and enriched my knowledge. Your passion for knowledge and dedication to excellence have been a constant source of inspiration.

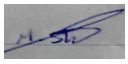
Lastly, I dedicate this work to all those who strive for knowledge and seek to make a difference in the world.

With heartfelt gratitude,

Maha Rashad Joma AlSharabati

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 thereof has not been submitted for a higher degree to any other university or institution.

Signature: 

Maha Rashad Joma AlSharabati

Date: 11.07.2024

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I am grateful to Allah, who granted me the power and courage to finish this study.

I would like to deeply thank and appreciate Dr. Suheir Ereqat, my supervisor, for her skillful help and support. Special thanks to Dr. Abedelmajeed Nassereddin, my co-supervisor, for his support and help.

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Abstract

The high risk of cardiovascular events in diabetic patients is associated with dyslipidemia, a common concomitant disease of diabetes. Dyslipidemia comprises of a wide range of lipid disorders including over production of lipids or its deficiency. It is characterized by the presence of either elevated low density lipoprotein cholesterol (LDL-C) or elevated high total cholesterol (Hypercholesterolemia) or elevated triglycerides (Hypertriglyceridemia) or decreased high density lipoprotein cholesterol (HDL-C). Several studies have found that dyslipidemia can be associated by single nucleotide variations in specific genes. Therefore, the present study aimed to investigate GLP-1R gene polymorphisms rs10305420 and rs3765467 and its association with the risk of dyslipidemia in Palestinian patients with type 2 diabetes mellitus (T2DM). A cross sectional study was conducted between October 2023 and February 2024 at Jericho health center MOH. Herein, we used next generation sequencing (NGS) to study two single nucleotide polymorphisms (SNPs), rs10305420 and rs3765467 within the GLP-1R gene in one multiplex PCR tube. Bioinformatics analysis was done using free online galaxy program (https://usegalaxy.org.au/). A total of 216 T2DM patients were enrolled in this study and divided into two groups: The control group (patients without dyslipidemia) and case group (patients with dyslipidemia). The control group included patients aged 40 years or older with confirmed T2DM (diagnosed according to WHO criteria) and had no history of dyslipidemia (TC < 200 mg/dl, TG < 150 mg/dl, and no use of lipid-lowering agents), while the case group included patients who aged 40 years or older with confirmed T2DM and dyslipidemia. Dyslipidemia was defined by TC ≥ 240 mg/dl and/or TG ≥ 150 mg/dl, LDL-C ≥ 140 mg/dl, HDL-C < 40 mg/dl, and/or use of lipid-lowering drugs. Participants

under 40 years old, individuals with confirmed diagnosis of Type 1 Diabetes Mellitus, patients with incomplete medical records, patients with severe comorbidities (e.g., severe liver disease or cancer), and pregnant women were excluded from the study. The results showed higher rates of diabetic neuropathy, retinopathy, and hypertension among patients with dyslipidemia compared to those without dyslipidemia ($p < 0.05$). However, no statistically significant differences were observed in the prevalence of nephropathy, diabetic foot, or tobacco smoking status between the two groups, whereas female patients were more prevalent in the dyslipidemia group ($p = 0.04$). The genotype distributions of rs10305420 in the studied groups showed that the most frequent genotype in the dyslipidemia group was CC (27.8%), followed by CT (15.3%) and TT (7.9%). For those without dyslipidemia, the CC, CT, and TT genotypes were 26.4%, 17.6%, and 5.1%, respectively. The study showed no significant difference in the distribution of the rs10305420 genotype between the T2DM patients with and without dyslipidemia. For the second SNP, the GLP-1R rs3765467 polymorphism, almost all samples exhibited the GG genotype. The mean (TC, TG, and LDL) of T2DM patients with dyslipidemia were respectively higher than those without dyslipidemia (178.7 ± 39.2 vs. 155.5 ± 33.6, $p < 0.001$), (189.1 ± 140.1 vs. 106.7 ± 40.6, $p < 0.001$), and (103.1 ± 32.3 vs. 92.9 ± 27.6, $p < 0.015$). Mean age, BMI, systolic blood presure of T2DM patients with dyslipidemia were higher than those without dyslipidemia. In conclusion, the rs10305420 and rs3765467 polymorphisms in the GLP-1R gene were not significantly associate with dyslipidemia in this population. Further studies with larger sample sizes are needed to confirm these findings.

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Abbreviations

Abbreviation Term

GLP-1R : The glucagon-like peptide-1 receptor

HDL: high-density lipoprotein

LDL: low-density lipoprotein

BMI: body mass index

TC: total cholesterol

LDL-C: low density lipoprotein cholesterol

TG: triglyceride

HDL-C: high density lipoprotein cholesterol

CVD: cardiovascular disease

T2DM: type 2 diabetes mellitus

T1DM: Type 1 Diabetes Mellitus

GLP-1: Glucagon-like peptide 1

GWAS: genome-wide association studies

FPG: fasting plasma glucose

SBP: systolic blood pressure

DBP: diastolic blood pressure

HbA_{1c}: Glycosylated hemoglobin A_{1c}

RT-PCR: Real-time polymerase chain reaction

CAD: coronary artery disease

SNP: single nucleotide polymorphism

ASCVD: Atherosclerotic Cardiovascular Disease

AHEAD: Action for Health in Diabetes

Chapter One

1. Introduction

1.1 Dyslipidemia

Dyslipidemia is one of the most frequently diagnosed and treated chronic disorders. It is characterized by abnormal serum levels of cholesterol, triglycerides, or both, as well as abnormal levels of lipoproteins. An increased risk of atherosclerotic cardiovascular disease (ASCVD) is the most common clinical consequence of dyslipidemia, which is accompanied by elevated levels of total and low-density lipoprotein (LDL) cholesterol, triglycerides (TGs), lipoprotein(a) (Lp(a)), as well as decreased high-density lipoprotein (HDL) levels. A lipid, such as cholesterol or triglycerides, is absorbed from the intestines and then carried throughout the body via lipoproteins, causing the body to produce energy, steroid hormones, or bile acids. Cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides, and high-density lipoprotein (HDL) are major contributors to these pathways. Therefore, imbalances in any one or more of these organic or

nonorganic factors may lead to dyslipidemia (Pappan & Rehman, 2023). Dyslipidemia is classified into two types based on its causes: primary dyslipidemia, which is inherited and induced by genetic factors, and secondary dyslipidemia, which is caused by an underlying condition or factor, rather than being primary or genetic. Common causes include diabetes, obesity, hypothyroidism, kidney disease, liver disorders, and certain medications. Familial combined hyperlipidemia is a common cause of primary dyslipidemia in youth and teenagers which can lead to high cholesterol. Hyperapobetalipoproteinemia is caused by mutations in specific apolipoproteins found in LDL lipoproteins, as well as familial hypertriglyceridemia and homozygous familial or polygenic hypercholesterolemia, in which the LDL receptors are mutated. During an individual's lifetime, secondary dyslipidemia can be caused by a number of lifestyle factors or medical conditions that interfere with their blood lipid levels including alcoholism, diabetes, obesity and others. It is common to have secondary predisposing factors, such as obesity and type 2 diabetes. It has also been found that rare dyslipidemias are associated with clinical consequences, including pancreatitis with severe levels of triglycerides (TGs), hepatosteatosis and fat-soluble vitamin deficiencies in individuals with genetically compromised production of apolipoprotein (apo) B-containing lipoproteins. (Berberich & Hegele, 2022).

Several studies investigated the genetic basis of dyslipidemias and how various molecular factors contribute to dyslipidemias. Many large-scale randomized clinical trials have also provided a new information regarding the safety and efficacy of intensive lipid-lowering therapies. Dyslipidemias are a rapidly growing field of research, with studies providing a better understanding of their molecular basis and genetic origins, outlining how they contribute to atherosclerosis development, and clarifying how pharmacologic agents can help individuals with ASCVD reduce their risks (Merćep et al., 2022).

1.2 Diabetes mellitus

The Egyptians were the first to diagnose diabetes. Diabetes is characterized by weight loss and polyuria. Yet the Greek physician Aretaeus coined the term diabetes mellitus. Diabetics are known to be characterized by weight loss and polyuria, but their term is derived from Greek. Diabetes refers to "passing through" and mellitus, which is Latin for honey (meaning sweetness). As a chronic disease, diabetes mellitus is caused by an inherited or acquired inability of the pancreas to produce insulin, or by an ineffective insulin production. The result of such a deficiency is increased glucose concentrations in the blood, which, in turn, damages the body's systems, especially the blood vessels and nerves. During diabetes, the liver, skeletal muscle, and adipose tissue are not able to effectively meet insulin's requirements, which causes metabolic disturbances in biomolecules like carbohydrate, proteins, and fats (Goyal et al., 2023).

Among the metabolic diseases of diabetes mellitus are chronic hyperglycemia caused by defects in insulin secretion and action or both. Metabolic abnormalities in carbohydrates, lipids, and proteins caused by insulin's role as an anabolic hormone. As a result of low levels of insulin to achieve adequate responses or insulin resistance of target tissues, primarily skeletal muscles, adipose tissue, and to a lesser extent, the liver, lead to these metabolic abnormalities. These issues are caused by dysfunctions insulin receptors, signal transduction system, and effector enzymes or genes. Some diabetes patients are asymptomatic during the early stages of the disease, especially those with type 2 diabetes. The severity of symptoms depends on the type and duration of the disease. The symptoms of hyperglycemia, especially in children with absolute insulin deficiency, can include polyuria, polydipsia, polyphagia, weight loss, and blurry vision in children. Ketoacidosis or rare nonketotic hyperosmolar syndrome are caused by uncontrolled diabetes that lead to stupor, coma and if not treated death (Thongnak et al., 2020). A study conducted from

eleven primary health care clinics offering diabetes care, Ramallah and El-Bireh governorate, reported that among 517 diabetic patients; 80% of them showed raised HbA1c levels, 62% were obese, 50% had uncontrolled hypertension, and 87% abnormal lipid levels. More than one-third of the patients had diabetes for longer than 10 years, and 76% had at least one complication. 40% of the patients took insulin, 85% self-reported not to smoke, and 30% were physically active (Kharroubi & Darwish, 2015).

1.2.1 Types of diabetes mellitus

The most common classifications of diabetes include type 1, type 2 diabetes mellitus, and gestational diabetes, all of which are heterogeneous, complex metabolic disorders characterized by elevated blood glucose levels as a result of resistance to insulin, insufficient insulin secretion, or both. Insulin resistance and a relative deficiency of insulin secretion are characteristics of Type 2 diabetes (T2DM). As a result of insulin resistance, there is usually an increase in absolute plasma insulin concentrations (both fasting and meal-stimulated), but "relative" to the severity of insulin resistance, the plasma insulin concentrations are insufficient to maintain normal glucose homeostasis. Diabetes patients with T2DM tend to lose insulin secretion capacity over time. Most people with type 1 diabetes mellitus have an absolute deficiency in beta-cell function as a result of an autoimmune attack on beta-cells. This disease remains classified as idiopathic, though it may be a result of autoimmune attack on beta-cells (Tan et al., 2019). A woman with gestational diabetes mellitus (GDM) develops glucose intolerance during pregnancy, which is defined as a form of diabetes mellitus. A woman with GDM is more likely to develop type 2 diabetes later in life if she develops the disorder during the third trimester. An excess of certain hormones like growth hormone and glucocorticoids may also contribute to diabetes. Genetic disorders, diseases

that damage the pancreas are all possible causes of diabetes. Besides drugs, chemicals, and infections. Classification of diabetes helps determine the appropriate treatment.) Solis-Herrera, Triplitt, Reasner, DeFronzo, & Cersosimo, 2015).

