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Association of Vitamin D Receptor Gene Polymorphisms with Type 2 Diabetes Mellitus in Hebron, Palestine

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Association of Vitamin D Receptor Gene Polymorphisms with Type 2 Diabetes Mellitus in Hebron, Palestine

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Dedication

To Allah, the source of all strength and guidance

To myself, for never giving up, for the effort and patience that made this possible, and for the commitment to keep growing and learning

To my beloved parents, for their unconditional love and support, who have always believed in me to reach all my dreams

To my dear siblings, Nour, Rand, Mariam, Mohammad, and Abd Al-Rahman whose constant support and motivation have meant the world to me. To my nephew Moein, whose presence brings joy and inspiration to my life

To my friends, who have stood by me through every challenge and celebration

Lastly, I dedicate this thesis to all those who strive for knowledge and insist to make a positive impact in the world….

Marah Imad Al-Atrash

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

Marah Imad Ali Al-Atrash

Date

January 10,2026

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Abstract

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from insulin resistance and decreased insulin secretion. Vitamin D receptor (VDR) mediates the actions of vitamin D, whichisnecessary for glucose metabolism, insulin secretion, and modulation of inflammation. Variations in the VDR gene, including FokI polymorphism which may alter the VDR protein structure, and the non-functional ApaI, TaqI, and BsmI polymorphisms, which may influence VDR activity or expression and thereby affect susceptibility to T2DM. However, data on the relationship between these VDR variants and T2DM in the Palestinian population are limited. This study aimed to examine the relationship of four VDR polymorphisms and the risk of developing T2DM in the West Bank, Palestine specifically in Hebron.

In this case-control study, a total of 300 participants, including 200 T2DM patients and 100 non-diabetic controls aged 40 years and above, were recruited from Alia Governmental Hospital and the Palestinian Diabetes Institute in Hebron between January and April 2025. Demographic, clinical, and biochemical data were collected from medical records. Blood samples were used for DNA extraction, PCR amplification, and genotyping of FokI (rs2228570), TaqI (rs731236), BsmI (rs1544410), andApaI (rs7975232) polymorphisms using next-generation sequencing.

Diabetic patients had significantly higher age, BMI, weight, HbA1c, fasting blood glucose, and triglycerides, and lower HDL cholesterol compared to controls. In diabetic patients, the minor allele frequencies were 45.7% for rs7975232 (C), 41.1% for rs731236 (G), 58.6% for rs1544410 (C), and 73.5% for rs2228570 (C). Among these, only TaqI (rs731236) showed a statisticallysignificant association with type 2 diabetes ($p = 0.030$), while the other polymorphisms were not significantlyassociated.Logistic Regression showed asignificant association between rs731236 (TaqI) and T2DM under the recessive model (GG genotype: $OR = 1.5$, $p = 0.016$), with GG carriers exhibiting higher HbA1c in diabetic participants and elevated triglycerides in non-diabetics. Haplotype analysis showed no significant correlations with T2DM risk. These findings suggest that the TaqI (rs731236) variant may serve as a genetic marker for T2DM susceptibility in Palestinian population and highlight the potential role of vitamin D signaling in the disease development.

In Conclusion, among the four VDR polymorphisms investigated, only rs731236 (TaqI) showed a statistic significant association with T2DM in the study population from Hebron West Bank, Palestine,indicating its potential utility as a genetic marker and supporting a role for vitamin D signaling in T2DM development in our population. Further studies with larger sample size are needed to confirm these findings and explore additional VDR-related variants.

Keywords: Type 2 Diabetes Mellitus(T2DM), Vitamin D Receptor(VDR) Gene, Single Nucleotide Polymorphisms, Next Generation Sequencing(NGS), Palestinian Population.

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List of Abbreviations

Abbreviation	Term
1,25(OH)2D	1,25-dihydroxyvitamin D
7-DHC	7-dehydrocholesterol
25(OH)D	25-hydroxyvitamin D
AD	Alzheimer's Disease
ADA	American Diabetes Association
ApaI	Vitamin D Receptor ApaI Polymorphism
BFDP	Bayesian False Discovery Probability
BMI	Body Mass Index
Bp	base pair
BsmI	Vitamin D Receptor BsmI Polymorphism
Ca^{+2}	Calcium
DBP	Diastolic Blood Pressure
DM	Diabetes Mellitus
DR	Diabetic Retinopathy
D2	Ergocalciferol
D3	Cholecalciferol
EDTA	Ethylenediamine tetra acetic Acid
ER	Endoplasmic Reticulum
FokI	Vitamin D Receptor FokI Polymorphism
FPRP	False Positive Report Probabilities
FPG	Fasting Plasma Glucose
GDM	Gestational Diabetes
GLUT4	Glucose Transporter Type 4
GSIS	Glucose-stimulated Insulin Secretion
HbA1c	Glycated Hemoglobin
HDL	High-Density Lipoprotein
HNF-α	Hepatocyte Nuclear Factor Alpha
IL-1β	Interleukin 1 Beta
IL-6	Interleukin 6
IR	Insulin Resistance

IQR	Interquartile Range
LD	Linkage Disequilibrium
LDL	Low-Density Lipoprotein
LOAD	Late-onset of Alzheimer's Disease
MAPK	Mitogen-Activated Protein Kinase
MCI	Mild Cognitive Impairment
MODY	Maturity-onset Diabetes of Youth
mRNA	Messenger Ribonucleic Acid
NAFLD	Nonalcoholic Fatty Liver Disease
NIDDM	Non-Insulin-Dependent Diabetes Mellitus
NF-κB	Nuclear Factor Kappa B
NGS	Next-Generation Sequencing
OGTT	Oral Glucose Tolerance Test
PCOS	Polycystic Ovary Syndrome
PCR	Polymerase Chain Reaction
PD	Parkinson's Disease
RDA	Recommended Dietary Allowance
RPG	Random Plasma Glucose
RXR	Retinoid X Receptor
SBP	Systolic Blood Pressure
SNP	Single Nucleotide Polymorphism
SPSS	Statistical Package for the Social Sciences
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TaqI	Vitamin D Receptor TaqI Polymorphism
TG	Triglycerides
TC	Total Cholesterol
VDR	Vitamin D Receptor
VDRE	Vitamin D-Responsive Elements
VGCC	Voltage-Gated Calcium Channels
WHO	World Health Organization

Chapter One
Introduction

1.1 Diabetes Mellitus

Diabetes mellitus is a major global health problem characterized by chronic persistent hyperglycemia.

1.1.1 Definition of Diabetes Mellitus

DMcomprises a group of carbohydrates metabolism disorders, characterized by persistent hyperglycemia resulting from impaired insulin secretion, defective insulin action, or a combination of both. Insulin serves as akey anabolic hormone, regulating the metabolism of carbohydrates,proteins, and lipids (Darenskaya et al., 2021). Metabolic abnormalities observed in this disease may result from decreased insulin secretion and/or resistance to insulin action in target tissues. These abnormalities primarily affect skeletal muscle, adipose tissue, and the liver, where insulin resistance disrupts normal metabolic processes. The severity of symptoms differs according to the type and duration of diabetes. Individuals with markedly elevated blood glucose levels, especially those with a complete insulin deficiency such as children, may experience symptoms including increased appetite, unexplained weight loss, polydipsia, frequent urination, and blurred vision. However, some individuals especially those with early stage type 2 diabetic may remain asymptomatic (Mansour et al., 2023). If left untreated, uncontrolled diabetes can result inserious complications such as coma, confusion, vision loss, diabetic foot, and in rare casesit may lead to death (Darenskaya et al., 2021).

In recent decades, the prevalence of diabetes has risen across all regions of the world, with more than 830 million people currently affected (Ogurtsova et al., 2017). This growing is most concerning because an increase in diabetes prevalence will eventually increase the number of chronic and acute diseases inthe general population, negatively impacting quality of life,demand on health services and economic costs. The macrovascularcomplications of diabetes, such as coronary heart disease,peripheral vascular disease, and stroke, along with microvascular complications, such as end-stage renal disease (ESRD), neuropathy,

retinopathy, and lower-extremity amputations(LEA), are responsible for much of the burden associated withdiabetes. Furthermore, diabetes has been increasingly associated with a range of other conditions, including certain cancers, ageing-related disorders (e.g. dementia), infections and liver disease. Therefore, understanding this disease and its underlying causes are important.

1.1.2 Classification of Diabetes Mellitus

DM is generally divided into three primary known types. The classification mainly based on their causes and clinical features, including type 1 diabetes, type 2 diabetes, and gestational diabetes. In addition to these well-studied forms, there are another less common forms, which are not highly recognized include monogenic and secondary diabetes(Malek et al., 2019).

1.1.2.1 Type 1 Diabetes Mellitus

Type 1 diabetes mellitus (T1DM) results from the autoimmune destruction of insulin-producing pancreatic β-cells, and accounts for approximately 10% of all diabetes cases (Akil et al., 2021; Green et al., 2021). T1DM is a potentially life-threatening, multifactorial autoimmune disorder, and its incidence has been increasing globally since the 1950s, with an average annual increase of 3–4% over the past three decades (Patterson et al., 2019). In Palestine, the overall prevalence of diabetes was 15.3% in 2023, compared to a global prevalence of 6%, with T1DM representing as estimated 4.4% of cases(Ayed et al., 2025). Whileβ-cell destruction isprimarily mediated by T cells, B-cell-produced autoantibodies against islet antigens serve as both markers and contributors to the disease. For research purposes, the presence of one or more of these autoantibodies is required for T1DM diagnosis, and they are detected in 70–80% of patients at the time of diagnosis(Cosentino et al., 2020). Immunosuppressive and immune-targeted interventions aimed at preventing T1DM did not result in preservation of β-cell function or provided only temporary effects.

There are specific and classic symptoms that associated with T1D including polydipsia (excessive thirst), polyuria (frequent urination), and polyphagia (increased hunger).This type of diabetes ismost commonly diagnosed and identified during childhood or adolescence, when patients commonly have these manifestations and markedly elevated blood glucose levels, that indicate that they require lifelong exogenous insulin replacement (Quarta et al., 2023). This type of diabetes is heterogeneous disorder, although several genes associated with susceptibility and protection are well-characterized, individual patient genotypes vary widely. Environmental factors can trigger and/or modulate the severity of the autoimmune attack on the β-cells, and the specific immune mechanisms differ between patients. At diagnosis, the clinical presentation ranges from severe insulin deficiency with pronounced hyperglycemia and ketoacidosis to asymptomatic or mild postprandial hyperglycemia. The rate of β-cell decline both before and after diagnosis is highly variable.

The American Diabetes Association (ADA)classifiesT1DMinto two forms: type 1a and type 1b diabetes (Diabetes, 2013). Type 1a or autoimmune T1DM, is diagnosed when antibodies are present alongside insulin deficiency and ketosis. Type 1b, also called idiopathic T1DM, describes patients with a clinical presentation consistent with T1DM but lacking detectableautoantibodies. The use of the term "type 1 diabetes" in Diabetes in America, 3rd edition, generally refers to the autoimmune form (type 1a) unless otherwise specified.

1.1.2.2 Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM), as the name implies, refers to diabetes diagnosed during pregnancy, specifically during the second or third trimester of pregnancy(Elsayed et al., 2023). It is characterized by glucose intolerance resulting in hyperglycemia with onset orfirst recognition during pregnancy, it is seriously harmful to both the woman and the fetus(Fan et al., 2023; Wicklow & Retnakaran, 2023). The most frequent perinatal complications of GDM include primary cesarean delivery, neonatal hypoglycemia, macrosomia, pretermbirht, fetal hyperinsulinemia, birth injury, hyperbilirubinemia, and preeclampsia(Wendland et al., 2012; Liu et al., 2021). The global prevalence of GDM varies depending on geographical location, ethnicity, and the screening and diagnostic methods used, ranging from 7.1 % to 27.6 % (Wang et al., 2022; Filardi et al., 2022; Galdikaitė et al., 2023).

In severe cases, this type of diabetes can result in prenatal death, making it essential to identify potential risk factors to protect the health of mothers and their offspring. Furthermore, children born to mothers with GDM have higher likelihood of developing obesity and/or develop T2DM in their early years (Shashikadze et al., 2021; Moon & Jang, 2022; Mantzorou et al., 2023). Several factors can increase the risk of GDM, including maternal obesity, advanced age, polycystic ovarian syndrome, a family history of the condition, and exposure to environmental pollutants (Wang et al., 2022). Diagnosis of GDM is based on specific criteria, which is typically involve assessing fasting blood sugar levels, blood sugar levels following a 75g oral glucose tolerance test, and other relevant measurements (Moon & Jang, 2022).

1.1.2.3 Other Specific Types of Diabetes

This category of diabetes includes secondary or other specific types, such as monogenic defects of β-cell function, genetic defects of insulin action, exocrine pancreatic diseases, endocrinopathies, infectious, drug or chemical induced diabetes, and rare immune-mediated and genetic syndromes associated with the disorder. Previously, monogenic defects of β-cell function were collectively known as maturity-onset diabetes of youth (MODY)(Ojala et al., 2020; Tosur & Philipson, 2022). In recent years, specific gene mutations have been identified and classified accordingly. For example, MODY1 is linked to mutations in the hepatocyte nuclear factor 4-alpha (HNF4α) gene on chromosome 20, while MODY2 is associated with mutations in the glucokinase gene on chromosome 7 (Tosur & Philipson, 2022).

1.1.2.4 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus (T2DM), also referred to as non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes, represents 90%–95% of all diabetes cases (Banday et al., 2020). A central feature in the pathogenesis of T2DM is impaired insulin secretion, which

varies widely according to insulin sensitivity to maintain appropriate glucose levels. The rising prevalence of T2DM, now affecting over 370 million people worldwide, is largely driven by increasing rates of obesity. Diagnosed is based on fasting and two-hour glucose measurements following a standardized oral glucose tolerance test, while pre-diabetes state is oftenidentified by impaired fasting glucose and/or impaired glucose tolerance (Poznyak et al., 2020). T2DM is best understood as a continuum of disease stages of increasing severity, where the degree of hyperglycemia dependson the magnitude of β-cell dysfunction. Insulin resistance is already well established once impaired glucose tolerance appears, and further rises in glucose, even within the normal range, result from progressive decline in β-cell function (Bellary et al., 2021). Hyperglycemia generally worsen over time, and theprogressive deterioration of β-cell function is another feature of T2D progression(Bellary et al., 2021).

Similar to T1DM, T2DM is highly heterogeneous. It can develop in children and adolescents, as well as in adults, affecting both lean and obese individuals. Patients may range from asymptomatic to presenting with ketoacidosis or nonketotic hyperosmolar coma, and the occurrence of diabetic complications over a patients' lifetime is highly variable. Microvascular complications such as retinopathy, and nephropathy as well as neuropathy are qualitatively similar in both T1DM and T2DM, with diabetes duration and glycemic levels being major determinants in their development(Liccini & Malmstrom, 2016). Both types of diabetes raise the likelihood of developing atherosclerotic macrovascular complications,although the older age at onset of individuals with T2DM results in a greater overall absolute risk(Kazi& Blonde, 2001).

1.1.3 Prevalence of Type 2 Diabetes Mellitus

Diabetes mellitus generally is a major public health concern, with a sharp rise in prevalence and significant impacts on patients' quality of life. The Global Burden of Disease Study 2017 estimated that approximately 476 million people worldwide live with diabetes(Webber, 2013). According to the latest International Diabetes Federation (IDF) data, 11.1% of adults aged 20-79 years roughly 1 in 9 have diabetes, with over 40% unaware of theircondition. By 2050, IDF projections indicate that 1 in 8 adults, around 853 million, will have diabetes, which means an increase of 46%(IDF, 2021). Over 90% of diabetes cases are T2DM, which is influenced by socio-economic, demographic, environmental, and genetic factors. In 2021, the global prevalence of T2DM among adults was 536.6 million people (10.5%), and it is projected to reach 783.2 million people (12.2%) by 2045 (James et al., 2018).

1.1.3.1 Prevalence of Type 2 Diabetes Mellitus in Palestine

The Middle East and Palestine, is experiencing a rising in prevalence of diabetes (Abu-Rmeileh et al., 2013; (Tietjen et al., 2021). A meta-analysis of 80 studies on T2DMacrossdifferent Arab countries,conducted between 1980 and 2015, estimated a mean prevalence of16.2% (Meo et al., 2017). Specifically, in Palestine, prevalence of the disease is projected to increase from 18.4% in 2015 to 21.5% by 2030 (Tietjen et al., 2021), with

approximately 20.8% of individuals aged 40–69 years affected by 2022(Abukhalil et al., 2024). Palestine is undergoing an epidemiological transition, with diabetes and considering as one of the leading causes of morbidity and mortality in the region (Abdul Rahim et al., 2014).

1.1.3.2 Diabetes Research in Palestine

Genetic factors have been increasingly studied in the Palestinian population to understand susceptibility to T2DM. In 2019, (Sabarneh et al., 2018)examined the role of the FTO gene (fat mass and obesity-associated gene), which is involved in regulating energy balance, appetite, and fat metabolism. They found that the rs9939609 variant was associated with an increased risk of T2DM and may partially influence body mass index, suggesting that FTO contributes to both diabetes susceptibility and obesity-related metabolic traits. Later, (Ereqat et al., 2019)investigated polymorphisms in the estrogen receptor 1 (ESR1) gene, specifically PvuII and XbaI in a case-control study among Palestinian women, and found associations with T2DM risk, highlighting a potential role for estrogen signaling in glucose metabolism and cardiovascular risk. In 2020, (Elqadi et al., 2021) conducted a cross sectional study examining the MTHFR gene (Methyelnetetrahydrofolate reductase), which is involved in folate metabolism, and the regulation of homocysteine levels. They found that the C677T variant influenced lipid profiles and blood pressure among diabetic patients, suggesting that alterations in folate metabolism may contribute to cardiovascular complications in T2DM. More recently, (Ereqat et al., 2022)explored APOE gene variations and DNA methylation, showing links with dyslipidemia in diabetic patients, indicating that epigenetic regulation contributes to metabolic complications.

Beyond genetics, clinical research has examined common diabetes-related complications in Palestine. (Dweib & Sharif, 2023) conducted a cross sectional study and reported that microvascular complications, including retinopathy, nephropathy, and neuropathy, affect a substantial proportion of T2DM patients, with risk factors including longer disease duration, poor glycemic control, and low adherence to follow-up guidelines. Similarly,(Sweity& Fanoun, 2023) found that older age, hypertension, and longer diabetes duration were associated with decreased kidney function. More recently, (Shrateh et al., 2024) performed a large retrospective cross-sectional study including 1,163 adults with T2DM investigated risk factors for diabetic retinopathy using fundus camera screening. The researchers found that longer disease duration, higher HbA1c levels, physical inactivity, and insulin use were independently associated with the presence of retinopathy, while higher education and obesity showed an inverse relationship. The impact of diabetes on other health challenges was also investigated; (Abu-helu et al., 2024)showed that diabetic patients experienced more severe COVID-19 outcomes and higher mortality than non-diabetic individuals in their case-control study.

Despite these risks, adherence to clinical guidelines in diabetes management remains suboptimal. (Abukhalil et al., 2024) performed a retrospective cross-sectional study and found gaps in medication optimization, glycemic monitoring, and preventive care such as foot and eye examinations. Collectively, these studies demonstrates that T2DM in Palestine is shaped

by a combination of genetic susceptibility, metabolic dysregulation, and clinical management factors.

1.1.4 Risk Factors for Type 2 Diabetes Mellitus

Multiple and various factors contribute to early development of T2DM, which gives it a multficatorial character and complex etiology. One of the most contributing factors is obesity and overweight especially for unhealthy adults and young people who depend mainly on fast food with low exercise rate. (Lascar et al., n.d.) reported that 95% of individuals younger than 40 years old unfortunately were overweight or obese, their study focused on investigation the characteristics of young diabetic patients and their life style habits. Early-onset of T2DM is highly associated with family history and genetic predisposition. A recent study, (Diabetes, 2024) found that around 60% of patients with T2DM have at least one of their parents living with the disease, while 90% of patients with T1DM have no family history and none of their parents had T1DM, concluding that T2DM is a genetic disease while the first type is not(Rasmussen, 2022). By focusing on pediatric and children population, (Strati et al., 2024) reported that around 74–100% of diabetic children have at least first or second-degree relative that had been diagnosed with the disease. In addition to life style habits and genetics as discussed, ethnicity has an important role in the early-onset of T2DM and considered a risk factor for the disease. As studies shown, there are specific ethnic groups highly affected with T2DM across generations including Indigenous Australians, Canada's First Nations, Native Americans and, Pima Indians, suggesting that both genetic predisposition and lower socioeconomic status contribute to the development of the disease (Diabetes, 2024).

Research also shows that early-life conditions within the uterus, such as maternal obesity and GDM can increase the likelihood of developing T2DM at a younger age (Pettitt et al., 2008). Other risk factors including being a female, especially women suffering from polycystic ovarian syndrome (PCOS), seems to have a higher risk to develop T2DM in comparison to healthy ones who not diagnosed with PCOS (Diabetes, 2024). Additionally, nonalcoholic fatty liver disease (NAFLD), and hypertension are well studied factors linked to late-onset of T2DM, and these diseases could affect young patients as well as adults. Dyslipidemia and albuminuria are frequently reported in studies as well (Lascar et al., n.d.). As shown, table 1.1 summarizesbothmodifiable and non-modifiable risk factors associated with T2DM.

Table 1.1 Modifiable and non-modifiable risk factors associated with T2DM

Modifiable Factors	Non-modifiable Factors
▪Obesity ▪ Lifestyle habits ▪ Hypertension ▪ Socioeconomic disadvantage ▪ Dyslipidemia ▪ NAFLD ▪ Albuminuria	▪ Genetics ▪ Female sex ▪PCOS ▪ Specific ethnicity ▪ Maternal obesity ▪Strong family history of T2DM

1.1.5 DiagnosisofType 2 Diabetes Mellitus

Uncontrolled DM can cause serious complications, affect multiple organs and lead to poor health outcomes if not managed appropriately. Early detection is essential to prevent these complications and to improve the patient's quality of life (Jiang et al., 2023). The diagnosis of DM is commonly made through the measurement of hemoglobin (HbA1c) plasma glucose levels or fasting plasma glucose (FPG) or oral glucose tolerance test (OGTT). T2DM is diagnosed based on established criteria set by organizations such as ADA (Diabetes, 2013) and the World Health Organization (WHO) (Kazi & Blonde, 2001). Diagnosis is confirmed if any of the following are present on two separate occasions, unless there is a clear clinical diagnosis: the level of FPG is more than 126 mg/dL (7.0 mmol/L) after at least 8 hours of fasting, the 2 hours plasma glucose level is more than 126 mg/dL (7.0 mmol/L) during a 75 g OGTT, and HbA1clevel is more than 6.5% (48 mmol/mol), or random plasma glucose (RPG) level is higher than 200 mg/dL (11.1 mmol/L) in a patient with classic hyperglycemia symptoms. These criteria are very important for identifying T2DM in early time, allowing proper management and reducing the risk of complications.

1.1.6 Physiological Regulation of Glucose Homeostasis

Glucose homeostasis is a closely controlled process maintained through complex network of hormonal, enzymatic, and nutrient-based regulators (Tadashi, 2023). Key hormones such as insulin, glucagon, and incretins coordinate glucose uptake, storage, and release, while enzymes involved in glycolysis, gluconeogenesis, and glycogen metabolism ensure metabolic flexibility (Dimitriadis et al., 2021; Jais & Brüning, 2022). The liver, skeletal muscle, and adipose tissue play central roles, influenced by neural signals and adipokines that further refine glucose balance. Counter regulatory hormones including cortisol, growth hormone, catecholamine's, and thyroid hormones which modulate glucose availability under stress or fasting states (McCormick et al., 2020). Importantly, vitamin D and its receptor are integrated into this regulatory network that supports pancreatic β-cell function, enhances insulin

responsiveness, and regulates inflammation pathways, thereby linking vitamin D status to glucose homeostasis, metabolism and the risk of developing T2DM(Argano et al., 2023).

1.2 Vitamin D

Vitamin D refers to a group of fat-soluble secosteroids, primarily including vitamin D2 (ergocalciferol) and vitamin D3(cholecalciferol).

1.2.1 Synthesis and Activation of Vitamin D

Vitamin D2is derived from plant sources, such as UV-exposed mushrooms, and fortified foods, while vitamin D3 is an animal-based food such as fatty fish and egg yolks. A small proportionof vitamin Dcan be obtained from dietary intake, including vitamin D2 and D3. The majority of vitamin D we have in our bodies is in the form of vitamin D3, and synthesized in the skin from 7-dehydrocholesterol (7-DHC) when the body exposed to sunlight (Mohammadi et al., 2022). Physiologically, after exposing skin to sun, vitamin D in bodies goes through two important hydroxylation steps by different organs to become biologically active, this form is the key in binding with the receptor as we will discuss later to perform various functions in the body, depending on the target tissue. These hydroxylation steps take place in the liver and the kidney, and any defect of these organs affecting this activations process, and consequently, the overall functioning of the body. First, in the liver vitamin D is converted by a hepatic enzyme which is 25-hydroxylase into 25-hydroxyvitamin (25(OH)D), and the second step is in the kidney, which works on the hepatic product (25(OH)D) and converts it to 1,25-dihydroxyvitamin D (1,25(OH)2D by using1α-hydroxylase enzyme (Dattola et al., 2020)Glencross et al., 2023) as shown in the upper part of figure 1.1. While (25(OH)D), the product of the first step in the liver is the primary form in the body circulation, and it is used as a biomarker for the overall vitamin D status, 1,25(OH)2Dis considered the metabolically active form and responsible for most of vitamin D's biological functions(Rochel, 2022).