1.3 Diabetic dyslipidemia

Defining dyslipidemia as increased total cholesterol levels, low density lipoprotein cholesterol levels (LDL-C), and plasma triglyceride levels (TG) or low plasma high density lipoprotein cholesterol levels (HDL-C) or a combination of these features. T2DM patients suffer from diabetic dyslipidemia, a complex metabolic disorder. Patients with T2DM are significantly more likely to develop cardiovascular disease (CVD) if they have both quantitative and qualitative changes in lipids and lipoproteins in their blood. Basically, dyslipidemia is a disorder of lipid metabolism that causes abnormal amounts of lipids to circulate in the bloodstream. This condition is clinically defined as elevated TC, TG, LDL-C, and decreased HDL-C levels (Ali et al., 2019).

So, diabetic dyslipidemia is a significant risk factor for CVD in T2DM patients and driven by insulin resistance and worsened by lipotoxicity, both of which contribute to abnormal lipid profiles and metabolic dysfunction. Diabetes patients with T2DM frequently exhibit lipid abnormalities before they develop diabetes as part of the insulin-resistant metabolic syndrome, which is characterized by an accumulation of triacylglycerol-rich lipoproteins in the plasma, as well as small, dense LDL particles with reduced HDL cholesterol levels. Cardiovascular disease is associated with these lipid abnormalities in individuals with the metabolic syndrome. It is clear that insulin resistance play an important role in the pathophysiology of diabetic dyslipidemia, as

evidenced by the presence of lipid abnormalities that are characteristic of diabetic dyslipidemia in non-diabetic insulin-resistant first-degree relatives of patients with T2DM (Wu & Parhofer, 2014)

1.3.1 Pathophysiology of diabetic dyslipidemia

In spite of the complexity and incomplete understanding of diabetic dyslipidemia, our understanding of how it develops is still limited. Low HDL-cholesterol, small dense LDL and hypertriglyceridemia can be detected years before the clinical diagnosis of type-2 diabetes in insulin-resistant, prediabetic individuals with normal glucose concentrations. As a result, hyperglycemia alone cannot fully explain lipid changes in diabetics. Insulin resistance is thought to be the main contributing factor to diabetic dyslipidemia. The dominant lipid abnormality in insulin resistance is hypertriglyceridemia, which plays a pivotal role in determining diabetic dyslipidemia's characteristic lipid profile. In both fasting and non-fasting states, elevated triglyceride levels are associated with increased production and decreased clearance of triglyceride-rich lipoproteins. The increased production of very low- density lipoprotein (VLDL), the main transporter of fasting triglycerides, is one of the prominent signs of insulin resistance. At all stages of VLDL secretion and production, insulin is involved. In adipose tissues, insulin suppresses lipolysis by inhibiting the activity of hormone sensitive lipase, which metabolizes stored triglycerides into free fatty acids. As a result, insulin regulates the amount of circulating free fatty acids, which serve as substrates and regulatory factors for VLDL formation and secretion. In the liver, insulin suppress microsomal triglyceride transfer protein transcription that mediates triglycerides transfer to nascent apolipoprotein B (apoB) which is the predominant surface protein

of VLDL. ApoB production rate is relatively constant, so that the amount of released apoB is determined by its degradation rate that depends on lipidation amount. Therefore, insulin-resistant individuals produce more VLDL because of the increased availability of free fatty acids in the liver. In addition to VLDL overproduction, hypertriglyceridemia can also be caused by a decrease in its clearance rate. A decrease in clearance rate is associated with impaired lipoprotein lipase activity, a decrease in VLDL uptake, and an increase in postprandial triglyceride-rich chylomicrons. In delipidation cascade, the key enzyme in the VLDL–intermediate density lipoprotein–LDL is Lipoprotein lipase. High free fatty acids can directly deteriorate lipoprotein lipase activity by detaching it from the endothelial surface (Wu & Parhofer, 2014).

Diabetes-induced dyslipidemia is characterized by postprandial hypertriglyceridemia, which occurs when both intestinal and liver triglyceride-rich lipoproteins are overproduced. Type-2 diabetes leads to an increased production rate of apoB-48 (Figure 1.1), which correlates with insulin levels. The mechanisms underlying these alterations are not known, However, elevated levels of free fatty acids may again determine the causative mechanisms. Also, insulin resistance may induce postprandial hypertriglyceridemia by affecting incretin (glucagon-like peptides-1,-2, and gastric inhibitory polypeptide) secretion and levels. Consequently, an elevated level of intestinal-derived chylomicrons can prolong the presence of hepatic-derived VLDL in plasma and vice versa. The result is that diabetic patients have higher triglyceride levels, regardless of whether they are fasting or not (Wu & Parhofer, 2014).

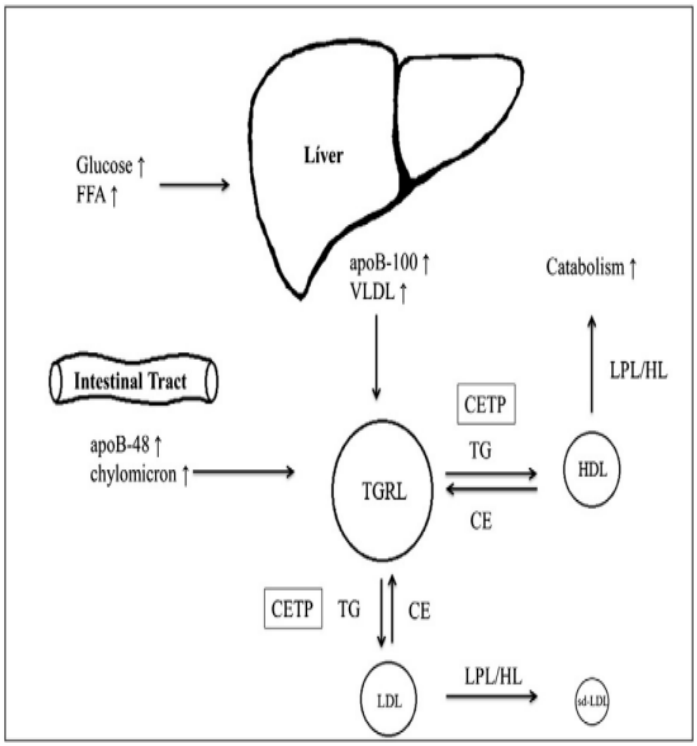

Figure 1. 1 Pathophysiology of diabetic dyslipidemia: Effects of diabetes on triglyceride-rich lipoproteins, HDL and LDL. Increased availability of glucose and free fatty acids in the liver leads to decreased degradation of apoB-100 and increased secretion of VLDL. In the postprandial state, apoB-48 and chylomicrons are produced at a higher rate in the intestinal tract. Both liver-derived VLDL and intestinal-derived chylomicrons contribute to the overabundance of triglyceride-rich lipoproteins in blood. CETP facilitates the exchange of triglycerides and cholesteryl esters between triglyceride-rich lipoproteins and LDL as well as HDL. Subsequently, triglyceride rich LDL and PW HDL are hydrolyzed by lipoprotein lipase or hepatic lipase. apoB: apolipoprotein; CE: cholesteryl ester, CETP: cholesterol transfer protein; FFA: free fatty acids; HDL: high-density lipoprotein; HL: hepatic lipase; LDL: low-density lipoprotein; LPL:tivate Wind lipoprotein lipase; sd-LDL: small-dense low-density lipoprotein; TG: triglyceride; TGRL: triglyceride-rich lipoprotein. (Wu & Parhofer, 2014).

1.3.2 Insulin resistance

Diabetes dyslipidemia is mainly caused by insulin resistance, a hallmark of Type 2 diabetes. Insulin resistance refers to a body's cells that do not respond effectively to insulin, leading to elevated blood sugar levels. The increased insulin resistance associated with diabetic dyslipidemia causes a series of abnormal changes in blood lipids and lipoproteins. Furthermore, lipotoxicity, an adverse effect of excess lipids or free fatty acids in non-fat tissues, intensifies diabetes' severity. As a result, lipotoxicity increases insulin resistance by activating protein kinase pathways. Diabetic dyslipidemia is a significant risk factor for cardiovascular disease in T2DM patients, as these pathways interfere with insulin signaling, reducing its effectiveness in regulating blood glucose and lipid levels (Thongnak et al., 2020). Having insulin resistance and lipotoxicity contributes to abnormal lipid profiles and metabolic dysfunction, which are both contributing factors. In order to reduce cardiovascular risk and improve overall health outcomes, it is essential to manage insulin resistance and dyslipidemia in the comprehensive care of individuals with T2DM. As an integral part of diabetes management, controlling diabetic dyslipidemia helps reduce the risk of cardiovascular disease (Wu & Parhofer, 2014). There are certain types of dyslipidemias that may remain clinically silent for many years before receiving genetic diagnosis and treatment, but diagnosing and treating them early can prevent or delay downstream clinical complications (Holst et al., 2018).

1.4 Prevalence of diabetic dyslipidemia in Palestinian population

There is an increase in the prevalence of dyslipidemia worldwide, and the risk of mixed forms of dyslipidemia is also on the rise due to the increase in the prevalence of other metabolic diseases such as diabetes mellitus, hypertension, and metabolic syndrome. There is an increased prevalence and incidence rates of diabetes mellitus among Arab populations, one of the most common noncommunicable diseases encountered in primary care clinics. Diabetes is an increasing health challenge throughout the world, with up to 422 million people been diagnosed with diabetes worldwide in 2014 and 80% of the population in developing countries being diagnosed with diabetes. According to the WHO reports, death caused by diabetes will double by the year 2030 (Temesgen & Syoum, 2017). Approximately, 285 million people worldwide were affected by diabetes in 2010, and by 2030, over 438 million people will be diagnosed with diabetes (Omodanisi et al., 2020). People in the Middle East and Palestine face an increasing prevalence of diabetes (Abu-Rmeileh et al., 2013). Based on estimates using 88 papers, including T2DM papers published between 1980 and 2015 from Arab states, the mean prevalence was estimated at 16.2%.

(Meo, Usmani, & Qalbani, 2017) .

In Palestine, the prevalence is projected to increase from 18.4% in 2015 to 21.5% in 2030 (Abu-Rmeileh et al., 2013). Palestine is in epidemiological transition with diabetes emerging as one of the leading causes of morbidity and mortality in the region (Husseini et al., 2009), (Rahim et al., 2014). Diabetes mellitus has increased from the 10th ranking cause of all deaths in 2005 to the 5th ranking cause of all deaths in 2018 (Radwan et al., 2018). According to the Palestinian annual health report for 2019, there were 5671 new diabetic cases with a prevalence rate of 210.4 per

100,000. Furthermore, diabetes and its complications constitute 12.1% of all Palestinian deaths, followed by cardiovascular diseases (29.9%) and cancer (15.5%).