The vitamin D status is evaluated by measuring serum levels of calcifediol (25-hydroxyvitamin,25(OH)D), which has a longer half-life compared to other vitamin D metabolites and considers the most reliable indicator of vitamin D stores in the body. Aserum value more than 30ng/mL is generally considered sufficient for being healthy. The recommended dietary allowance(RDA) for vitaminD varies by different age groups: 400 IU for infants, 600 for children and adults up to 70 years, and 800 IU for individuals over 70 years (Fu et al., 2023).

1.2.2 Vitamin D Mechanisms of Action

After the activation steps of vitamin D in the body and having a biologically functioning form, vitamin D works in tow different mechanism including genomic and nongenomic, and both of these mechanisms has an important role in metabolism. The genomic pathway, as demonstrated in figure 1.1, starts with (1,25(OH)2D), the active form of the vitamin that acts as a ligand by binding specifically to its receptor, vitamin D receptor (VDR). This receptor is a nuclear receptor working as a transcription factor consequently regulating the expression of various genes. Upon activation, VDR forms a heterodimer with the retinoid X receptor (RXR) (Carlberg et al., 2023). VDR/RXR complex controls gene activity by binding to specific DNA regions called vitamin D-responsive elements (VDRE). VDRE are very crucial in the response since they are located in the regulatory regions of target genes and consists of direct consecutive repeats of hormone response elements. Depending on the state of the cell, this interaction leads to activate or repress the gene expression and these processes keep the cell well-balanced. The transcriptional effects regulated by VDR are precisely controlled by a specific set of co-regulatoryspecific proteins, such as chromatin remodelers, co-activators, and co-repressors (Hanel et al., 2020). In addition to its genomic actions, 1,25(OH)2D also has an important role in the nongenomic effects by binding to membrane-associated VDRs or other membrane receptors, to regulate the activity of signaling molecules and accelerate these signals or the production of second messengers.

1.2.3 Functions of Vitamin D

Vitamin D plays a central role in regulating calcium and phosphorus metabolism, which is essential for maintaining bone mineralization, skeletal integrity, and overall bone health. By promoting calcium absorption in the intestines and reducing its excretion through the kidneys, vitamin D ensures proper bone formation and remodeling. Beyond its classical role in bone physiology, vitamin D also exerts multiple non-skeletal effects. It contributes to the regulation of immune system function by modulating both innate and adaptive immune responses(Huynh, 2020). Additionally, vitamin D influences cellular proliferation, differentiation, and apoptosis, which may impact the development of various chronic diseases, including autoimmune disorders, cardiovascular diseases, certain cancers, and metabolic conditions such as T2DM. 1,25(OH)2D carries out these effects by binding to VDR, which regulates the expression of numerous genes involved in various physiological processes.

1.2.4 The Protective Role of Vitamin D against T2DM

Vitamin D, in its active form 1,25 (OH)2 D regulates insulin secretion and sensitivity across multiple tissues relevant to T2DM. In pancreatic β cells, which express the VDR and 1α-hydroxylase (Cyp27b1), 25(OH)D is converted 1,25 (OH)2 D. Active vitamin D binds to VDR to modulate insulin gene expression, enhance glucose-stimulated insulin secretion (GSIS), and regulate intracellular calcium (Ca^{2}) via voltage-gated calcium channels (VGCC)

and calbindin, facilitating insulin vesicle exocytosis. Vitamin D also reduces endoplasmic reticulum (ER) stress and inhibits cytokine-induced β cell apoptosis through suppression of NF-κB and mitogen-activated protein kinase (MAPK) signaling (Morró et al., 2020; Kjalarsdottir et al., 2019).

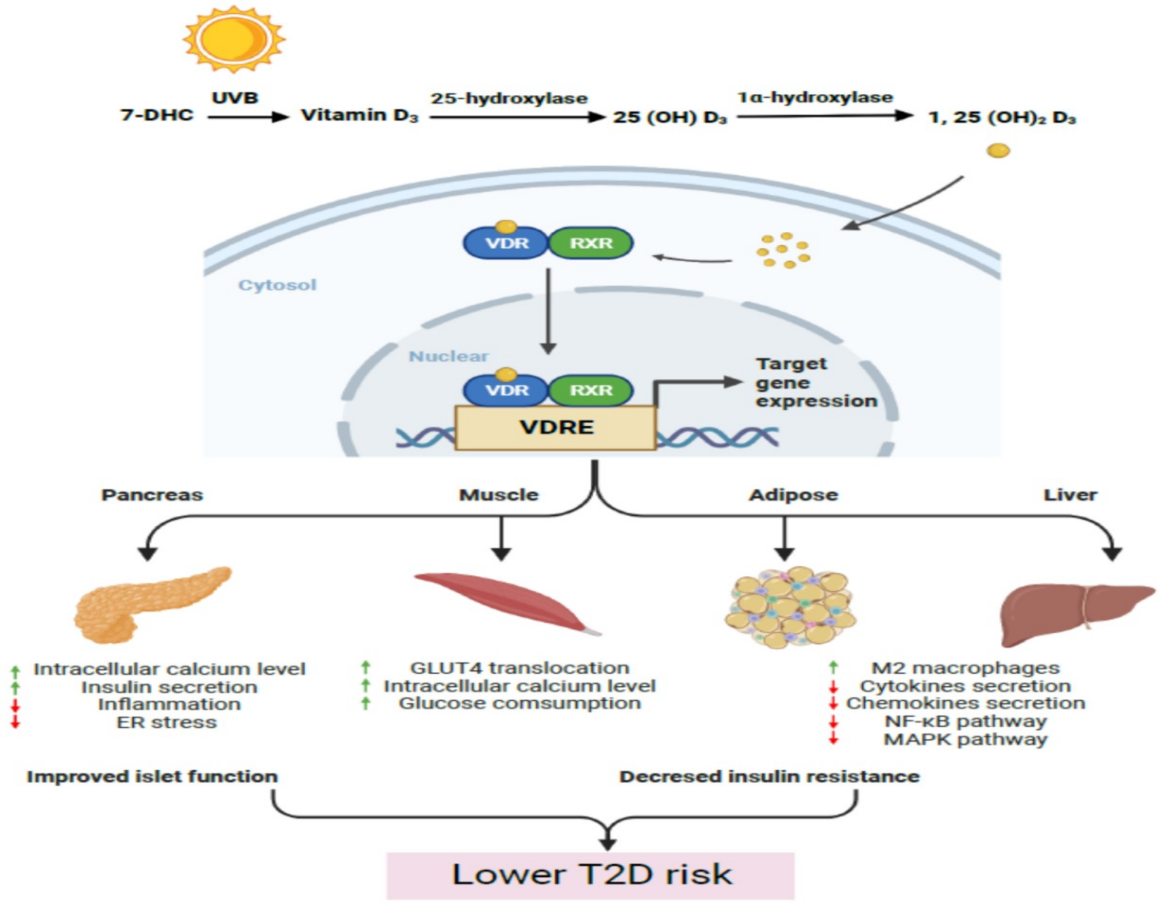

Figure 1.1 Vitamin D metabolism, signaling through VDR, and its role in

glucoseregulation.(Wu et al., 2023).

In peripheral tissues, as shown in figure 1.1, vitamin D improves insulin sensitivity. In skeletal muscle, 1,25(OH)2D-VDR signaling increases insulin receptor (IR) expression, and promotes GLUT4 translocation, enhancing glucose uptake (Liu et al., 2019). In adipose tissue, VDR activation down regulates pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) and chemokines (CCL2, CCL5, CXCL10, CXCL11), inhibits monocyte recruitment, and promotes anti-inflammatory M2 macrophages via NF-κB/MAPK pathway modulation (Marziou et al., 2020). In the liver, vitamin D reduces inflammation in macrophages and may restore insulin receptor expression, contributing to improved hepatic glucose metabolism (Dong et al., 2020).

Epidemiological and interventional studies support these mechanistic roles. Low serum 25(OH)D levels are linked to increased T2DM risk, insulin resistance, and impaired β cell function, while supplementation may improve insulin secretion and peripheral glucose uptake.

Collectively, vitamin D has pleiotropic effects across the pancreas, skeletal muscle, adipose tissue, and liver, focusing on its potential role in preventing T2DM progression.

1.2.5 Vitamin D Receptor Gene

VDR gene is located on the long arm of human chromosome 12 (12q13.11), as shown in figure 1.2. It spans approximately 75kb and consists of nine exons. The VDR gene is a member of the nuclear receptor superfamily of transcriptional regulators which plays a significant role in mediating the biological actions of 1,25(OH)2D (Pike, 2011).

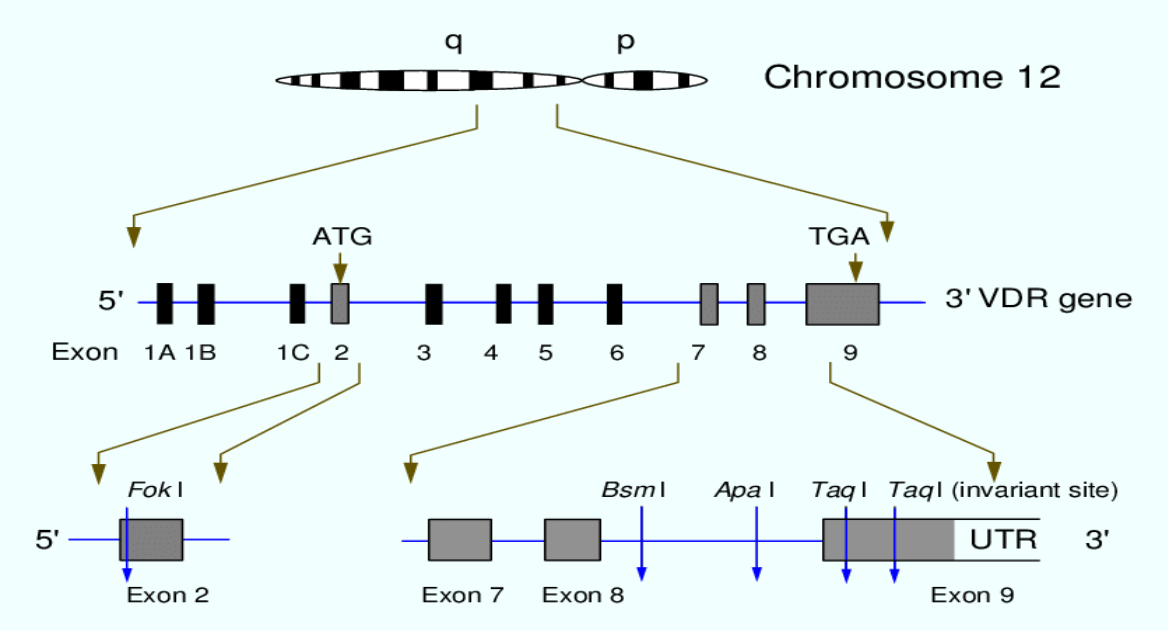

Figure 1.2 Vitamin D Receptor (VDR) genomic organization and key polymorphisms(Kohama et al., 2000).

Functionally, the VDR forms a heterodimer with one of the three RXRisoforms, RXRα, (NR2B1), RXRβ (NR2B2), or RXRγ, (NR2B3) to regulate gene transcription. VDR and its ligand control and influence a wide range of physiological processes, includingcalciumand phosphate homeostasis, cell proliferation, differentiation, apoptosis and both innate and adaptive immune responses (Bikle & Christakos, 2020; Rochel, 2022). VDR is expressed in different tissues throughout the body, many of these tissues were not originally considered as classical targets for 1,25D3, emphasizing the broad and systematic role of vitamin D signaling beyond its important role in bone metabolism.Deregulation of VDR function can contribute to the development of a wide range of pathological conditions reflecting its important role in cellular regulation and immune balance. These diseases due to impaired VDR signaling are considered serious conditions, including, various cancers, rickets (a childhood bone disorder), psoriasis, renal osteodystrophy, diabetes,and several autoimmune disorders such as multiple sclerosis, inflammatory bowel diseases, and rheumatoid arthritis (Umar et al., 2018).

1.2.6 Vitamin D Receptor Gene Polymorphisms

VDR gene polymorphisms have recently gained attention due to their association with susceptibility of various clinical conditions (Ansari et al., 2021). The most studied variants of VDR gene are ApaI (rs7975232), BsmI (rs1544410), TaqI (rs731236), and FokI (rs2228570). ApaI, BsmI, and TaqIare located within the 3′ untranslated regions (3′ UTR) of the gene.Variants in this region can influence mRNA stability, and consequently the efficiency of protein translation(Faghfouri et al., 2022). In contrast, the FokI polymorphism is located near the 5′ end of the VDR gene, close tothe promoter region as presented in figure 1.2(Al-Daghri et al., 2014). This single nucleotide polymorphism (SNP), FokI, is caused by a T > C base substitution in the start codon of exon 2, resulting in an altered translation initiation site. The mutation produces a shorter VDR protein consisting of 424 amino acids, rather than 427 amino acids (full-length). Functionally, this truncated version shows increased transcriptional activity, suggesting that the FokI variant can modify VDR-mediated gene regulation. Overall, these polymorphisms whether occurring in intronic or exonic regions, can influence the expression, stability, and functionality of the VDR protein, consequently altering the cellular response to vitamin D and contributing to various complex disorders.

1.2.7Role of Vitamin D Receptor Gene Polymorphisms in Human Diseases

Polymorphisms in the vitamin D receptor (VDR) gene can alter vitamin D signaling and have been associated with susceptibility to various diseases, including metabolic, autoimmune, cardiovascular, and neurodegenerative disorders.

1.2.7.1 VDR Gene Polymorphisms in Neurodegenerative Diseases

VDR gene polymorphisms have been associated with Alzheimer's disease (AD), especially the late-onset AD (LOAD). Polymorphisms such as ApaI, BsmI, TaqI, FokI, and Cdx2 have been linked to altered vitamin D signaling, affecting neuroprotection through β-amyloid clearance, neurogenesis, and reduced neuroinflammation (Janjusevic et al., 2022; Liu et al., 2021). Studies report population-specific associations: ApaI and BsmI predict mild cognitive impairment (MCI), while TaqI is linked with AD risk, though results vary across ethnic groups (Dimitrakis et al., 2022; Jeong et al., 2024; Khorshid et al., 2013). Haplotypes formed by BsmI, ApaI, and TaqI polymorphisms have also been linked with cognitive decline, suggesting combined effects of genetic and environmental factors.

Similarly, VDR polymorphisms are implicated in Parkinson's disease (PD). The FokI (rs2228570) SNP, in particular, shows a strong association with PD risk, with the T allele and TT genotype linked to increased susceptibility, while the C allele produces a shorter, more transcriptionally active VDR protein that may confer protection (Agliardi et al., 2021). VDR regulates neurotrophic factors, which support dopaminergic neurons, and vitamin D deficiency is frequently observed in PD patients (Zheng et al., 2024). These findings suggest that VDR genetic variation can modulate the neuroprotective effects of vitamin D, influencing both AD and PD risk, and underscore the potential value of personalized vitamin D-based preventive or therapeutic strategies (Usategui-Martín et al., 2022).

1.2.7.2 VDR Gene Polymorphisms in Cancer

Vitamin D deficiency is common among breast cancer patients and has been linked to increased disease risk. In women with breast cancer, serum vitamin D levels were often significantly lower compared to healthy controls (Imtiaz et al., 2012; Shrestha & Bista, 2023). Variants in the VDR gene, such as the FokI polymorphism, were associated with higher susceptibility to breast cancer, with certain genotypes conferring greater risk (Xu et al., 2018). Low circulating vitamin D may reduce local production of the active hormone within breast tissue, potentially impairing anti-proliferative effects and promoting tumor development.

Similarly, VDR gene polymorphisms have been associated with colorectal cancer. While individual SNPs often showed mixed results(Serrano et al., 2016), haplotype analyses showed specific combinations that significantly increased colorectal cancer risk(Messaritakis et al., 2020; Pan et al., 2018). Polymorphisms located in regulatory regions, such as the 3′-UTR, may affect gene expression and disrupt vitamin D signaling. Epidemiological evidence also supports a role for vitamin D and calcium intake in modulating colorectal cancer risk, highlighting the interaction between genetic and environmental factors (Al-Ghafari et al., 2019). Genotyping and haplotype analysis of VDR may help identify high-risk individuals and guide targeted prevention strategies.

1.2.7.3 VDR Gene Polymorphisms in Osteoporosis

VDR plays an important role in bone metabolism through regulation of calcium and phosphate homeostasis, and polymorphisms within the VDR gene have been linked to susceptibility to osteoporosis. Several studies have shown that ApaI, BsmI, and FokI polymorphisms are significantly associated with osteoporosis risk in the overall population, while TaqIshows inconsistent(Jiang et al., 2022; Zhang et al., 2018). Subgroup analyses reflect population-specific effects, with ApaI, BsmI, and TaqI variants associated with osteoporosis risk in Caucasians, whereas BsmI and FokI are associated with risk in Asians. Mechanistically, these variants may alter VDR mRNA expression or protein function, thereby impairing vitamin D activity and affecting bone mineral(Fu et al., 2020). Moreover, certain polymorphisms such as ApaI, BsmI, and FokI have been implicated in type I osteoporosis, while FokI and TaqI are linked to type II disease, though evidence remains limited by small sample sizes. These various findings indicate that genetic variation in VDR contributes to osteoporosis pathogenesis, with ethnicity and disease subtype influencing the strength of this association(Jiang et al., 2022).

1.2.7.4 VDR Gene Polymorphisms in Obesity

VDR and its gene polymorphisms may influence obesity risk and related traits, although results vary across populations due to differences in ethnicity, age, gender, and study design (Bagheri et al., 2017; Faghfouri et al., 2022). VDR regulates adipose tissue function by mediating vitamin D effects on adipogenesis, energy metabolism, and gene expression, and its expression is often reduced in obese patients (Beydoun et al., 2018). Polymorphisms such as FokI, ApaI, BsmI, and TaqI can alter VDR structure and function, potentially affecting these processes, but studies worldwide report conflicting associations with BMI, fat mass, and other obesity(Karonova et al., 2018; Khan et al., 2018; Khan et al., 2016). Overall, current evidence

does not establish a definitive role for VDR polymorphisms in obesity, highlighting the need for large, well-controlled studies.

1.2.7.5 VDR Gene Polymorphisms in DM

VDR plays a central role in mediating the effects of vitamin D, which is important for glucose metabolism, immune regulation, and endocrine function as discussed earlier. In T1DM, patients often exhibit lower 25(OH)D levels, and vitamin D insufficiency has been linked to increased disease susceptibility (Cheng et al., 2016; Ferraz et al., 2022). VDR is expressed in pancreatic β-cells, activated T cells, and antigen-presenting cells, suggesting a protective role in T1DM, though associations between common VDR polymorphisms such as ApaI, TaqI, and FokI and T1DM risk have demonstrated population-specific variability(Ahmed et al., 2019; Zhai et al., 2020). In GDM, meta-analyses indicate that VDR polymorphisms such as ApaI and FokIare associated with increased risk in certain ethnic groups, while BsmI and TaqI show variable associations depending on population background (Liu, 2021; Zhu et al., 2019). For T2DM, low vitamin D levels have been implicated in disease pathogenesis, and VDR polymorphisms, particularly TaqI and FokI, have been associated with increased T2DM susceptibility in overall and ethnic-specific populations, while the association with BsmIis less consistent (Salehizadeh et al., 2024). These findings collectively highlight that VDR polymorphisms affect vitamin D function, influence glucose homeostasis, and contribute to diabetes risk. These effects are population- and ethnicity-specific, emphasizing the need for further multi-ethnic studies with larger sample sizes to clarify these genetic associationsTherefore, these polymorphisms are associated with various diseases, indicating their potential role in disease susceptibility and progression.

1.3 Objectives of the study

The relationship between VDR gene polymorphisms including (FokI, ApaI, TaqI, and BsmI) and T2DM is not well understood, particularly here in Palestine. Investigating this gap is crucial for identifying genetic factors that may contribute to the risk of developing T2DM. Therefore, our study aims to investigate and explore the link between specific VDR gene polymorphisms and T2DM among Palestinian patients in Hebron, West Bank.

The specific objectives are:

- To determine allele and genotype frequencies of VDR gene polymorphisms (FokI, ApaI, TaqI, and BsmI) in patients diagnosed with T2DM compared to non-diabetic controls in Hebron, Palestine.
- To examine the relationship between the significant VDR variants and clinical parameters (e.g., age, BMI, and blood pressure) as well as biochemical parameters, including fasting blood glucose, HbA1c, and lipid profile in diabetic patients.
- To investigate the combined effects of these polymorphisms on T2DM susceptibility through haplotype analyses.

1.4 Significance of the Study

Our study aims to investigate the association between well-known VDR gene polymorphisms and T2DM patients from Hebron district in Palestine. This is the first study in the region to explore these associations. Understanding the relationship will help identify genetic risk factors for T2DM, supporting improved screening and personalized treatment strategies and enhancing health outcomes in Palestine. Additionally, the methodology introduced in this study offers a precise, cost-effective, and labor-efficient approach, which can benefit researchers studying various VDR-related diseases.

1.5 Literature Review

Many studies have investigated the role of various VDR polymorphisms in T2DM whether a single SNP or multiple SNPs in the same research. It is reported four well-characterized di-allelic variants: ApaI (A > C, rs7975232), BsmI(T > C, rs1544410), TaqI (A> G, rs731236), and FokI (T > C, rs2228570). However, the results of these studies are still uncertain and controversial, since different research teams, designs and ethnicity lead to different results.

Several studies on the Chinese Han population have highlighted varying associations between VDR polymorphisms and T2DM with its complications. The first study, (Jia et al., 2015) found no significant association between FokIpolymorphism and T2DM. In contrast, the second one (Zhong et al., 2015) focused on diabetic retinopathy (DR) as a complication of T2DM and identified a significant association between FokI and increased DR risk along with diabetes duration. However, no significant association between DR and BsmI or ApaI polymorphisms. A recent prospective cohort study (Fu et al., 2024) in the same population reported a significant interaction between VDR polymorphisms and the risk of T2DM.

In the Emirati population, (Safar et al., 2018)identified a significant association between VDR polymorphisms (specifically FokI and BsmI) and T2DM, while no link was found with TaqI SNP. Meanwhile, (Al-Daghri et al., 2014) in Saudi Arabia reported that BsmI polymorphism increased the risk of T2DM and lower HDL cholesterol levels, while FokI appeared to reduce the risk of developing the disease Building on this, a 2022 Saudi study (Shafie et al., 2022) found that BsmI and TaqI polymorphisms were significantly associated with an increased risk of coronary artery disease in patients with T2DM, with the TaqI t allele showing the strongest association. Conversely, (Rodrigues et al., 2019) found no significant associations between various VDR polymorphisms, including ApaI, BsmI, FokI, and TaqI with T2DM in a cohort Brazilian study. Additionally, (Bertoccini et al., 2017) in Italy concluded that FokI was not significantly associated.

In an Egyptian study, (Gendy et al., 2019) explored a significant association between one of the detected SNPs which is FokI polymorphism and susceptibility to T2DM, while no differences were noted for BsmI and Taq polymorphisms. In the same country, another study in 2023, (Mohammed et al., 2023) reported significant differences in allelic discrimination for VDR polymorphisms (FokI and BsmI), indicating a positive association with T2DM risk, while no significant difference for ApaI. In a more recent investigation, (Fteah et al., 2025)

focused on the association of FokI, and ApaI variants with nephropathy stages in Egyptian T2DM patients. They found that the FokI ff genotype and ApaI aa genotype were significantly more prevalent in diabetic individuals, with the f and a alleles associated with a 17-fold and 13-fold increased risk of end-stage renal disease (ESRD), respectively. These findings suggest a potential role for VDR polymorphisms as genetic markers for both T2DM and its complications.

A pilot study in Jordan by (Alhawari et al., 2022), found ApaI and BsmI are associated with an increased risk of developing retinopathy in T2DM patients, therefore, these variants are considered risk factors for complications related to the disease in Arab population. Later (Alfaqih et al., 2023) noted a significant link between FokI SNP and the risk of T2DM, while no similar association was found with the other variants among Jordanian population.

In Pakistan, they investigated the association between BsmI SNP with T2DM (Khan et al., 2019). In their cohort study, they found a significant association, considering BsmI variant as a risk factor for the disease in this population. In the same country, (Memon et al., 2022) investigated another variant, which is FokI and found a significant association between FokI polymorphism and T2DM. As well as in the Kashmir Valley by (Malik et al., 2018) in thier hospital-based-case-control study found a significant association between the TaqI (t allele) and BsmI (b allele) polymorphisms and the risk of T2DM. In contrast, studies performed on different populations in Malaysia, North England, and South Africa found no considerable differences of the FokI, TaqI, and BsmI genotypes between T2DM patients and healthy controls in these populations (Erasmus et al., 2022; Sattar et al., 2021; Zakaria et al., 2021).