Based on a study conducted by the Diabetes Care Center at Augusta Victoria Hospital in Jerusalem, 23% and 37.3% of 1308 diabetic patients had hypertension and dyslipidemia, respectively. Furthermore, 16.3% had previously suffered from macrovascular disease (myocardial infarction or stroke), and 25.9% had suffered microvascular complications.

It is most common among adults and leads to serious complications in central organs such as cardiovascular, neuronal, retinal, and renal systems. Diabetes mellitus is indeed a complex condition influenced by both genetic and environmental factors. Changing dietary and lifestyle patterns, reducing physical activity, and working long hours sedentary are all factors that may contribute to dyslipidemia, so lifestyle modification is considered the best way to manage the disease. It can be difficult to maintain, and achieve acceptable compliance in the elderly. In these cases, lifestyle changes and drug therapy are the best ways to achieve a successful treatment outcome (Ali et al., 2019).

1.5 Therapeutic strategies of diabetic dyslipidemia

A diabetic's dyslipidemia can be treated non-pharmacologically and pharmacologically. Non-pharmacological treatments include medical nutrition therapy, weight loss, and physical activity. Patients with diabetes should consume more plant stanols/sterols, viscous fiber (legumes, citrus, oats), omega-3 fatty acids, and reduce saturated fats and trans fats. The consumption of walnut-rich diet in a randomized study improved non-HDL cholesterol and apolipoprotein B levels. Tree

nuts, peanuts, grains are good sources of unsaturated fat that can help reduce cholesterol, blood pressure, and CVD and diabetes risks (Jialal & Vikram, 2017) (Wu et al., 2014).

The reduction of body weight by about 5% leads to an improvement in lipid profile, insulin resistance, and glycemic control (Klein et al., 2004). Despite the fact that weight loss has been shown to improve multiple risk factors, including hemoglobin A1C and blood pressure, weight loss decreases triglycerides, raises HDL-C levels, and can also reduce blood pressure (Wing et al., 2011). According to the action for health in diabetes (AHEAD) study, long-term weight loss with intensive lifestyle change did not result in a reduction in cardiovascular events (CVEs), which indicates that pharmacotherapy and lifestyle modification are needed to reduce Atherosclerotic Cardiovascular Disease (ASCVD) (Jialal & Vikram, 2017).The pharmacological therapy such as statins, cholesterol absorption inhibitors, niacin, fibrates, PCSK9 inhibitors and omega-3 fatty acids. The drugs that lower LDL-cholesterol effectively and safely are illustrated in Table 1.1.

Table 1: Summary of low- density lipoprotein cholesterol lowering medications.

Drug class	Mechanism of action	Clinical eficacy	Adverse reactions
Statins	Inhibition of HMG coenzyme A Reductase	Highly effective	Myalgia, myositis, rhabdomyolysis, elevation in liver enzymes, new onset diabetes
Ezetimibe	Decrease intestinal cholesterol absorption by binding to Niemann-Pick C1-like 1 protein	Moderately effective; Safe addition to statin therapy	Worsening of liver function, myopathy or rhabdomyolysis if added to statins; Nasopharyngitis, diarrhea, upper respiratory tract infection
PCSK9 inhibitors	Inhibition of Proprotein Convertase Subtilisin/Kexin Type 9	Very highly effective in combination with statin therapy	Injection site reaction including itching, swelling, erythema and pain
Bile acid sequestrants	Bind bile acids in the small intestine and prevent reabsorption	Moderately effective, safe addition to statin therapy, not desirable if triglycerides are > 300 mg/dL	Constipation, abdominal pain, bloating, drug malabsorption

HMG: Hydroxymethylglutaryl; PCSK9: Proprotein convertase subtilisin/kexin type 9.

Among the primary and secondary preventive measures of CVD and stroke are statins, which inhibit 3-hydroxymethylglutaryl coenzyme A, a rate-limiting step in cholesterol synthesis. In addition to lowering the level of plasma LDL cholesterol, statins also lower the level of TG and

increase HDL levels. By decreasing cholesterol levels in the liver, LDL receptors are upregulated, leading to a reduction in plasma LDL cholesterol (Wu et al., 2014).

Ezetimibe is a medication reduces the level of cholesterol by inhibiting intestinal cholesterol absorption and when ezetimibe combined with statins, LDL-C will be significantly reduced. It is also useful in patients who are unable to tolerate statin therapy(Bays, Neff, Tomassini, & Tershakovec, 2008).

The fibrates include bezafibrate, gemfibrozil, ciprofibrate, and fenofibrate that stimulate lipoprotein lipase activity and reduce triglyceride levels by activating nuclear peroxisome proliferator-activated receptor alpha. The fasting plasma triglycerides level can be decreased by 30%-50% by fibrates, while the postprandial lipemia can be decreased by reducing fatty acid synthesis (Staels & Auwerx, 1998).

The drug niacin is very effective at improving HDL-cholesterol levels. TG and LDL-cholesterol levels are also lower with niacin, but a combined statin-niacin treatment showed no additional benefit when compared to statin treatment alone (Miller, 2003).

Alirocumab and Evolocumab, both powerful proprotein convertase subtilisin/kexin 9 inhibitors, can significantly lower LDL-C when used as monotherapy or combined with statins. Inhibitors of PCSK9 prevent PCSK9 from binding to LDL receptors and removing them from the lysosomal system of the liver by binding to PCSK9. Expression of LDL receptors will be increased causing LDL-C level reduction (Orringer et al., 2017).

In addition to lowering triglyceride levels, omega-3 fatty acid formulations, which contain eicosapentaenoic acid (EPA) and docosahexaenoic acid, are often used as additional therapy (Oikawa et al., 2009).

1.6 Glucagon-like peptide 1 (GLP-1) and its Role

A biologically active hormone, glucagon -like peptide 1 (GLP-1) is involved in glucose and lipid regulation and metabolism. As a result of nutrient intake, specialized cells in the intestinal tract secrete glucagon-like peptide 1 (GLP-1), primarily in the distal small intestine and ileum. Known as incretins, GLP-1 belongs to a family of hormones released postprandially (after meals) to modulate metabolism. Among the most significant actions of GLP-1 is its ability to boost pancreatic beta cell insulin secretion in a glucose-dependent manner. As a result, it reduces blood sugar spikes after meals by promoting insulin release when blood glucose levels are elevated. In addition, GLP-1 inhibits pancreatic alpha cells from producing glucagon which is a hormone that raises blood sugar (Baggio & Drucker, 2007). GLP-1R, encoded by the GLP-1R gene, is a G-protein-coupled receptor (GPCR) belonging to the B1 class with the characteristic seven-transmembrane helical core domains and one extracellular domain (Parthier et al., 2009). It has a role in receptor activation and hormone signaling by conjugation with heterotrimeric G-proteins (de Graaf et al., 2016). GLP-1 or GLP-1R agonists display a diverse range of physiological functions as shown in the figure below.

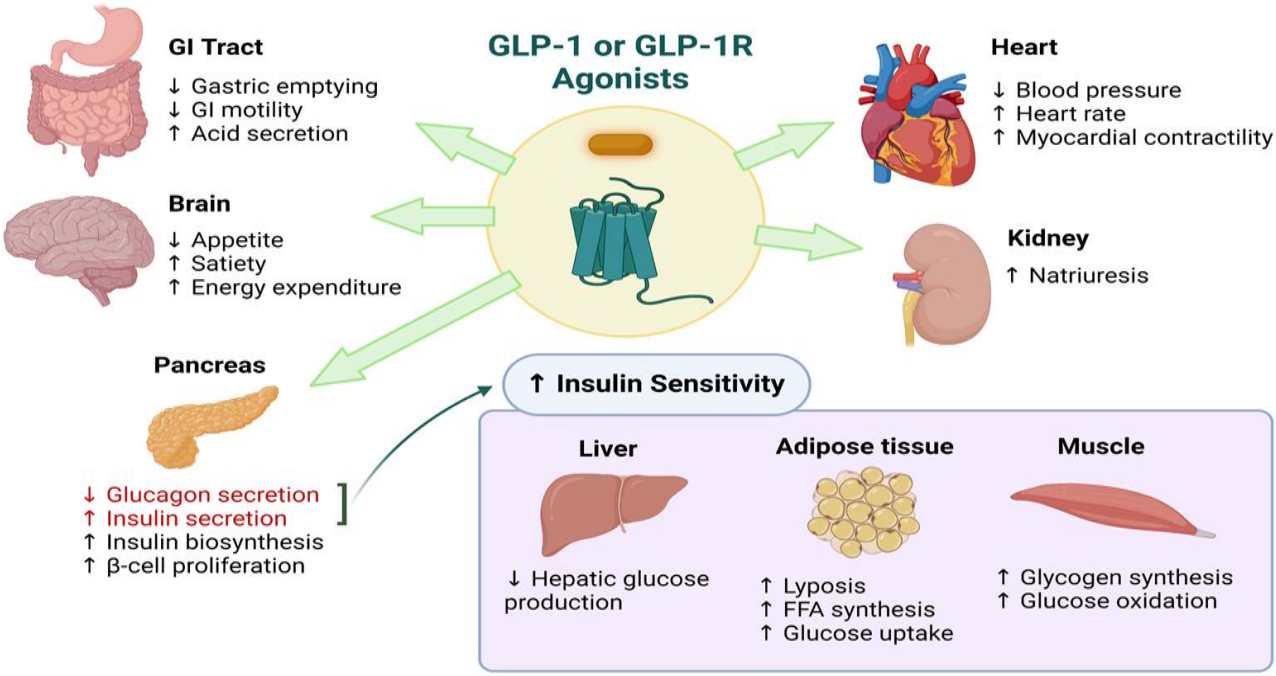

Figure 1. 2: Pleitropic effects of GLP-1 or GLP-1R agonists.

Cardiovascular Diabetology. 2014, 13:142 http://www.cardiab.com/content/13/1/142.

1.6.1 Genomic location

GLP-1R, encoded by the GLP-1R gene, is a B1 class G-protein-coupled receptor (GPCR) with a typical seven-transmembrane α-helical core domain and one extracellular domain(Parthier, Reedtz-Runge, Rudolph, & Stubbs, 2009), which plays an important role in hormone signaling and receptor activation through conjugation with heterotrimer G-proteins (De Graaf et al., 2016).GLP-1R gene is located on human chromosome 6p21 and contains 13 exons encoding 463 amino acids with a total length of 42 500 base pairs (Parthier et al., 2009).

The polymorphism of rs10305420 results in the substitution of proline with leucine (CCG-CTG) at position 7 in exon 1, and rs3765467 polymorphism results in the mutation of arginine to glutamate at position 131 of exon 4(Tokuyama et al., 2004).