A comprehensive meta-analysis including 47 case-control studies (Aravindhan et al., 2021) found significant association between certain VDR polymorphisms and susceptibility to T2DM. Specifically, FokI and BsmI (under the heterozygous model) showed a significant link with increased T2DM risk, while no overall association was observed with other variants. Conversely, (Han et al., 2017) included 28 studies and reported no overall significant associations between these variants with T2DM. However, their subgroup analyses found significant associations of the ApaI variant with T2DM in Asian populations, and associations of BsmI and TaqI variants with insulin resistance-related diseases in dark-pigmented Caucasians. Building upon these findings, (Liu et al., 2021)conducted an updated and more rigorous meta-analysis, integrating data from various global databases and applying robust statistical tests including false-positive report probabilities (FPRP) and Bayesian false discovery probability (BFDP). The results showed a significantly decreased T2DM risk associated with BsmI and FokI polymorphisms in Asian and African populations, and with ApaI polymorphism in Caucasians and North Americans. Conversely, ApaI was linked to increased risk in mixed populations. However, their credibility analysis (FPRP > 0.2, BFDP >0.8) indicated that these associations were likely false positives. The authors concluded that while statistical associations were observed, they are likely false positives rather than true genetic associations. This shows the importance of evaluating the credibility of statistically significant findings in genetic association studies, especially when interpreting marginal or ethnicity-specific results. These meta-analyses underscore the complexity of the relationship

between VDR polymorphisms and T2DM, highlighting the potential roles of ethnicity, genetic heterogeneity, and study quality in shaping outcomes.

Therefore, these findings show the variability of VDR gene polymorphism associations with T2DM across different populations, suggesting that genetic factors interact differently with environmental and lifestyle factors in diverse ethnic groups.In this study we aimto investigate the association between specific VDR gene polymorphisms and T2DM in Palestine.

Chapter Two

Methodology

2.1 Study Participants

This research study included 300 Palestinian participants from Hebron, comprising 200 diabetic patients (case group) and 100 non-diabetic ones (control group). All participants were older than 40 years at the time of data collection process. Patients were actively recruited from Alia Governmental Hospital, and Palestinian Diabetes Institute in Hebron. The medical records of each patient proved the diagnosis of T2DM. Patients were diagnosed according to the criteria of the WHO(Kazi & Blonde, 2001).The control participants were selected from patients visiting the internal medicine and emergency departments at the hospital.

2.2 Study Design and Participation Criteria

A case control study design was employed to evaluate the frequency of FokI (rs2228570), ApaI (rs7975232),TaqI (rs731236), and BsmI (rs1544410)VDR gene polymorphisms among patients with T2DM. Our study included unrelated participants, recruited within the period from January to April 2025.

2.2.1 Inclusion Criteria

- Palestinian participants aged 40 years and older.
- Participants who were willing to provide informed consent for participation in the study **(Appendix1)**.
- Group1: Unrelated diabetic patients diagnosed with T2DM for at least one year.
- Group2: Healthy individuals with no history of diabetes (controls).

2.2.2 Exclusion Criteria

- Individuals are younger than 40 years.
- Patients with incomplete medical records.
- Patients diagnosed with T1DM.
- Pregnant females with or without GDM.

2.3 Ethical Consideration

This study was approved by the Research Ethics Subcommittee of the Faculty of Medicine at Al-Quds University (Ref#: Re7-21-24)**(Appendix 2)**. Each Participant provided a written informed consent, confirming their approval to participate in the study.

2.4 Study Investigations

This study included the collection of demographic, clinical, and biochemical data to evaluate metabolic status and disease-related risk factors among participants.

2.4.1 Data Collection and Biochemical Analysis

Participant's Demographic and clinical information, such as age, gender, height, weight, blood pressure, diabetic-related complications, and treatment history, were checked and obtained from both paper-based and electronic medical records of the participants after obtaining their consent to participate in our study. Biochemical measurements including FPG, HbA1c, and lipid profile were analyzed using the Selectra Pro automatic biochemistry analyzer at the laboratory of the Palestinian Diabetes Institute, and the Abbott Alinity system at Alia Governmental Hospital laboratory. Prior to data collection, approval and permission to access medical records and to collect blood samples were obtained from the participating institutes.

2.4.2 Blood Sample Collection and Processing

From each participant, 5mLof venous blood was drawn in the laboratory phlebotomy room under strictbiosafety and quality control protocols after ensuring the comfort of the participants through the process. Of the collected volume, 2mL were placed into sterile ethylenediamine tetra acetic acid (EDTA) tubes for the determination of HbA1c and later for DNA extraction process, molecular analyses and SNPs genotyping.

The remaining3 mL were transferred into plain tubes and allowed to clot in the laboratory at room temperature. Once clotting was complete,thesamples were centrifuged at 3000 rpm for

10 minutes and the resulting serum was carefully separated into new tubes. The serum samples were used for measuring FPG levels at Alia Governmental Hospital laboratory and Palestinian Diabetes Institute using different analyzers as mentioned earlier. All biochemical tests we needed for our study were conducted as part of routine diagnostic procedures for diabetic patients, and no additional tests were requested from theses institutes to enrich our study.

2.5 Molecular Analysis

Molecular analysis was conducted to evaluate genetic variations through DNA extraction and genotyping of selected polymorphisms.

2.5.1DNA Extraction

DNA extraction was performed using whole blood samples collected from participants in EDTA tubes using the Wizard® Genomic DNA Purification Kit and according to the company recommendations (Promega).

The detailed instructions were provided in manual recommendations #TM050for human blood as shown in **(Appendix 3)**.DNA was extracted from 300µL of whole blood. Briefly, for each 300µLsample volume, 900µLof cell lysis solution was added in each tube to break down cell membranes, the mixture was incubated at room temperature for 10 minutes with inversion. Then tubes were centrifuged for 1 minute, the supernatant product was removed and the white pellet was kept. 300µLof nuclei cell lysis and 100µLof protein precipitation then samples were centrifuged. The supernatant in this step was needed, it contains DNA, then mixed with isopropanol until the white thread of DNA was visible. After this, a series of washing steps remove proteins and other impurities, using ethanol and other washing solutions. The DNA is then precipitated out, washed again, and dissolved in a buffer solution.

2.5.2 DNA Quantification

The concentration of extracted DNA was measured using a Qubit fluorometer, which employs a fluorescent dye specific for double-stranded. This method allows for accurate and sensitive quantification with minimal interference from contamination by measuring fluorescence proportional to the amount of double-stranded DNA present in each sample.

2.5.3 Primer's Selection

Primers were designed to target several gene regions including FokI(rs2228570), ApaI (rs7975232), TaqI (rs731236), and BsmI (rs1544410) sites. The National Library of Medicine was used to retrieve the genomic sequence database that includes the target SNPs. Primers were designed by Primer 3 software which ensured optimal primer sequences and accurate amplification for targeting the SNPs-containing region for subsequent analysis focusing on

parameters that minimize self-complementarity and secondary structures while maximizing annealing specificity. Table 2.1 presents the forward and reverse primer sequences designed for each target SNP in the VDR gene.

Table 2.1 Sequences of PCR primers for the selected VDR gene polymorphism

SNP ID	Region	Variant	Primers Sequence 5'-3'	PCR Product bp
ApaI rs79785232	Intron-8	A>C	F: GGCACGGGGATAGAGAAGAA R: GAGAAGTCACTGGAGGGCTT	172
BsmI rs1544410	Intron-8	T>C	F: AAAGTTTTGTACCCTGCCCG R: GTGTGTGGACGCTGAGGT	214
FokI rs2228570	Exon-2	T>C	F: CACGTTCCGGTCAAAGTCT R: GAGATGCCCACCCTTGCT	203
TaqI rs731236	Exon-9	A>G	F: GTACGTCTGCAGTGTGTTG R: AGTCATAGAGGGGTGGCCTA	201

SNP: Single Nucleotide Polymorphism; VDR: Vitamin D Receptor; PCR: Polymerase Chain Reaction; F: Forward primer sequence; R: Reverse primer sequence; bp: base pair.

Each forward primer attached to the Illumina overhang adapter sequences at the 5' end (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3') and each reverse primer modified by adding the Illumina overhang adapter sequences at the 5' end of it (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG). These sequences were added to the primers to enable the amplified DNA fragments to attach to the flow cell for NGS, ensuring platform compatibility and efficient data generation.

2.5.4 DNA Amplification, Gel Electrophoresis and Library Preparation

The DNA library was generated using two-stage PCR process. First-stage PCR to amplify target SNP regions, purification and cleaning the first PCR products, and the second-stage PCR to add sample specific barcodes. Each step is described in detail below.

2.5.4.1 First-Stage PCR

In the first stage, target SNP regions were amplified using a multiplex PCR reaction. Each reaction contained 2.5 µL of genomic DNA (from the extracted samples), 1 µL of a 10uM mix of forward and reverse primers for each target SNP, 12.5 µL of PCRBIO HS TaqPCRMixRed(PCR Biosystems), and 9µL of nuclease free distilled water. The PCRMixRed contains hot-start Taq DNA polymerase, dNTPs, $MgCl_2$, and red loading die

which improves amplification specificity and allows the PCR products to be loaded directly onto agarose gel without any additional dye.

The PCR was performed on a 96-Well Thermal Cycler (Applied Biosystems), amplification conditions were specifically optimized for the multiplex amplification for the selected VDR gene regions. The run began with an initial denaturation step at 95°C for 5 minutes (stage 1), ensuring complete separation of the DNA strands. This was followed by 28 amplification cycles (stage2), each consisting of denaturation at 95°C for 30 seconds, then annealing at 65°C for 30 seconds, and extension at 72°C for 40 seconds. A final extension step (stage 3) was carried out at 72°C for 6 minutes to ensure full elongation of all amplified fragments. At the end of the run, the reaction was held at 12°C until samples were collected for downstream purification.

2.5.4.2 Gel Electrophoresis

The PCR products of the first stage were subsequently loaded onto a 2% agarose gel. The gel was prepared by using 2g of agarose anddissolvedin 100 mL Tris-acetate-EDTA (TAE) buffer. The mixture was heated in a microwave for 2 minutes until the agarose was completely melted and clear. After slight cooling at room temperature, then three drops of a 10mg/ml (0.4-0.6ug/mL) ethidium bromide stock solution were added, this step was done to visualize DNA bands under UV light, the solution was then poured into a casting tray, allowed to solidify. Subsequently, 5µL of every PCR product were carefully loaded into the wells in parallel with 5µl of 100bp DNA molecular ladder (Bio-Helix, Taiwan). Finally, electrophoresis was conducted approximately for 40 minutes at 120 Volts. After the run, the amplified DNA fragments were visualized under UV light usingaUVITEC Gel Documentation System (GelDoc) to confirm successful amplification.

2.5.4.3Purification of First PCR Products

After amplification and confirmation the success of the process using gel electrophoresis, all PCR products were purified using the AgencourtAMPure XP system (X1, cat. no. A63881; Beckman Coulter Genomics) to remove residual primers, nucleotides, enzymes and other contaminations, ensuring that the DNA fragments were clean and suitable for the second PCR stage. For each reaction, 20µL of PCR product were combined with an equal volume of AMPure XP beads (20µL) and mixed vigorously to ensure effective efficient binding of DNA fragments. The mixture was incubated at room temperature for 10 minutes, after that the tubes were placed on a magnetic stand for approximately 5 minutes until the solution cleared and the beads were fully pelleted. The supernatant from each sample was carefully discarded, and the bead-bound DNA was washed twice with 180µL of freshly prepared 80% ethanol using a multichannel pipette, keeping the tubes on the magnetic stand throughout the washing steps. After the second wash, the beads were left to air-dry on the magnet for an additional 5 minutes to ensure complete evaporation of residual ethanol drops.

The tubes were then removed from the magnetic stand, and the DNA was eluted by adding 25µL of elution buffer, mixing and incubating for 5 minutes. The tubes were returned to the magnetic stand, and once the solution cleared, the elute containing the purified DNA and then transferred to new tubes to be used in the second PCR reaction.

2.5.4.4Second-Stage PCR

This stage was performed to incorporate indices and llumina sequencing adapters using Nextera XT Indexing system. Each reaction consisted of 7.5µL of purified first-stage PCR product, 12.5µL of the ready master mix, and 2.5µL of each index primer (i7 and i5), resulting in 2 unique indices for each sample. All these components were added into new PCR tubes, The prepared libraries were sequenced on a NextSeq 500/550 machine using the 150-cycle Mid Output Kit (Illumina, Inc). Then 5µL from each indexed sample were combined in a single tube to created the pooled library. 100µL of the pooled mixture was then purified using 100µL of AgencourtAMPure XP beads, after mixing the tube was incubated on the magnetic stand until the solution is clear, and the supernatant was removed. The bead-bound DNA was washed with ethanol, air-dried to eliminate residual alcohol, and finally eluted in the appropriate volume of elution buffer. As in the purification step, the elute was returned to the magnetic stand, and the clarified supernatant representing the cleaned and indexed sequencing library, that transferred into a fresh new tube for further quantification and sequencing.