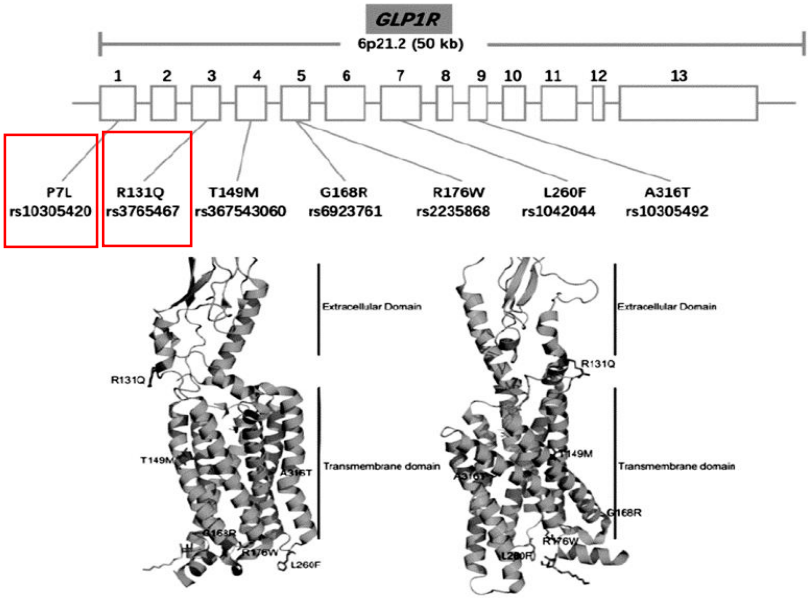

Figure 1. 3 :Genomic location of GLP-1R gene and single nucleotide polymorphisms DNA Cell Biol. doi:10.1089/dna2020.5424**.**

1.7 Objectives of the study

The relationship between Glucagon-Like Peptide 1 Receptor (GLP-1R) genetic polymorphisms and lipid levels remains poorly understood, with significant gaps in our knowledge. In this study, the relationship between GLP-1R genetic polymorphisms and the risk of dyslipidemia will be investigated in Palestinian T2DM patients, so as to provide reference for further revealing the clinical significance of GLP-1R genetic polymorphism. The project focuses on using advanced sequencing technologies to study two genetic variants within the GLP-1R gene in Type 2 Diabetes Mellitus Palestinian patients.

General objective

to investigate the association between GLP-1R gene polymorphism rs10305420 and rs3765467 and the presence of dyslipidemia in Palestinian patients with type 2 diabetes mellitus.

Specific objectives:

1. To assess the prevalence and characteristics of dyslipidemia in in type 2 diabetic patients.

2. To identify potential subgroups of patients at higher risk of dyslipidemia based on two genetic polymorphisms in the GLP-1R gene.

3. To explore any associations between GLP-1R gene variants and specific lipid profile abnormalities.

1.8 Literature review

1.8.1 Genetic variations in glucagon-like peptide 1 receptor and dyslipidemia in T2DM

There are many SNPs with mutation patterns that can change the amino acid sequences of GLP-1R gene, and most five common genetic variation loci are rs10305420, rs3765467, rs6923761, rs2235868 and rs1042044 (Ma et al., 2018). Several studies investigated GLP-1R polymorphisms related to glucose levels and insulin secretion and the pathogenesis of obesity and T2DM diabetes. A study in China conducted by Li et al. (2023), examined the relationship between GLP-1R gene polymorphisms and type 2 diabetes mellitus with and without dyslipidemia. The study involved 200 diabetics with type 2 diabetes mellitus (T2DM), of whom 115 had dyslipidemia and 85 did not. Using Sanger double deoxygenation terminal sequencing assay and (PCR-Restriction fragment length polymorphism), PCR-RFLP, genotypes were determined for GLP-1R rs10305420 and rs3765467 loci. The number of alleles and genotype distribution of rs3765467 differed significantly between patients with and without dyslipidemia GG 69.6%, GA + AA 30.4%; P = 0.017) vs. (GG 52.9%, GA + AA 47.1%. Variant (G/A) rs3765467 is associated with dyslipidemia, and G allele is associated with dyslipidemia risk.

A pilot study in 88 non-diabetic subjects investigated 21 GLP1-R tag single nucleotide polymorphisms (SNPs). Among them, rs6923761 (Gly168Ser) and rs3765467 (Arg131Gln) were nominally associated with decreased insulin secretion stimulated by hyperglycemia and GLP-1 infusion (Sathananthan, Dalla Man, et al., 2010). A mouse ß-cell model system study showed that besides rs3765467 (Arg131Gln), rs10305492 (Ala316Thr) also significantly reduced glucose-stimulated insulin secretion. Furthermore, these SNPs also decreased β cells viability and mass by promoting β cell apoptosis (Li et al., 2020).

Another study found that the nonsynonymous SNP locus rs367543060 is associated with T2DM susceptibility (Tokuyama et al., 2004) and its expression can reduce the receptor affinity and intracellular signaling of GLP-1R (Beinborn et al., 2005). Mutations at the rs10305420 locus affect glycemic response and body weight in patients with T2DM treated with exenatide, a GLP-1 receptor agonist (Yu et al., 2019) . Patients with the rs10305420 T allele have a worse treatment response to liraglutide for weight loss than those with CC genotype (Corcos et al., 1989). As mentioned above, the polymorphisms of the GLP-1R gene has important reference value for predicting insulin secretion in vivo and the efficacy of GLP-1 receptor agonists (Berberich & Hegele, 2019). The rs3765467 (G/A) variant is associated with the incidence of dyslipidemia, and G allele may be a risk factor for dyslipidemia(Y. Li et al., 2023).

In another study done by Li et al. (2020) revealed that two SNPs within the GLP1R gene, rs3765467 and rs10305492, could significantly reduce insulin secretion and cyclic AMP concentration, while promoting apoptosis in cells. Both rs3765467 and rs10305492 affected pancreatic cell insulin secretion and viability under high glucose conditions; accordingly, these variants suppress insulin secretion following glucose stimulation. Furthermore, the GLP-1 agonist Exendin (9-39) augmented, whereas the GLP-1 agonist Exendin-4 moderately reduced the effects of SNPs on cell functions and apoptosis. Taking these two SNPs into consideration, it is shown that rs3765467 and rs10305492 in GLP1R play a critical role in regulating insulin secretory capacity and cell mass. Through leading to cell dysfunction and apoptosis, GLP1R rs3765467 and rs10305492 may also alter the GLP-1-GLP1R interaction.

Skuratovskaia et al. (2019) examine the relationship between the Leu260Phe polymorphism of the GLP-1R gene (rs1042044) and postprandial hormone production in obese diabetics (C-peptide, insulin, ghrelin, GLP-1) was carried out in 174 obese patients with type 2 diabetes, of which 82

had obesity with type 2 diabetes. GLP-1R gene polymorphisms (rs1042044) were determined by PCR using the polymorphism sets for the GLP-1R gene, CC genotypes of the Leu260Phe polymorphism of GLP-1 receptor genes are associated with higher postprandial plasma levels of C-peptide and insulin in obese patients with type 2 diabetes, while CA genotypes are associated with lower levels.

A genetic risk assessment of dyslipidemia in diabetics has proven to be a helpful strategy in preventing or delaying diabetes complications. Since dyslipidemia is associated with mutations in specific genes, genetic risk assessment has become an important strategy in preventing diabetes complications from developing (Kathiresan et al., 2009). Since there are insufficient studies to analyze the association between GLP-1R gene and dyslipidemia, and there is a lack of clinical data on Palestinians, this study will investigate the role of GLP-1R in T2DM. We will study the effects of polymorphisms of GLP-1R rs10305420 and GLP-1R rs3765467 loci on glycolipid metabolism in Palestinian patients and the genetic risk factors for dyslipidemia will be explored.

Chapter two

2. Materials and Methods

2.1 Study design and study samples

A cross sectional descriptive study was conducted at Jericho Health MOH Center, Palestine on 216 T2DM participants who were recruited between October 2023 and February 2024. We used purposive sampling technique to select the study participants who met followed specific criteria. Study participants were divided into two groups: control group (patients without dyslipidemia, n = 106), included patients aged 40 years or older with confirmed T2DM (diagnosed according to WHO criteria) and had no history of dyslipidemia (TC < 200 mg/dl, TG < 150 mg/dl, and no use

of lipid- lowering agents), while the cases group (patients with dyslipidemia, n = 110) included patients who aged 40 years or older with confirmed T2DM and dyslipidemia. Dyslipidemia was defined by TC ≥ 240 mg/dl, and/or TG ≥ 150 mg/dl, LDL-cholesterol ≥ 140 mg/dl, HDL-cholesterol < 40 mg/dl, and/or use of lipid- lowering drugs. Participants under 40 years old, individuals with confirmed diagnosis of T1DM, patients with incomplete medical records, patients with severe comorbidities (e.g., severe liver disease or cancer) and pregnant women were excluded from the study. Individuals with a history of lipid- lowering drug use were also excluded from the control group. Demographic and clinical data, including age, gender, BMI, treatment regimens, and diabetic complications, were obtained from medical records. Biochemical measurements (FBG, HbA1c, TC, TG, HDL cholesterol, and LDL cholesterol) were analyzed using the ARCHITECT C4000 instrument. The study protocol was approved by the central research ethical committee-AQU (333\REC\2023). The approval also was obtained from Jericho Health Center to collect the samples. Written informed consent was obtained from all participants. All patients' names were restricted and shared between the supervisor and Jericho health MOH Center that provided these samples.

2.2 Study investigations

2.2.1 Biochemical analysis

About 5 ml venous blood samples were drawn from all study participants, under quality control and safety procedures. Two milliliters of the collected blood were placed into sterile ethylene diamine tetra acetic acid (EDTA) tubes to be used for the determination of hemoglobinA1c

(HbA1c) by ARCHITECT C4000 Instrument and for DNA extraction and consequent SNPs genotyping. Three milliliters were delivered in plain tubes and left for a while without anticoagulant to allow blood to clot. The tubes were been then centrifuged at 3000 rpm for 10 minutes and the serum was collected into fresh tubes. The obtained serum used for the determination of: FBS, TC, TG, HDL-C, LDL-C by ARCHITECT C4000 Instrument at MOH Jericho Health Laboratory. All of these tests are part of routine diagnostic procedures, and the data have been extracted from the patient's medical record and laboratory tests.

2.3Molecular analysis

2.3.1 DNA extraction

Genomic DNAs were extracted from whole blood using NucleoSpin® Blood kit as described by the manufacturers. In brief, 25 µl of proteinase K and 200 µl buffer 3 were added to 200 µl blood sample to digest any proteins present, then incubated for 15 minutes at 70°C. Then, 210 µl of ethanol was added and mixed by pipetting. For each preparation, one NucleoSpin® Blood Column placed in a collection tube was taken and samples were loaded. Then centrifugation for 1 min at 11,000 x g was done. Collection tube containing flow-through was discarded; the column was placed into a new 2ml collection tube. A wash buffer (BW- 500 µl) was added then centrifuged at 11000 x g, the flow-through was discarded and column was placed back into the collection tube. A Wash buffer B5 (600 µl) was added then centrifuged at 11000 x g. The collection tube containing the flow-through solution was discarded. NucleoSpin® Blood Column was placed back into the collection tube and centrifuged for 1 minute at 11,000 x g. Column was transferred to sterile 1.5 ml microcentrifuge tube. Finally, 100 µl of preheated Buffer (BE) was added to elute genomic

DNA, then incubated for 1 minute and centrifuged at 11000 x g. At last, the concentration of DNA samples was measured by nanodrop spectrophotometer 1000 (ND-1000), representative samples are shown in Table 2.1.