2.6 Bioinformatics Analysis

The DNA sequence results as FASTQ files that obtained via Next Generation Sequencing (NGS), and by using Galaxy platform for the analysis and processing. The analysis process was designed as one workflow that consists of sequences upload as collection, then filtering the high-quality sequence and trimming the adaptors, followed with SNPs specific sequence selection, and finally counting the identical ones as shown in figure 2.1. The selected SNPs (TaqI, FokI, ApaI, and BsmI) of VDR gene were successfully identified ina single reaction tube within NGS approach. FASTQ files that we had were considered as raw data, and were processed using the platform's default quality filtering parameters to ensure the retention of only high-quality reads. The detailed procedure of the workflow that was used for identifying and detecting SNPs is presented in figure 2.1. The collapsed sequence was analyzed usingBLAST tool to ensure amplifying the targets regions. To further validate the sequencing results, the presence of the both allele at the studied polymorphic sites was confirmed by performing multiple sequence alignment using a free online alignment program (MultiAlin tool),which allowed comparison of the obtained sequences with the reference sequence.

2.7 Statistical Analysis

Data analysis was performed using IBM SPSS Statistics for Windows, Version 29. Differences between cases and controls for categorical variables were assessed and analyzed using the Pearson's chi-square. Continuous variables that did not follow a normal distribution were reported and expressed as the median with the Interquartile range(IQR). Mann-Whitney U test was used to evaluate the significant differences between the studied groups. Logistic regression was employed to evaluate the association between VDR genotypes and T2DM risk under different genetic models.

SNPStatswas used to calculate haplotype frequencies and their associations with the disease. Statistical significance defined as $p<0.05$.

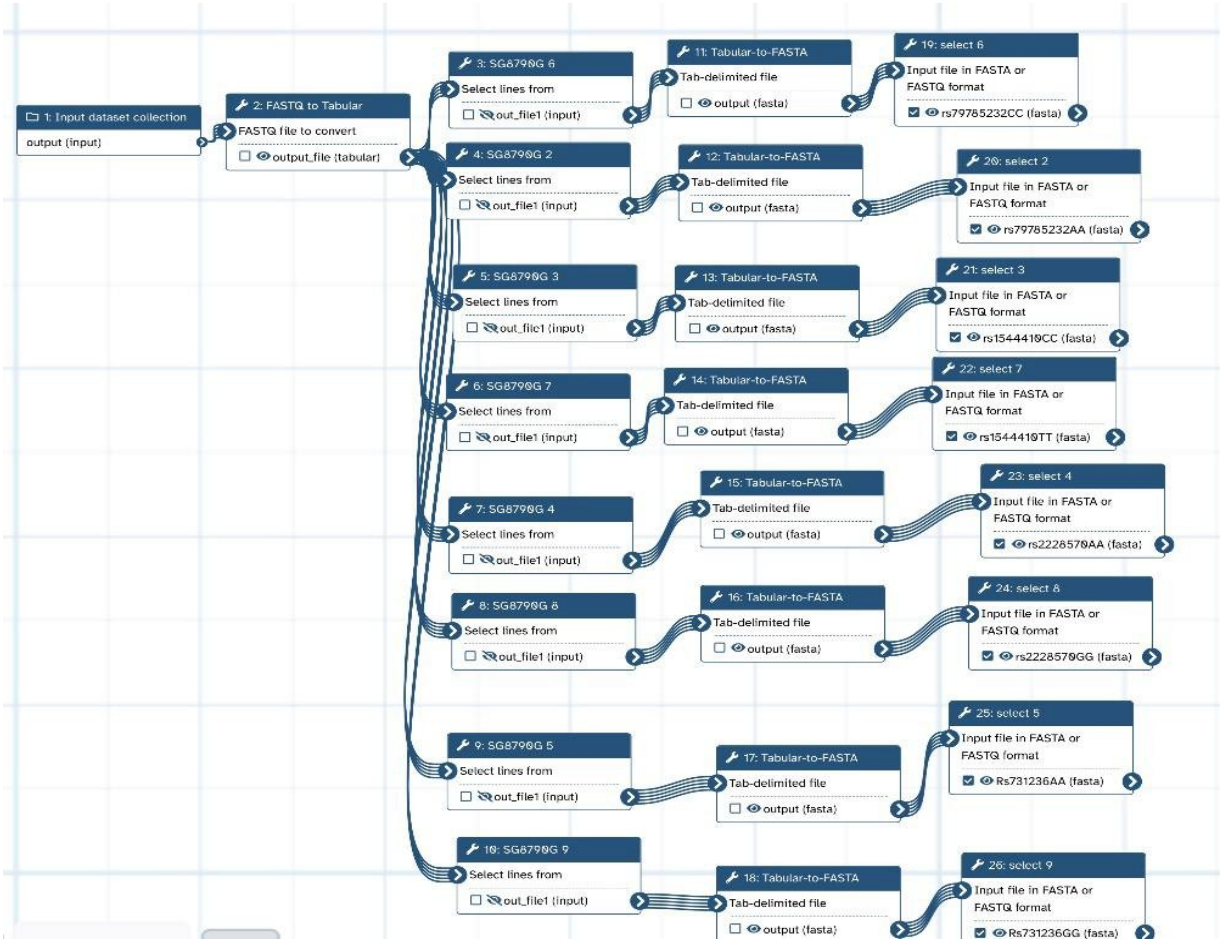

Figure 2.1 Diagram of the NGS workflow.

Chapter Three

Results

3.1 Demographic and Clinical Characteristics of the Study Participants

A total of 300 Palestinian participants were recruited between January to April 2025 from Alia Governmental Hospital and Palestinian Diabetes Institute in Hebron, Palestine. Of them, 200 were cases (T2DM patients) and 100 were controls (without T2DM). The continuous variables were not normally distributed, data is presented as median with interquartile range (IQR) as shown in table 3.1. The proportion of females was 47% in the diabetic group compared to 44% in the control group, showing a significant association between gender and T2DM status ($p = 0.0001$). The median age of diabetic patients was significantly higher compared to controls (60 vs. 54 years, $p = 0.0001$). Diabetic patients also had a significantly higher BMI (30.1 vs. 28.0 kg/m², $p = 0.001$) and weight (85 vs. 79 kg, $p = 0.01$), while their height was slightly lower than that of controls (165 vs. 167.2 cm, $p = 0.039$). No significant difference was found in systolic blood pressure ($p = 0.237$), whereas diastolic pressure was significantly lower in the diabetic group (81 vs. 84 mmHg, $p = 0.035$). As expected, HbA1c and fasting plasma glucose levels were markedly elevated among diabetic patients compared to controls ($p < 0.001$). Regarding the lipid profile, the median of triglyceride levels was significantly higher and HDL cholesterol significantly lower in the diabetic group ($p < 0.001$), while total cholesterol and LDL cholesterol did not differ significantly between the two groups as shown in table 3.1.

3.2 Concentration of DNA Samples

DNA concentrations were measured in randomly selected 45 DNA samples (15% of the total 300 samples) to ensure enough quantity for downstream analysis. As shown in tables 3.2 (A-B), DNA concentrations of eight representative samples ranged from 22.8 to 52.7ng/µL, with values adequate for PCR and genotyping procedures. These results confirm the reliability of the DNA extraction process across the selected samples.

Table 3.1 The demographic and clinical data for all study participants

Variable	Type 2 diabetic patients n=200	Non-diabetic controls n= 100	P-value
	Median (IQR)		
Age (years)	60 (53-66.7)	54 (45.3-64)	0.0001*
Gender, n (%) Female	94 (47%)	44 (44%)	0.0001 *
BMI (kg/m2)	30.11 (27.8-32.4)	28.02(26.5-29.9)	0.001*
Height (cm)	165(158-173)	167.2 (160-175)	0.039*
Weight (kg)	85 (74.3- 93)	79 (73-87)	0.01*
SBP (mmHg)	127 (120-134)	125 (120-130)	0.237*
DBP (mmHg)	81 (75-87)	84 (80-87.8)	0.035 *
HbA1c (%)	7.95 (7-9.4)	5.4 (5.1-5.7)	0.0001*
FPG (mg/dL)	158 (129-200)	88.7 (75-99)	0.0001*
TG (mg/dL)	144.5 (100-199.7)	104.5 (86-155)	0.001 *
TC (mg/dL)	154.5 (132-185)	160.1 (130-197)	0.099
LDL (mg/dL)	100 (75-132.7)	100 (78-146)	0.354
HDL (mg/dL)	35 (30-40)	39.5 (34-48)	0.001 *

Values for all continuous variables are expressed and presented as the median (interquartile range, IQR). P-values were calculated using the Pearson's chi-square test for discrete variable (gender) and Mann–Whitney U test for all continuous variables. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HbA1c: glycated hemoglobin; FPG: fasting plasma glucose; TG: triglycerides; TC: total cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein; n: number; *Significant p-value < 0.05.

Table 3.2-A DNA concentrations of representative study samples

Sample ID	Group	DNA Concentration (ng/μL)
25	Diabetic	34.2
34	Diabetic	50.6
57	Diabetic	22.8
68	Diabetic	27.9

Table 3.2-B DNA concentrations of representative study samples

86	Control	33.4
90	Control	38.5
98	Control	43.4

3.3 PCR Amplification and Gel Electrophoresis of VDR Gene Polymorphisms

Multiplex PCR amplification of the selected VDR gene polymorphisms (BsmI,TaqI,ApaI, andFokI) was successfully performed using the designed primers. The PCR products were visualized on 2% agarose gel electrophoresis, and it appeared as four distinct and specific bands, ranging from approximately 200–250 bp, corresponding to the targeted fragments of the VDR gene. Sixteenrepresentative samples were shown in figure 3.1. The presence of clear, specific bands confirmed the success of the PCR reactions. Negative controls were included to rule out contamination and validate the reactions. Band intensities varied among samples, reflecting differences in the quantity of the extracted DNA.

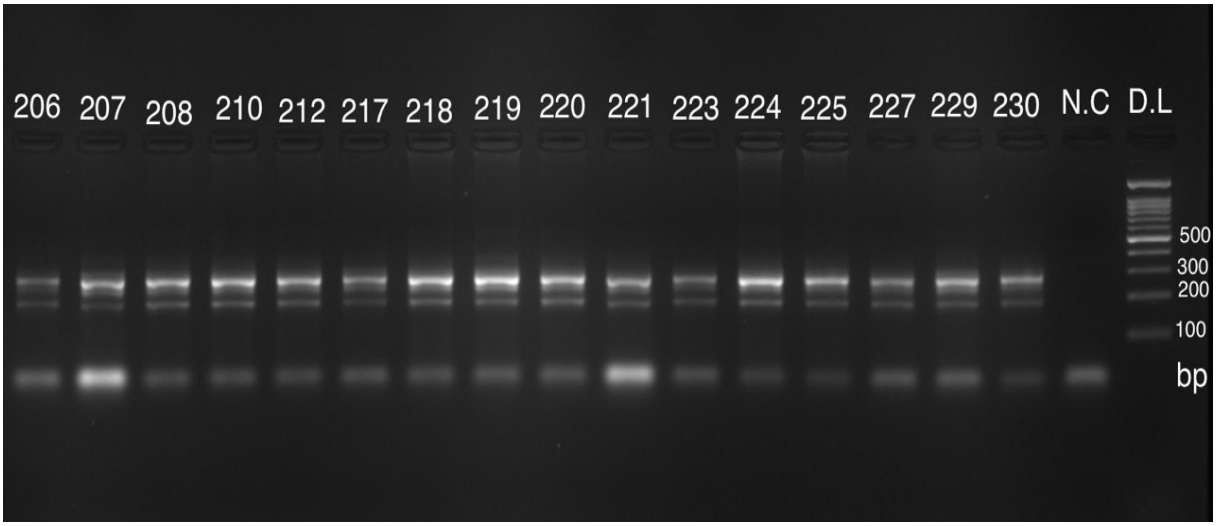

Figure 3.1 Agarose gel electrophoresis of multiplex PCR products for VDR gene polymorphisms. N.C: Negative Control, D.L: 100 bp DNA Ladder.

3.4 Genotyping for VDR Gene Polymorphisms Using Galaxy Program

Genotyping of VDR SNPs (rs7975232, rs731236, rs1544410, and rs2228570) was performed using allele read counts obtained from NGSdata processed through Galaxy. Of the 300 samples analyzed, 285 (95%) had adequate sequencing coverage. The remaining 5% showed low coverage (<1000 sequencing reads) and were excluded from further analysis. All excluded samples were from case group, totaling 185 cases and 100 controls included in the

final genotyping dataset. Figure 3.2 presents the BLAST results for sample #162 for the rs731236 SNP (TaqI) which was used as a representative sample. The collapsed sequence ensured the amplification of the target's regions with 100% identical sequence.