Table 2.1: The concentration of DNA in representative samples measured by nanodrop spectrophotometer 1000 (ND-1000).

Sample number	**Sample code**	**Concentration ng/ul**
1	**1**	**31.29**
2	**23**	**35.16**
3	**34**	**30.01**
4	**14**	**39.30**
5	**22**	**37.71**
6	**28**	**39.74**
7	**7**	**33.79**
8	**12**	**43.71**
9	**5**	**45.71**
10	**4**	**29.95**

2.3.2 Primer selection

In this study, to design new primers targeting the studied SNPs, fragments of GLP-1R gene were selected, including the target SNPS rs10305420 and rs3765467. The NCBI database (https://www.ncbi.nlm.nih.gov/snp) was queried to retrieve the GLP-1R gene sequence housing the SNPs of interest. Following sequence retrieval, through identification of the flanking regions adjacent to each SNP was performed. The primers were designed using the online software, Primer3 (https://primer3.ut.ee/). For each SNP (rs10305420 and rs3765467), two primers (forward and reverse) were designed to amplify fragments of 206bp and 170bp, respectively as shown in figures 2.1and 2.2.

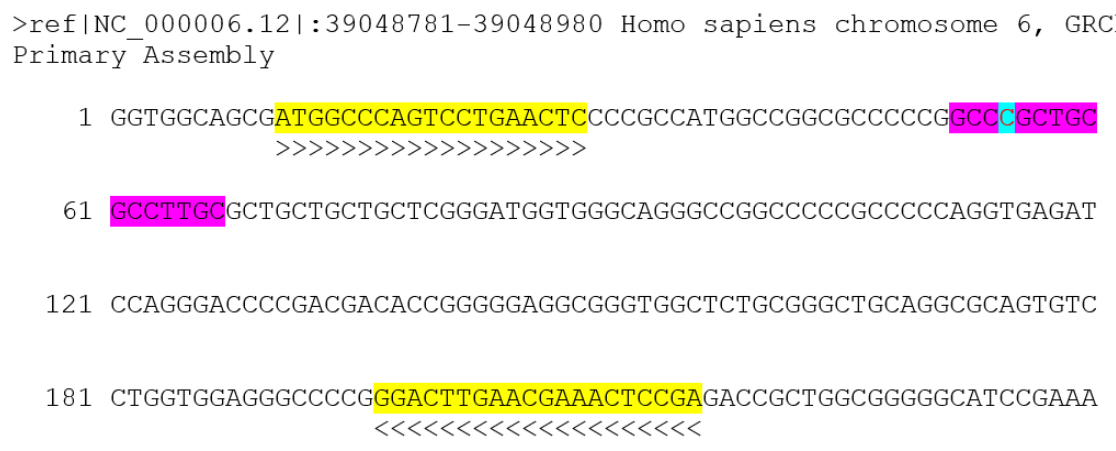

Figure 2.1 :Primer sequences of rs10305420 in GLP-1R. The primers targeting 206 bp PCR product, yellow shade show the primers localization, purple is the virtual probe and the blue is the target SNP.

>ref|NC_000006.12|:39065701-39066060 Homo sapiens chromosome 6, GRCh38.p14 Primary Assembly
CCCACCCCAGTGCCGCAGGGCCACGTGTACCGGTTCTGCACAGCTGAAGGCCTCTGGCTGCAGAAGGACA
ACTCCAGCCTGCCCTGGAGGGACTTGTCGGAGTGCGAGGAGTCCAAGCGAGGGGAAAGAGTGAGTTGAGG
CGGGGTTCTGAGCCAGGGAGCGGGGAGCCATGTCTTGGAGCACTTCACTGGAGCAAAGACCCTTGGCTTT

Figure 2.2 :Primer sequences of rs3765467 in GLP-1R. The primers targeting 170 bp PCR product, yellow shade show the primers localization, purple is the virtual probe and the blue is the target SNP.

Illumina adaptor sequences (76bp) were added to the forward ends of the 5' ends (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3') and reverse (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'). Two specific virtual probe sequences were used to identify the polymorphisms at this locus as shown in figures 2.1 and 2.2. The primers and probe sequences and the expected PCR product in bp are shown in Table2.2

Table 2.2: Primer sequences and virtual probes of rs10305420 and rs3765467 in GLP-1R.

SNP	Primer sequence	PCR product size plus76bp adaptors	Probe name	Probe sequence	Targeted SNP
rs10305420	**F: ATGGCCCAGTCCTGAACTC**	**282 bp**	**rs10305420C**	**GCCCGCTGCGCCTTGC**	**C**
	R: TCGGAGTTTCGTTCAAGTCC		**rs10305420T**	**GCCTGCTGCGCCTTGC**	**T**
rs3765467	**F: ACGTGTACCGGTTCTGCAC**	**246bp**	**rs3765467G**	**CCAAGCGAGG**	**G**
	R: TCCAGTGAAGTGCTCCAAGA		**rs3765467A**	**CCAAGCAAGG**	**A**

2.3.3 DNA amplification

The DNA library was prepared using two PCR steps. The first one, is a multiplex PCR, which was performed with four primers (two forward and two reverse), targeting the regions contained the two SNPs of GLP-1R (rs10305420 and rs3765467). Amplification of GLP-1R rs10305420 and rs3765467 were performed using primers shown in Table 2.2. The reaction was carried out using 2.5 µl of the extracted DNA in a final volume of 25µl, which contained 12.5 µl X2 PCRBIO HS Taq Mix Red (PCR Biosystems, Ltd.), 6 µl double distilled water (dH2O) and 1 µl of each primer (10 uM). The amplification conditions were as follows: Initial denaturation at 95˚C for 5 minutes followed by cycles 25 cycles of 95˚C for 30 seconds, 58˚C for 30 seconds, and 72˚C for 40 seconds, with a final extension step of 72˚C for 6 minutes.

2.3.4 Gel Electrophoresis

The product was loaded on 2% agarose gel, two bands at the DNA molecular size around 200 bp (170 bp and the Illumina adaptors) and 250 bp (206 bp and the Illumina adaptors) for GLP-1R showed a successful amplification.

For gel preparation, approximately 2g of agarose was added to 100 ml Tris-acetate-EDTA (TAE) buffer, then the mixture was boiled for two minutes, followed by the addition of 2-3 drops (10mg/ml) ethidium bromide. The mixture was poured into an agarose gel casting system (Bio-Rad, SUBCELL®GT). In each well, 5 µl of each PCR product was loaded. Samples were then run at 120 volts for 30 minutes. PCR products were visualized on gel documentation system (GelDoc).

2.3.5 Cleaning PCR product

The PCR products (20 µl) were cleaned by AMPure XP beads, Beckman Coulter (X1), and eluted in 25 µl elution buffer (EB). In order to purify samples, AMPure XP beads (20 µl) were added to 20µl of each sample at room temperature and incubated for 10 minutes. On a magnetic plate, samples were left for two minutes until they became clear, After the supernatant was discarded, the pellets were washed two times with ethanol 80%(180 µl) during their stand on the magnet plate and left for 5 minutes to dry. A final step involved adding 25 µl of EB buffer to each sample and incubating it at room temperature for 5 minutes. Then, transferring the supernatant (25µl) to new collection tubes and keep frozen at -20C until further use.

2.3.6 Second PCR amplification and Library preparation

The purified products were subjected to a second PCR, the purpose of this PCR is to give DNA sequence barcode for each sample, using special PCR primers with specific barcodes (indexes). The reaction was carried out using 7.5 µl cleaned DNA with 12.5 µl X2 PCRBIO HS Taq Mix Red (PCR Biosystems, Ltd.) and 2µl of 2 different indexes (barcodes) using Nextera XT Index Kit (Illumina, San Diego, CA, USA). The PCR conditions for barcoding were: 72°C for 3 minutes, 95°C for 30 seconds, followed with 10 cycles of 95°C for 10 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and final extension 72°C for 5 minutes. Then, 5 µl from each barcoded sample was pooled together, mixed and spun down. Finally, 100 µl of pooled DNA was purified using X1 AMPure XP beads as described above. Samples were deep-sequenced (Macrogen company) with

the Nextseq500 machine using the 150-cycle mid output kit (Illumina, Inc.) from the forward read direction. At least 10,000 reads for each sample were targeted.

2.4 Bioinformatics analysis

The obtained DNA sequences from next-generation sequencing (NGS) were then analyzed using the Galaxy program. (https://usegalaxy.eu/).The NGS method was able to detect two SNPs in one tube. The obtained sequences as fastq files were trimmed using default parameters to retain the highest quality DNA sequence reads. The filtered data were captured by virtual specific SNPs virtual probes. The outlined workflow for SNP detection and identification is shown in Table 2.3.

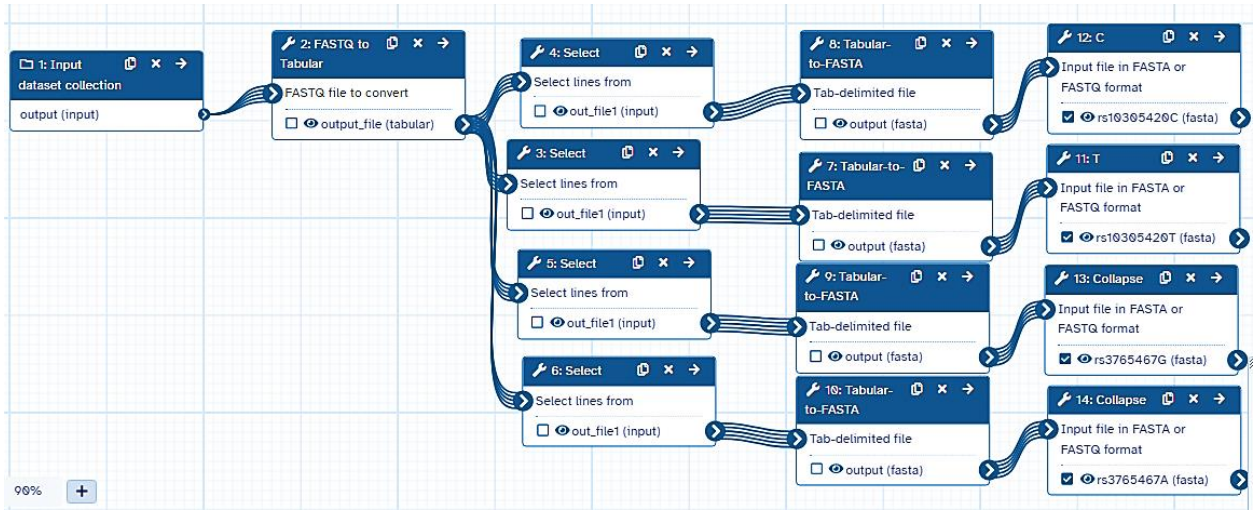

Table 2. 3 Workflow pipelines used in galaxy free online program for the fastq files for NGS analysis (https://usegalaxy.org.au).