To further confirm the presence of two alleles as output from the collapsed sequence, multiple supporting reads were observed for each allele. A multiple alignment was performed for sample #162 as an example of heterozygote for rs731236 (TaqI). As presented in figure 3.3, the alignment demonstrated both G (11752 sequencing reads) and A (11423 sequencing read), with a single SNP detected in this region of the VDR gene.

Figure 3.2BLAST results for sample #162.

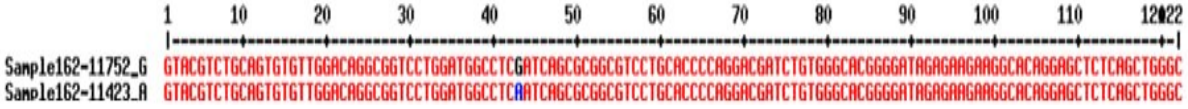

Figure 3.3 Multiple sequence alignment representing the heterozygous genotype in sample #162.

Tables 3.3 (A-D) shows representative samples for each VDR Polymorphism in diabetic and control participants. Genotyping was determined by analyzing allele-specific read counts from the sequencing data obtained from Galaxy. For each sample, the number of reads for each allele was counted, and the allele ratio was calculated. Samples with a ratio close to zero were classified as homozygous for the minor allele, ratios much greater than one were classified as homozygous for the major allele, and ratios near one were classified as heterozygous. These results obtain the distribution of homozygous and heterozygous genotypes across diabetic and control samples for all four VDR polymorphisms.

Table 3.3- A Genotyping for VDR rs7975232 (ApaI) in representative samples

Sample ID		VDR rs7975232			
		A Allele	C Allele	A:C Ratio	Genotype
Diabetic patients	25	153	32163	0.0	CC
	34	42492	3402	12.5	AA
	68	31290	31534	1.0	AC
Controls	86	37211	4212	8.8	AA
	91	4056	3542	1.1	AC
	100	40716	4042	10.1	AA

Table 3.3- B Genotyping for VDR rs731236 (TaqI) in representative samples

Sample ID		VDR rs731236			
		A Allele	G Allele	A:G Ratio	Genotype
Diabetic patients	25	10390	30	346.3	AA
	34	281	15142	0.0	GG
	68	8492	8578	1.0	AG
Controls	86	835	15817	0.0	GG
	91	10671	12488	0.9	AG
	100	12045	412	29.2	AA

Table 3.3- C Genotyping for VDR rs1544410 (BsmI) in representative samples

Sample ID		VDR rs1544410			
		T Allele	C Allele	T:C Ratio	Genotype
Diabetic patients	25	35	27730	0.0	CC
	34	17968	18633	1.0	TC
	68	18524	2278	8.1	TT
Controls	86	24009	1560	15.4	TT
	91	19896	20207	1.0	TC
	100	13549	14053	1.0	TC

Table 3.3- D Genotyping for VDR rs2228570 (FokI) in representative samples

Sample ID		VDR rs2228570			
		T Allele	C Allele	T:C Ratio	Genotype
Diabetic patients	25	20482	109	188	TT
	34	18111	154	117.6	TT
	68	12295	11454	1.1	TC
Controls	86	13107	426	30.7	TT
	91	11001	11010	1.0	TC
	100	17605	286	61.6	TT

3.5 Allele and Genotype Frequencies of VDR Gene Polymorphisms in T2DM Patients and Controls

The allele and genotype distributions of four VDR gene polymorphisms (rs7975232, rs731236, rs1544410, and rs2228570) were analyzed in 185 patients with T2DM and 100 healthy controls. Table 3.4 summarizes the allele frequencies of these SNPs. Among the studied variants, only rs731236 (TaqI) showed a significant difference in allele distribution between cases and controls, with the A and G alleles observed at 58.9% and 41.1% in patients versus 66.5% and 33.5% in controls, respectively ($p = 0.03$). The other SNPs—rs7975232,

rs1544410, and rs2228570—did not show significant differences in allele frequencies between the two groups ($p > 0.05$).

Genotype frequencies of the same VDR SNPs are presented in table 3.5. Consistent with the allele analysis, rs731236 genotypes were significantly associated with T2DM. The distribution of AA, AG, and GG genotypes was 39.5%, 38.9%, and 21.6% in patients, compared to 43%, 47%, and 10% in controls ($p = 0.041$). In contrast, rs7975232 (ApaI), rs1544410 (BsmI), and rs2228570 (FokI) showed no significant differences in genotype frequencies between patients and controls ($p > 0.05$).

Overall, these results suggest that among the four studied VDR polymorphisms, only rs731236 is significantly associated with T2DM, while the other three variants do not appear to influence susceptibility.

Table.3.4 Allele frequencies of rs7975232, rs731236, rs1544410, and rs2228570 VDR SNPs in controls and patients with T2DM

SNP ID	Allele	Cases n (%)	Controls n (%)	P-value
rs7975232	A C	200 (54.3%) 168 (45.7%)	113 (56.5%) 87 (43.5%)	0.455
rs731236	A G	218 (58.9%) 152 (41.1%)	133 (66.5%) 67 (33.5%)	0.03*
rs1544410	T C	153 (41.4%) 217 (58.6%)	68 (34%) 132 (66%)	0.213
rs2228570	T C	98 (26.5%) 272 (73.5%)	50 (25%) 150 (75%)	0.741

P-values were calculated using the Pearson's chi square test of association. SNP: Single Nucleotide Polymorphism; T2DM: type 2 diabetes mellitus; VDR: vitamin D receptor; n: number; *significant p-value < 0.05.

Table.3.5 Genotype frequencies of rs7975232, rs731236, rs2228570, and rs1544410 VDR SNPs in controls and patients with T2DM

SNP ID	Genotype	Cases (n=185) (%)	Controls (n=100) (%)	P-value
rs7975232	AA AC CC	63 (34.1%) 74 (40%) 48 (25.9%)	33 (33%) 47 (47%) 20 (20%)	0.5480
rs731236	AA AG GG	73 (39.5%) 72 (38.9%) 40 (21.6%)	43 (43%) 47 (47%) 10 (10%)	0.041*
rs1544410	CC TC TT	64 (34.6%) 89 (48.1%) 32 (17.3%)	44 (44%) 44 (44%) 12 (12%)	0.231
rs2228570	TT TC CC	18 (9.7%) 62 (33.5%) 105 (56.8%)	11 (11%) 28 (28%) 61 (61%)	0.629

3.6 Association of rs731236 with T2DM under Different Genetic Models

As shown in table 3.6, the association of rs731236 with the risk of T2DM was further evaluated under different inheritance models using logistic regression analysis. In the co-dominant and dominant models, no significant associations were observed between cases and controls ($p > 0.05$). Similarly, the overdominant model showed no significant effect ($p = 0.187$). However, under the recessive model, individuals carrying the GG genotype had a significantly higher risk of T2DM compared to carriers of the AA or AG genotypes (OR = 1.5, $p = 0.016$). These results suggest that the recessive model best explains the contribution of rs731236 to T2DM susceptibility in this population.

Table.3.6 Logistic regression analysis for the association of rs731236 with risk of T2DM under different inheritance models

Model	Genotype	Cases n (%)	Controls n (%)	OR (95% CI)	P-value
Co dominant	AA AG GG	73 (39.5%) 72 (38.9%) 40 (21.6%)	43 (43%) 47 (47%) 10 (10%)	1 0.795(0.38-1.036) 0.656(0.21-1.05)	0.503
Dominant	AA AG-GG	73 (39.5%) 112 (60.5%)	43 (43%) 57 (57%)	1 0.864 (0.527-1.415)	0.5620
Recessive	AG-AA GG	145 (78.4%) 40 (21.6%)	90 (90%) 10 (10%)	1 1.5(1.15-2)	0.016*
Overdominant	GG-AA AG	113 (61.1%) 72 (38.9%)	53 (53%) 47 (47%)	1 1.392(1.057-1.9)	0.187

3.7 Association of rs731236 Genotypes Recessive Model with Biomedical Parameters

Since the recessive model of rs731236 was significant,biomedicalparameters were compared between GG and AG+AA carriers in diabetic and non-diabetic participants as presented in tables 3.7 (A-B). In diabetics, GG carriers had significantly higher HbA1c levels (median: 9.5 vs. 7.8, $p = 0.042$), while other parameters showed no significant differences. Among non-diabetics, triglyceride levels were significantly higher in GG carriers ($p = 0.031$), with no other significant variations. These findings indicate a potential association of the GG genotype with poorer glycemic control in diabetics and elevated triglycerides in non-diabetics.

Table3.7-A Biomedical parameters according to recessive rs731236 genotype in diabetic and non-diabetic participants

	Genotypes of diabetic participants			Genotypes of non-diabetic participants		
	AG+AA	**GG**	**P-value**	**AG+AA**	**GG**	**P-value**
Variables	Median (IQR)					
Age (years)	58(51-65)	60.5(53-66)	0.928	53 (44-64)	60.5 (57.3-65)	0.138
BMI (kg/m^2)	30.1(27.8-32.5)	30.1(28-32.2)	0.709	28.1 (26.8-30)	26.7 (25.3-29)	0.215

Table3.7-B Biomedical parameters according to recessive rs731236 genotype in diabetic and non-diabetic participants

SBP (mmHg)	127 (120-136)	115 (110-126)	0.076	125 (120-130)	129 (120-135)	0.225
DBP (mmHg	80 (75-86)	80.5 (70.5-85)	0.428	84(80-87.3)	85(82-90)	0.240
HbA1c (%)	7.8 (7-8.9)	9.5 (7.2-10.1)	0.042*	5.4 (5.1-5.7)	5.35 (5.25-5.7)	0.604
FPG (mg/dL)	162 (131-201)	165 (133-205)	0.523	92(86.5-105)	90.5(85.5-97.8)	0.451
TG (mg/dL)	144 (105-200)	150 (98-1950	0.726	104.5 (86-151.8)	127 (87-188)	0.031*
TC (mg/dL)	150 (130-187)	155 (135-184)	0.508	169 (137.5-205)	151(100-201)	0.250
LDL (mg/dL)	100 (75-130)	101 (85-134)	0.705	100.5(79.8-144)	94 (85-100)	0.343
HDL (mg/dL)	35 (30-40)	34.7 (29-38)	0.374	40 (34-49)	38.5 (35-44.2)	0.428

Values for all continuous variables are expressed and presented as the median (interquartile range, IQR). P-values were calculated using the Pearson's chi-square test for discrete variable (gender) and Mann–Whitney U test for all continuous variables. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HbA1c: glycated hemoglobin; FPG: fasting plasma glucose; TG: triglycerides; TC: total cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein; n: number; *Significant p-value < 0.05.

Table.3.8 Frequency of diabetic complications according to recessivers731236 genotypes in diabetic participants

Diabetic complications		Genotypes of diabetic participants		
		AG+AA n= 145 (%)	GG n=40 (%)	p-value
Retinopathy	yes	24 (16.6%)	4 (10%)	0.306
	No	121(83.4%)	36 (90%)	
Nephropathy	yes	5 (3.4%)	1(2.5%)	0.764
	No	140 (96.6%)	39 (97.5%)	
Neuropathy	yes	3 (2.1%)	1 (2.5%)	0.868
	No	142 (97.9%)	39 (97.5%)	
Diabetic foot	yes	13 (9%)	3 (7.5%)	0.770
	No	132 (91%)	37 (92.5%)	

P-values were calculated using the Pearson's chi square test.

3.9 Haplotype Analysis of VDR Polymorphisms in Relation to T2DM Risk

Table 3.9 presents the haplotype analysis of four VDR polymorphisms in T2DM patients and controls. The most common haplotype was GATC, with similar frequencies in patients (25.9%) and controls (26.4%) (OR = 1.15, 95% CI = 0.66–2.01, p = 0.62). Other haplotypes,

such as GATT (11.6% vs. 8.0%) and ACCT (9.5% vs. 13.7%), also showed no statistically significant association with T2DM ($p > 0.05$). Although the GACC haplotype had a higher odds ratio (OR = 3.25, 95% CI = 0.66–15.90), the association was not significant ($p = 0.15$) as presented in table 3.9. Overall, none of the haplotypes showed a significant association with the risk of T2DM.