2.5 Statistical Analysis

The genotype frequencies were tested for Hardy–Weinberg equilibrium by calculating a chi-square statistic and corresponding p-value. Pearson's Chi-square analysis was performed to test genotype frequency differences between the two studied groups (with and without dyslipidemia). ANOVA was used to assess the association between GLP-1R genotypes and continuous variables. Inter-group comparison was evaluated by independent sample t test and or Mann-Whitney test. Logistic regression was used to measure odd ratio (OR) for GLP-1R gene polymorphism and dyslipidemia. P-value less than 0.05 was considered significant. Analysis was performed using SPSS program version 23.

Chapter Three

3. Results

3.1 Demographic, clinical, and biochemical characteristics of the study groups

A total of 216 T2DM patients were recruited between October 2023 and February 2024 from the Jericho Health MOH Center in Palestine. Of them, 51% were diagnosed with dyslipidemia (n=110) and 49% without dyslipidemia (n=106).

The mean (SD) age of the study participants was 59.6 (10.57) years. Almost 56.5% of participants were females. The mean (SD) of BMI was 31.9 (5.8) kg/m^2.

The demographic, clinical parameters and T2DM complication were compared among the study groups as shown in Table 3.1. Results showed higher rates of diabetic Neuropathy, Retinopathy, and hypertension among patients with dyslipidemia compared to those without dyslipidemia (p <

0.05. However, no statistically differences were observed in the prevalence of nephropathy, diabetic foot and the status of tobacco smoking among the two groups. Whereas, female patients were more prevalent among dyslipidemia group (p =0.04).

Table 3. 1: Demographic and clinical parameters of T2DM patients with and without dyslipidemia.

Parameter		**T2DM without Dyslipidemia n=106**		**T2DM with Dyslipidemia n=110**		**p_value**
		Frequency	%	Frequency	%	
Gender	Female	53	50%	69	62.7%	0.04
	Male	53	50%	41	37.3%	
Nephropathy	No	103	97.2%	107	97.3%	0.6
	Yes	3	2.8%	3	2.7%	
Neuropathy	No	98	92.5%	86	78.2%	.003
	Yes	8	7.5%	24	21.8%	
Retinopathy	No	102	96.2%	97	88.2%	.024
	Yes	4	3.8%	13	11.8%	
Diabetic Foot	No	105	99.1%	107	97.3%	0.32
	Yes	1	0.9%	3	2.7%	
Hypertension	No	54	50.9%	29	26.4%	0.01
	Yes	52	49.1%	81	73.6%	
Tobacco Smoking	No	70	66%	81	73.6%	0.14
	Yes	36	34%	29	26.4%	

Note: P $<$ 0.05 was considered significant (obtained by $\chi^2 -$ test for categorical variables). % from the grand total.

The mean values of the demographic, clinical and biochemical measurements were compared between the two studied groups and presented in Table 3.2. Results show that the mean age of T2DM patients with dyslipidemia were higher than those without dyslipidemia (62.0 ± 9.9 vs. 57.1 ± 10.6, p =0.001). Also, the mean BMI of T2DM patients with dyslipidemia were higher than those without dyslipidemia (31.9 ± 5.7 vs. 30.3 ± 5.9, p =0.044), the same for systolic blood pressure (SBP): it was found that the mean SBP of T2DM patients with dyslipidemia were higher than those without dyslipidemia (138.6 ± 21.6 vs. 132.1 ± 22.8, p =0.032). Moreover, the mean values of TC, TG, and LDL-C in T2DM patients with dyslipidemia were, respectively, higher than those without dyslipidemia (178.7± 39.2 vs. 155.5 ± 33.6, p < 0.001), (189.1 ± 40.1 vs. 106.7 ± 43.5, p < 0.001), and (103.1 ± 32.3 vs. 92.9 ± 27.6, p < 0.015). While, no differences were found in the mean values of diastolic blood pressure (DBP), HbA1C, HDL-C, and FBS in T2DM patients with dyslipidemia and without dyslipidemia (p > 0.05).

Table 3.2: Comparison of the mean values of demographic, clinical, and biochemical parameter of T2DM patients with and without dyslipidemia.

Parameter	**T2DM without Dyslipidemia**	**T2DM with Dyslipidemia**	P-value
Age (Years)	57.1 ± 10.6	62.0 ± 9.9	0.001
BMI (kg/m²)	30.3 ± 5.9	31.9 ± 5.7	0.044
SBP (mmHg)	132.1 ± 22.8	138.6 ± 21.6	0.032
HbA1C (%)	7.9 ± 2.1	8.0 ± 1.8	0.812
TC (mg/dL)	155.5 ± 33.6	178.7± 39.2	< 0.001

TG (mg/dL)	106.7 ± 43.5	189.1 ± 40.1	< 0.001
LDL-C (mg/dL)	92.9 ± 27.6	103.1 ± 32.3	0.015
HDL-C (mg/dL)	41.1 ± 11.6	42.3 ± 11.3	0.423
FBS: mg/dL	169.9 ± 81.9	181.5 ± 76.9	0.283

Note: $P < 0.05$ was considered significant (obtained by $t-$ tests), data are expressed as mean ± standard deviation.

3.3 Amplification of GLP-1R target sequences and Gel electrophoresis

PCR products revealed two bands with around 250-300bp targeting the two GLP-1R gene targeted fragments. Figure 3.1 shows a picture of representative samples on agarose gel electrophoresis to visualize the PCR products.

A B

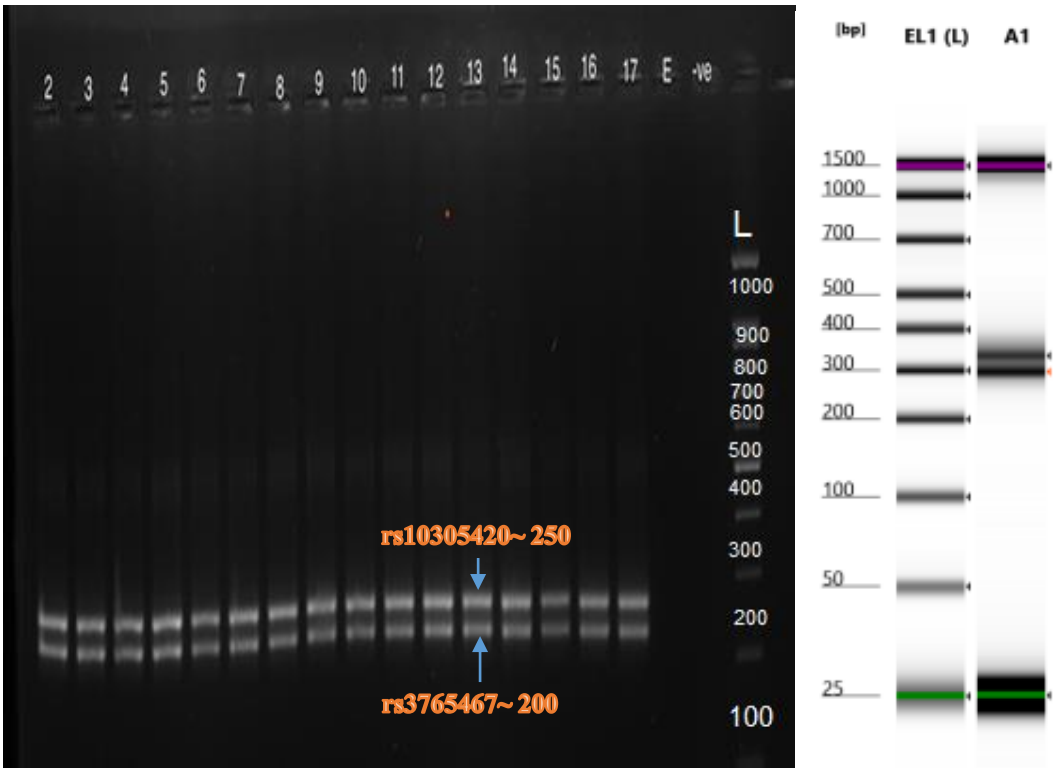

Figure 3.1: A- Agarose gel electrophoresis showing Multiplex-PCR products of the GLP-1R gene (rs10305420 and rs3765467). Lane L refers to DNA ladder (100bp),.–ve : Negative control, the size of the upper and lower band were about (246 and 282bp) for PCR products. Panel B- Representative sample (A1); the purified pooled samples after adding the indices and loading on gel using Tapestation machine and High sensitivity D100 kit (Agilent company) provided by Macrogen company.

3.4 Determination of GLP-1R genotypes by Galaxy program

The genotypes were determined based on the calculated ratio between the read counts for each allele in each individual sample as shown in table 3.3. For instance, the expectation of specific

allele (example, T) abundance is 0 (ratio <0.1), 50% (ratio around 1), or 100% (ratio >10). (CC, CT, TT) / homozygous or heterozygous.

Table 3.3: Genotyping results, count reads and ratios of GLP-1R rs10305420 and rs3765467 in representative samples.

Sample ID	rs10305420 (C)	rs10305420 (T)	rs10305420 Ratio	rs10305420 Genotyping	rs3765467 (G)	rs3765467 (A)	rs3765467 Ratio	rs3765467 Genotyping
1	7413	7779	1	CT	8620	2155	4	GG
2	15795	26	608	CC	15750	1125	14	GG
3	18004	15	1200	CC	16653	925	18	GG
4	7701	7957	1	CT	15744	874	18	GG
5	77	18091	0.0	TT	17667	803	22	GG
9	12894	24	537	CC	13350	834	16	GG
18	7580	8198	1	CT	8818	8414	1.0	GA
241	291585	17764	16	CC	221435	164133	1.349119	GA

3.5 Multiple sequence alignment

The results are confirmed by showing the multiple alignment against the reference gene using http://multalin.toulouse.inra.fr/multalin/ website.

Sample ID	C	T	Ratio	Genotyping
127	16091	493	32.63895	CC

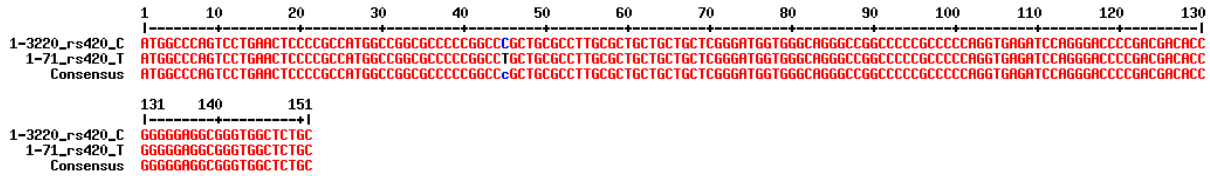

Figure 3. 2: An example of the multiple sequence alignment of homozygous sample (127).

Sample ID	C	T	Ratio	Genotyping
126	9963	8696	1.145699	CT

Figure 3. 3: An example of the multiple sequence alignment of heterozygous sample (126).

Sample ID	C	T	Ratio	Genotyping
116	6053	18753	0.322775	TT

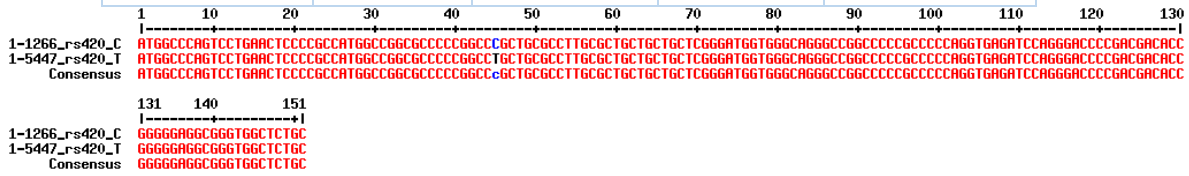

Figure 3. 4: An example of the multiple sequence alignment of homozygous sample (116).

3.6. GLP-1R genotypic distribution in all the study participants

Among all participants, the genotype distribution of rs10305420 was found to be consistent with Hardy-Weinberg equilibrium (P = 0.187) (Table 3.4). This implies that the population that was included in the genetic analysis could be representative of the entire population. For the second SNP, almost all samples analyzed for the rs3765467 polymorphism in the GLP-1R gene exhibited a homozygous genotype (GG) (n= 214, 99.09%) Therefore, no further analysis has been done on this SNP.

Table 3.4: The genotype distribution and Hardy-Weinberg equilibrium of rs10305420 in the included population.

SNP	Genotype	N (%)	χ^2	P-value
rs10305420	CC	117 (54.1)	3.779	0.187
	CT	71 (32.9)		
	TT	28 (13)		

Note: P > 0.05, indicating the genetic balance of the population, the data are from the same mondel population.

3.7. Distributions of GLP-1R rs10305420 Genotype among T2DM patients with and without dyslipidemia

The allele and genotype frequencies for rs10305420 in both groups are shown in Table 3.5. The distribution of the GLP-1R rs10305420 genotype among T2DM patients shows that there was no statistically differences between the genotype and allele frequency among the tested groups (P>0.05). The CC genotype was the most common, present in approximately 26-28% of patients in both groups, followed by the CT genotype, found in about 15-18% of patients, while the TT genotype was the least common, appearing in around 5-8% of patients.

Table 3.5: The genotype distribution of the T2DM patients with or without dyslipidemia.

SNP GLP-IR rs10305420		**T2DM without Dyslipidemia**		**T2DM with Dyslipidemia**		**P-value**
		N	%	N	%	
Genotype	CC	57	26.4%	60	27.8%	0.44
	CT	38	17.6%	33	15.3%	
	TT	11	5.1%	17	7.9%	
		Frequency	%	Frequency	%	
Alleles	C	163	58%	170	57%	0.87
	T	117	42%	127	43%	

Note: P $<$ 0.05 was considered significant (obtained by $\chi^2 -$ test for categorical variables). % from the grand total.

To investigate if there is an association of GLP-1R rs10305420 polymorphism with clinical and biochemical parameters. The data was stratified according to genotypes in each group. The results in tables 3.6 and 3.7 revealed that there are no significant differences between GLP-1R rs10305420 and the mean values of each of age, BMI, SBP, DBP, HbA1C, and lipid profile in the T2DM patients within each group ($p > 0.05$).

Table 3. 6: Differences between GLP-1R rs10305420 gene polymorphisms and BMI, blood pressure, HbA1C and lipid profile in T2DM patients with dyslipidemia.

Parameter	**SNP** GLP-IR rs10305420			P-value
	CC	CT	TT	
BMI (kg/m2)	32.5 ± 5.91	30.7 ± 6.11	32 ± 4.30	.336
SBP (mmHg)	139.1 ± 21.62	134.3 7 ± 20.73	145.5 ± 22.41	.213
DBP (mmHg)	75.9 ± 17.51	72.6 ± 10.88	75.1 ± 17.04	.638
HbA1C (%)	8.1 ± 1.97	7.9 ± 1.86	7.4 ± 1.51	.374
TC (mg/dl)	180.8 ± 34.47	173.3 ± 45.62	181.7 ± 42.88	.642
TG (mg/dl)	183.2 ± 109.45	177.7 ± 163.86	231.2 ± 183.29	.398
LDL-C (mg/dl)	103.1 ± 30.58	101.5 ± 35.42	105.7 ± 33.62	.911
HDL-C (mg/dl)	41.1 ± 10.37	44.1 ± 13.12	43.0 ± 10.83	.459
FBS (mg/dl)	182.2 ± 78.53	184.5 ± 72.69	173.1 ± 83.58	.882
X represents the mean, and SD represents the standard deviation.				

Note: $P < 0.05$ was considered significant (obtained by ANOVA – tests).

Table 3.7: Differences between GLP-1R rs10305420 gene polymorphisms and age, BMI, blood pressure, HbA1C and lipid profile in T2DM patients without dyslipidemia.

Parameter	**SNP** GLP-IR rs10305420			P-value
	CC	CT	TT	
Age (years)	56.2	57.5	60.6	0.452
BMI (kg/m2)	30.4	29.8	30.8	0.848

SBP (mmHg)	131.4	130.3	142.2	.296
DBP (mmHg)	73.2	74.3	81.4	.277
HbA1C (%)	8.1	7.6	7.6	.487
TC (mg/dL)	154.0	156.0	161.4	.797
TG (mg/dL)	101.9	114.2	105.4	.400
LDL (mg/dL)	93.8	92.1	91.7	.948
HDL (mg/dL)	41.5	39.5	43.7	.531
FBS (mg/dL)	180.5	156.5	161.1	.354
X represents the mean, and SD represents the standard deviation.				

Note: $P < 0.05$ was considered significant (obtained by ANOVA – tests).

Furthermore, logistic regression analysis adjusted for age, gender, and BMI confirmed that there was no association between GLP-1R rs10305420 polymorphism and the risk of dyslipidemia. (OR=1.043, CI 0.595-1.828) and the P value (0.884).

Chapter Four

4.1 Discussion

The high risk of cardiovascular events in diabetic patients is associated with dyslipidemia, a common concomitant disease of diabetes (Wu & Parhofer, 2014). To reduce the risk of cardiovascular disease in patients with T2DM, it is crucial to manage lipid levels effectively (Low Wang, Hess, Hiatt, & Goldfine, 2016).

There are many common DNA variants associated with dyslipidemia. The cumulative effect of multiple common variants can cause polygenic dyslipidemia. In recent years, several studies have found that dyslipidemia can be affected with specific genes. Therefore, assessing genetic risk with dyslipidemia in diabetes patients can prevent or delay health complications associated with the condition(Carrasquilla, Christiansen, & Kilpeläinen, 2021). In this context, we investigated the relationship between GLP-1R rs10305420 and rs3765467 polymorphisms and

the risk of dyslipidemia among diabetic patients. For this purpose, two groups of diabetic patients with and without dyslipidemia were included.

The results of the present study showed that hypertension, neuropathy, retinopathy, were more prevalent in T2DM patients with dyslipidemia compared to those without dyslipidemia ($p < 0.001$). Findings by Hammer and Busik demonstrated In a related study, that diabetic patients with dyslipidemia are more likely to develop retinal abnormalities(Hammer & Busik, 2017). A study about the association between hyperlipidemia, lipid-lowering drugs and diabetic peripheral neuropathy in patients with T2DM showed an opposite results to our findings suggesting that hyperlipidemia were not associated with neuropathy in adults with T2DM diabetes. Moreover, hyperlipidemia and the resulting alterations in lipoprotein metabolism are associated with nephropathy and the severity of proteinuria, such as glomerulus albuminuria (Chang et al., 2023) (Vaziri, 2016). Herein, we could not find any differences in the occurrence of nephropathy between patients with and without dyslipidemia. A previous study showed that dyslipidemia are associated with infection, severity, and delayed healing of diabetic foot ulcers (Zhang et al., 2023). Another study on the association between cigarette smoking and dyslipidemia found that smoking induces changes in the lipid profile, particularly a decrease in HDL-C and an increase in TG, which can contribute to atherosclerosis (Haj Mouhamed et al., 2013). However, findings revealed no statistically differences in the association of diabetic foot and tobacco smoking among patients with and without dyslipidemia. Also, the lack of data on the duration of diabetes and comorbidities of the studied participants may have an impact on our findings about the prevalence of diabetes complications among the studied groups.

In the present study, dyslipidemia was more prevalent in females than males (31.9% vs 19.0%). Moreover, our results showed that mean age of T2DM patients with dyslipidemia was higher

than those without dyslipidemia (62.0 ± 9.9 vs. 57.1 ± 10.6, p <0.001). Also, the mean BMI of T2DM patients with dyslipidemia was higher than those without dyslipidemia (31.9 ± 5.7 vs. 30.3 ± 5.9, p =0.044). In contrast to a study done in Lalitpur - Nepal, male patients have higher diabetic dyslipidemia than females, with (28.0%) being overweight and (22.0%) being obese (Thapa et al., 2017). The mean of cholesterol, TG, and LDL in T2DM patients with dyslipidemia were higher than those without dyslipidemia, respectively (178.7± 39.2 vs. 155.5 ± 33.6, p < 0.001), (189.1 ± 40.1 vs. 106.7 ± 43.5, p < 0.001), and (103.1 ± 32.3 vs. 92.9 ± 27.6, p = 0.015) while no differences were found in the mean of DBP, HbA1C, HDL, and FBS (p > 0.05) among the two groups. In a similar study, lipid profile was analyzed among patients with T2DM. At different age groups, the results showed that T2DM patients with dyslipidemia have a high level of cholesterol, serum LDL cholesterol and triglycerides and as the age increases total cholesterol, serum LDL, TG also increases (Thapa et al., 2017). Notably, the mean of SBP was higher in T2DM patients with dyslipidemia than those without dyslipidemia (138.6 ± 21.6 vs. 132.1 ± 22.8, p =0.032), which could be attributed to several factors, including insulin resistance, endothelial dysfunction, inflammation, renal disease, and lifestyle factors. Hypertension and dyslipidemia, both classical components of metabolic syndrome are caused by insulin resistance and obesity.

On the other hand, when we examined the potential role of the GLP-1R rs10305420 polymorphism on dyslipidemia, we found no significant difference in the allele and genotype distribution of rs10305420 in the T2DM patients with and without dyslipidemia.

The newly introduced NGS method successfully detects two SNPs in a single tube, reducing costs and simplifying procedures. This approach eliminates the need for gels, stains, and enzymes typically required in traditional analysis methods. It offers high sensitivity and

specificity, allowing for efficient genotyping with a minimal number of sequences (Satam et al., 2023).

The differences in reported genotypes, observed in studies primarily conducted in China, contrasts with the consistent findings across numerous studies from these regions, which have consistently linked a specific SNP with (T2DM). This discrepancy suggests that the A allele is more prevalent in different populations, such as the Chinese, as reported in studies like (Y. Li et al., 2023), which found an association between GLP-1R rs3765467 polymorphism and dyslipidemia in Chinese patients with T2DM. Similarly, (Fang et al., 2023), identified the GLP1R rs3765467 polymorphism as associated with the risk of early onset T2DM in both Chinese and American populations. Another study by Sathananthan group supported the abundance of the A allele, showing common genetic variation in GLP1R and its influence on insulin secretion in response to exogenous GLP-1 in nondiabetic subjects. These findings suggest a higher prevalence of the A allele in these populations, which contradicts our genotyping results (Sathananthan et al., 2010).

The present study analyzing if the GLP-1R rs10305420 polymorphism can explain some of the inter-individual differences in clinical and lipid profile, we stratified the data of the enrolled population according to the genotype and compared the association between genotypes and the metabolic indicators including age, BMI, blood pressure, HbA1C and blood lipid profile and there were no significant differences between GLP-1R rs10305420 and age, BMI, blood pressure, HbA1C and blood lipid profile ($p > 0.05$).

Logistic regression analysis, adjusting for age, gender, and BMI, also confirmed that there was no significant association between GLP-1R rs10305420 and the risk of dyslipidemia (OR=1.043, 95% CI 0.595-1.828, p=0.884).

4.2 Study limitations

In terms of the study limitation, the number of enrolled subjects was relatively small. Further studies with a larger sample size are needed to verify the effect of rs10305420 polymorphism on lipid profile in Palestinian T2DM patients. As dyslipidemia have multifactorial etiologies, both genetic and environmental factors play a role in its pathogenesis. The lack of information on environmental factors (e.g physical activity, high-fat diets,) disabled us to understand the gene-environment interaction because of some people their genotype may only become evident or manifest in combination with exposure to certain environmental factors. Moreover, we only investigated two common variants (rs10305420 and rs3765467), other polymorphisms within the GLP1R gene may exert synergistic effect dyslipidemia's genetic polymorphisms must be screen extensively to uncover their underlying mechanisms, which need to be better understood.

4.3 Study recommendation

Given the lack of association between GLP-1R genetic polymorphisms and lipid metabolism observed in Palestinian T2DM patients in this study, it's important to explore other avenues for understanding dyslipidemia in this population. Instead of focusing solely on GLP-1R rs10305420 and rs3765467 variants, future research could investigate other polymorphisms within the GLP-1R or other genetic factors such as PPAR gene variants, LPL gene polymorphisms, LDLR gene mutations, ABCA1 gene variants, CETP gene polymorphisms, HMGCR gene variants, FTO gene polymorphisms, TCF7L2 gene variants, and ANGPTL family gene polymorphisms that may contribute to dyslipidemia in T2DM patients. Additionally, regular screening for dyslipidemia

among individuals with T2DM, personalized treatment strategies tailored to their lipid profiles and medical history, lifestyle adjustments, support for quitting smoking, and proactive measures to prevent complications should be implemented.

4.4 Conclusions

In conclusion, there is no significant differences in the polymorphism of GLP-1R rs10305420, but with increasing the sample size and identifying more SNP variants, we may find an association between polymorphism and the risk of dyslipidemia. This can be used as a useful marker or tool to predict the risk of dyslipidemia in T2DM patients.

Despite the high prevalence of dyslipidemia-related complications such as hypertension, neuropathy, and retinopathy in T2DM patients, the specific GLP-1R variant studied did not contribute to these lipid abnormalities. The observed higher prevalence of dyslipidemia in females, older age groups, and individuals with higher BMI underscores the multifactorial nature of dyslipidemia. Future research should explore other genetic factors and polymorphisms to better understand the genetic risk and develop targeted interventions. Additionally, implementing regular screening and personalized treatment strategies remains crucial for managing dyslipidemia and reducing cardiovascular risks in T2DM patients.

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Appendices

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

Al-Quds University
Jerusalem
Deanship of Scientific Research

بسم الله الرحمن الرحيم

جامعة القدس
القدس
عمادة البحث العلمي

Research Ethics Committee
Committee's Decision Letter

Date: October 21, 2023
Ref No: 333/REC/2023

Dears Dr. Suheir Eriqat, Dr. Abedelmajeed Nasereddin,

Thank you for submitting your application seeking approval for research ethics. After a thorough examination of your submission titled "Association between GLP-1R Gene Polymorphism and Dyslipidemia in Palestinian Patients with Type 2 Diabetes Mellitus", the Research Ethics Committee (REC) at Al-Quds University is pleased to confirm that your application is in accordance with our research ethics guidelines.
This ethical approval will remain valid as long as there are no alterations to the data collection procedure or modifications to any aspect of the research protocol. Please be aware that while this approval authorizes your research, however, please keep in mind that this approval does not substitute for any departmental or other approvals that may be necessary, including but not limited to sample shipment, data sharing permissions or administrative approval to distribute questionnaires.

In addition, we kindly request that you provide us with a copy of your final research report or publication once it becomes available.
Thank you once again for your commitment to conducting ethical research, and we extend our best wishes for a productive research endeavor that serves the best interests of your research subjects.

PS: Please note that this ethical approval letter will remain valid for a period of two years from the date of issuance. Should your research extend beyond this timeframe, a request for renewal will be necessary.

Sincerely,

Elham Kateeb, BDS MPH PhD

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Al-Quds University
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Cc. Prof. Imad Abu Kishek - President
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Appendix2: Consent form

Al-Quds University
Faculty of Graduate Studies

Consent Form

Dear/ patient:

You are invited to participate in a research study conducted by student of graduate studies from AL-Quds University in order to fulfill master degree of Biochemistry and Molecular biology. This study aims to investigate the association between GLP-1R gene polymorphism and the presence of dyslipidemia in Palestinian patients with type 2 diabetes mellitus at Jericho health center.

The purpose of this research is to enhance our understanding of genetic factors that may contribute to dyslipidemia 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.

• 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: Maha Al-Sharbati

ارتباط تعدد الأشكال الجيني لجين GLP-1R بخطر الإصابة بعسر شحميات الدم لدى المرضى الفلسطينيين المصابين بمرض السكري من النوع الثاني.

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الملخص

يرتبط ارتفاع خطر الإصابة بأمراض القلب والأوعية الدموية لدى مرضى السكري باضطراب المعدلات الطبيعية لشحميات الدم، والذي يتضمن ارتفاع مستوى الكوليسترول و/أو ثلاثيات الغليسيريد أو انخفاض مستوى كوليسترول البروتين الشحمي عالي الكثافة. يُعد هذا المرض شائعًا بين مرضى السكري. لتقليل خطر الإصابة بأمراض القلب والأوعية الدموية في مرضى السكري، يجب التحكم في مستويات الدهون في الجسم بشكل فعال. أظهرت العديد من الدراسات أن عسر شحميات الدم يمكن أن يكون مرتبطًا بتغيرات في جينات معينة.لهذا، كان الهدف الرئيسي من هذه الدراسة هو الكشف عن تعدد الأشكال الجينية rs10305420 و rs3765467 في جين GLP-1R وعلاقتهما بخطر الإصابة بعسر شحميات الدم لدى المرضى الفلسطينيين المصابين بداء السكري من النوع الثاني .(T2DM) استخدمت الدراسة تقنيات الجيل الجديد والتفاعل التسلسلي البوليميرازي في أنبوب مخبري واحد.

أجريت دراسة مقطعية بين أكتوبر ٢٠٢٣ وفبراير ٢٠٢٤ شملت ٢١٦ مريضًا بالسكري من النوع الثاني، وتم تقسيم المرضى إلى مجموعتين: مجموعة المرضى بدون عسر شحميات الدم ومجموعة المرضى المصابين بعسر شحميات الدم. شملت المجموعة

الأولى المرضى الذين تبلغ أعمارهم ٤٠ عامًا فأكثر وتم تأكيد إصابتهم بالسكري من النوع الثاني وفقًا لمعايير منظمة الصحة العالمية ولم يكن لديهم تاريخ من عسر شحميات الدم، ولم يستخدموا عوامل خفض الدهون. أما المجموعة الثانية، فقد شملت المرضى الذين تبلغ أعمارهم ٤٠ عامًا فأكثر وتم تأكيد إصابتهم بالسكري من النوع الثاني وعسر شحميات الدم. تم استبعاد المشاركين الذين تقل أعمارهم عن ٤٠ عامًا، الأفراد الذين تم تشخيصهم بالسكري من النوع الأول، المرضى الذين لديهم سجلات طبية غير مكتملة، المرضى الذين يعانون من أمراض مصاحبة شديدة (مثل أمراض الكبد الشديدة أو السرطان)، والنساء الحوامل. أظهرت النتائج معدلات أعلى من اعتلال الأعصاب السكري واعتلال الشبكية وارتفاع ضغط الدم بين المرضى المصابين بعسر شحميات الدم مقارنةً بأولئك الذين ليس لديهم عسر شحميات الدم .($p < 0.05$) ومع ذلك، لم تُظهر الدراسة أي اختلافات ذات دلالة إحصائية في انتشار اعتلال الكلى أو القدم السكري أو حالة التدخين بين المجموعتين، بينما كان المرضى الإناث أكثر انتشارًا في مجموعة عسر شحميات الدم($p = 0.04$).

أظهرت توزيعات النمط الجيني لـ rs10305420 في المجموعات المدروسة أن النمط الجيني الأكثر تكرارًا في مجموعة عسر شحميات الدم كان CC بنسبة ٢٧,٨%، يليه CT بنسبة ١٥,٣%، ثم TT بنسبة ٧,٩%. أما بالنسبة لأولئك الذين ليس لديهم عسر شحميات الدم، فقد كانت الأنماط الجينية CC وCT وTT بنسبة ٢٦,٤%، ١٧,٦%، و٥,١% على التوالي. لم تجد الدراسة اختلافًا ذا دلالة إحصائية في توزيع النمط الجيني rs10305420 بين مرضى السكري من النوع الثاني المصابين وغير المصابين بعسر شحميات الدم. أما بالنسبة لتعدد الأشكال الثاني GLP-1R rs3765467، فقد أظهرت معظم العينات النمط الجيني GG لتعدد الأشكال. GLP-1R rs3765467. كان متوسط مستويات الكوليسترول الكلي والدهون الثلاثية والدهون منخفضة الكثافة (LDL) لمرضى السكري من النوع الثاني الذين يعانون من عسر شحميات الدم أعلى بشكل ملحوظ مقارنة بأولئك الذين ليس لديهم عسر شحميات الدم. كان المتوسط ١٧٨,٧ ± ٣٩,٢ مقابل ١٥٥,٥ ± ٣٣,٦ للكوليسترول الكلي ($p < 0.001$، 189.1 ± 140.1 مقابل ١٠٦,٧ ± ٤٠,٦ للدهون الثلاثية ($p < 0.001$)، و١٠٣,١ ± ٣٢,٣ مقابل ٩٢,٩ ± ٢٧,٦ للـ LDL ($p <$

0.015). كان متوسط العمر ومؤشر كتلة الجسم والضغط الانقباضي لمرضى السكري من النوع الثاني المصابين بعسر شحميات الدم أعلى من أولئك الذين ليس لديهم عسر شحميات الدم. بناءً على النتائج، لا يبدو أن تعدد الأشكال الجينية rs10305420 و rs3765467 في جين GLP-1R مرتبطان بشكل كبير بعسر شحميات الدم في هذه العينة. لذا، ينبغي إجراء الدراسة على عينة أكبر لتأكيد هذه النتائج.