Table.3.9 Haplotype frequencies of VDR SNPs, rs731236, rs7975232, rs1544410, and rs2228570 in controls and diabetic patients

rs731236	rs7975232	rs1544410	rs2228570	Frequency in patients with T2DM	Frequency in control subjects	OR (95% CI)	P-value
G	A	T	C	0.259	0.264	1.15 (0.66 - 2.01)	0.62
G	A	T	T	0.1162	0.0802	0.80 (0.32 - 1.54)	0.38
G	A	C	C	0.0085	0.0208	3.25 (0.66 - 15.90)	0.15
A	C	C	T	0.0948	0.1371	1.76 (0.78 - 3.95)	0.18
A	A	C	C	0.0982	0.1186	1.30 (0.64 - 2.66)	0.47
A	A	C	T	0.0489	0.0556	1.31 (0.47 - 3.65)	0.6
A	A	T	C	0.0238	0.0237	1.09 (0.30 - 4.00)	0.89

P-values were calculated using SNPStats. OR: Odds Ratio; CI: Confidence Interval; VDR: vitamin D receptor; T2DM: type 2 diabetes mellitus.

Chapter Four
Discussion

4.1 Discussion

Our research exploring the genetic determinants of T2DM by investigating polymorphisms in the VDR gene including FokI (rs2228570), ApaI (rs7975232), TaqI (rs731236), and BsmI(rs1544410) and their association with T2DM risk in the Palestinian population. Despite significant advances in understanding the multifactorial pathophysiology of T2DM, involving complex interplay between genetic, environmental, and lifestyle factors, the role of VDR polymorphisms in disease susceptibility remains incompletely understood (Christakos et al., 2015). In Palestine, where vitamin D deficiency is widespread (Abukhalil et al., 2022; Lenz et al., 2023), the presence of genetic variants affecting VDR function may predispose individuals to impaired insulin secretion, reduced insulin sensitivity, and altered glucose homeostasis, thereby increasing T2DM risk (Liu et al., 2021). Earlier studies have demonstrated that VDR polymorphisms can influence disease susceptibility, progression, glycemic control, and lipid metabolism, potentially by altering VDR transcriptional activity, mRNA stability, or protein function, and consequently affecting pathways involved in pancreatic β-cell function, calcium homeostasis, and systemic inflammation.

In our case-control study, we aimed to examine the relationship between these specific polymorphisms and T2DM in the Palestinian population using NGS. Understanding these genetic risk factors is essential for clarifying mechanisms underlying disease susceptibility and guiding strategies for early detection, prevention, and personalized treatment. Our results indicate that the TaqI (rs731236) A > G variant is significantly associated with increased T2DM risk under the recessive genetic model, whereas FokI, ApaI, and BsmI did not show significant associations. Additionally, haplotypes analysis of all four SNPs showed no significant association with T2DM. To the best of our knowledge, this is the first study to explore these VDR genetic variants associations with T2DM here in Palestine, addressing acritical gap in understanding genetic contributions to T2DM in a population experiencing a rapidly rising prevalence of diabetes.

Demographic data showed that participants with T2DM in this case-control study were significantly older (median age 60 years) than controls (median 54 years), consistent with international reports showing an increased risk of T2DM with advancing age (Webber, 2013). In addition, diabetic patients had higher BMI, and weight, highlighting the well-established

association between excess adiposity and T2DM pathogenesis (Mehran et al., 2022). Additionally, glycemic control indicators including FPG, HbA1c levels were as expected higher in the diabetic compared to controls, confirming their diagnostic utility. Triglycerides were elevated, and HDL cholesterol levels were reduced in diabetic participants, whereas no significant differences were observed in total and LDL cholesterol levels between the two groups. These results are consistent with findings from multiple studies conducted in different populations, emphasizing the important role of dyslipidemia and central obesity as risk factors for T2DM development(Al-Sulaiti et al., 2019; Artasensi et al., 2023; Kaze et al., 2021). However, systolic blood pressure did not differ significantly between the two groups, possibly due to widespread antihypertensive medication use among patients.

The DNA concentration measured in the representative samples ranged from 22.8 to 52.7ng/µL, confirming the reliability of the extraction process and providing sufficient template for downstream PCR and genotyping analyses. Multiplex PCR successfully amplified the four targeted VDR polymorphisms (BsmI, TaqI, ApaI, and FokI), yielding clear bands of 200–250 bp on agarose gels, which demonstrates the specificity and efficiency of the designed primers. Genotyping using NGS data processed through the Galaxy program offered multiple advantages for this study. It provided high-throughput and highly accurate detection of both common and rare variants while minimizing human error compared to conventional molecular methods such as restriction fragment length polymorphism (RFLP) and Sanger sequencing (Saini et al., 2023), which are commonly used to detect known VDR SNPs. Sanger sequencing provides only a single read per direction, whereas NGS generates tens of thousands of reads per sample, making it roughly 20 times more efficient in accuracy (Eren et al., 2022; Lu et al., 2025). RFLP analysis requires restriction enzymes followed by high-concentration gel electrophoresis and can be labor-intensive, with limitations such as incomplete digestion and faint bands that make distinguishing heterozygotes from homozygotes difficult (Hashim & Al-Shuhaib, 2019). In comparison, the multiplex PCR approach followed by NGS is approximately 3–4 times more cost-effective, 5 times faster, and significantly less labor-intensive, while allowing simultaneous analysis of multiple SNPs and greater sensitivity for distinguishing genotypes (Muir et al., 2016; Schwarze et al., 2020). This technique allowed simultaneous analysis of multiple SNPs, and offered greater sensitivity for distinguishing homozygous and heterozygous genotypes (Pervez et al., 2022; Kumar et al., 2024), making it a powerful tool for investigating complex associations between VDR polymorphisms and T2DM in the Palestinian population. However, some variants showed lower sequencing coverage in certain samples representing only 5% of the total, which may reflect technical factors, such as variability in DNA quality or concentration, uneven PCR amplification, or differences in read depth during sequencing. Although this small percentage did not affect the overall results, it may limit detection of rare alleles. Future studies could improve coverage by optimizing library preparation or increasing sequence depth.

Among the four VDR SNPs analyzed, only rs731236 (TaqI) demonstrated a significant association with T2DM, with the GG genotype showing increased risk under the recessive model (OR = 1.5, p = 0.016). Similar associations have been reported in other populations. For instance (Shafie et al., 2022) reported a significant link between the rs731236

polymorphism and T2DM in Saudi Arabian cohort study. Similarly, (Malik et al., 2018) a hospital-based case-control study observed a correlation between TaqI and T2DM risk in Kashmiri individuals. In contrast, studies conducted in the United Arab Emirates(Safar et al., 2018), Brazil(Rodrigues et al., 2019), and Italy(Sentinelli et al., 2016) did not detect a significant association between this SNP and T2DM in their populations. This variability likely reflects ethnic differences, population structure, sample size, and environmental factors such as vitamin D status, dietary habits, cultural clothing practices, and sun exposure,which are known to influence VDR activity(Lezman& Riskin, 2023).

Notably, rs2228570 (FokI), rs7975232 (ApaI), and rs1544410 (BsmI) did not show any significant associations in our study. These findings considered consistent with studies in different populations which also reported no significant association between these polymorphisms and T2DM. In Brazil, (Rodrigues et al., 2019)found that ApaI, BsmI, and FokI were not associated with T2DM. Similarly, in Italy, (Bertoccini et al., 2017)reported no significant link between FokI and T2DM. In Malaysia, findings are in agreement with our results, where (Zakaria et al., 2021) found no differences in the distribution of FokI and BsmI genotypes between diabetic patients and controls. Supported results were also reported in North England(Sattar et al., 2021) and South Africa (Erasmus et al., 2022). However, several studies from other populations have reported significant associations between VDR polymorphisms and T2DM or related complications. For instance, (Al-Daghri et al., 2014)in Saudi Arabia found that the BsmI polymorphism increased T2DM risk and was linked to lower HDL cholesterol level. Similarly, in the Emirati population, (Safar et al., 2018)reported a significant association of VDR polymorphisms, particularly FokI and BsmI, with T2DM susceptibility.

In Pakistan, findings also contrast with our findings, (Khan et al., 2019)were the first to report that BsmI is a significant risk factor for T2DM in a hospital-based cohort. Three years later, (Memon et al., 2022) in another study confirmed a significant relation between FokI polymorphism and T2DM risk, strengthening evidence that these variants may play a key role in T2DM susceptibility among South Asian populations. The absence of associations for these three SNPs in our study may reflect differences in linkage disequilibrium (LD) structure between populations or limited sample power to detect modest effects. Functional studies indicate that some VDR variants, particularly FokI, may alter protein length and transcriptional activity(Meza et al., 2022),suggesting that population-specific gene-environment interactions may shape their effects on disease susceptibility.

Compared with neighboring countries, in Jordan, a pilot case-control study of 90 participants by (Alhawari et al., 2022)analyzed all the VDR SNPs investigated in thisstudy (FokI, ApaI, BsmI, and TaqI), they found that ApaI and BsmI associated with T2DM and its complications. Later, (Alfaqih et al., 2023)conducted a prospective case-control study with 250 participants investigating the same SNPs and reported a significant link between FokI and T2DM risk However, neither study detected a significant association with TaqI, which contrasts with our findings in the Palestinian population, where TaqI was the only SNP significantly associated with T2DM. While in Egypt, (Gendy et al., 2019)performed a case-control study with 100 participants (50 T2DM patients and 50 controls) and found FokI associated with T2DM, but

BsmI and TaqI showed no effect. This partially aligns with our study results in that BsmI was also non-significant; however, it contrasts with our findings since FokI showed no association with T2DM in Palestinians, whereas TaqI was significantly associated. Later, (Mohammed et al., 2023)conducted a prospective case-control study with 301 participants (156 diabetic patients and 145 controls), theyfoundsignificantassociations of FokI and BsmI with T2DM but not ApaI, again differing from our findings. Recently, (Fteah et al., 2025)reported in a hospital-based case-control study with 200 participants (150T2DM patients and 50 controls) that FokI and ApaIwere linked to nephropathy risk in Egyptian T2DM patients, suggesting roles in complications, while in our study, TaqI GG was associated with glycemic control but not with diabetic complications, showing distinct SNP effects across populations.

Building on the association of rs731236 (TaqI) with T2DM, analysis of diabetic complications under the recessive model (GG vs. AG+AA) showed no significant associations with retinopathy, nephropathy, neuropathy, or diabetic foot. Although the prevalence of retinopathy and diabetic foot was slightly lower in GG carriers (10% and 7.5%, respectively) compared to AG+AA carriers (16.6% and 9%), these differences were not statistically significant ($p > 0.05$ for all comparisons). Similarly, nephropathy and neuropathy rates were comparable between the two genotype groups. These findings suggest that while the GG genotype of rs731236 is associated with increased T2DM risk and altered glycemic control, it may not directly influence the occurrence of diabetes-related complications in our population. The lack of significant associations could be due to the relatively small number of participants with complications, limiting the statistical power to detect modest effects.

Haplotype analysis in our study, using the SNP order rs731236 – rs7975232 – rs1544410 – rs2228570 that was conducted with SNPStats, did not show significant associations with T2DM. Although the GACC haplotype showed an elevated odds ratio of 3.25, likely due to its low frequency in our population so it has a limited statistical power ($p = 0.15$). Other haplotypes, including GATC, and AACC also revealed no significant differences between patients and controls. In comparison, the Emirati study by (Safar et al., 2018), analyzing haplotypes in the order rs731236 – rs2228570 – rs1544410, reported that the AAT and GGC haplotypes were significantly more frequent in T2DM patients, whereas AAC and GAC were decreased, suggesting susceptibility and protective effects respectively. In Jordan(Alfaqih et al., 2023), haplotypes were analyzed in the order rs2228570 – rs1544410 – rs7975232 – rs731236, were GAAG haplotype was associated with increased T2DM, while ACAA with decreased risk and has a protective. These comparisons suggest that while rs731236 (TaqI) showed a significant SNP-level association in our study, haplotype effects differ across populations due to variations in SNP combinations, allele frequencies, and sample sizes.

Our findings highlight the need for further studies with larger and more geographically diverse samples. Future research may also consider investigating additional VDR polymorphisms or other genes involved in T2DM susceptibility, as well as evaluating the impact of these variants on vitamin D levels, glycemic control, and metabolic parameters.

4.2 Limitations

This research study has several limitations that should be considered and acknowledged. The sample size might have restricted the statistical power to identify modest associations for some SNPs. Additionally, the study population was restricted to patients from Hebron city, which may limit the generalizability of the findings to the whole Palestinian population. Vitamin D levels were not measured, which could have an important context for interpreting the effects of VDR polymorphisms, but this was not feasible due to limited resources and

logistical challenges. Data on environmental factors such as sun exposure, diet, and lifestyle were not available, although they are known to influence VDR activity and T2DM risk. Functional analyses, which test how a genetic variant affects the activity or function of the VDR protein, were not performed.Additionally, SNPs in the VDR gene or other genetic regions may contribute to disease susceptibility. Moreover, epigenetic factors such as DNA methylation, which were not examined in this study, could also play a role in disease progression.

4.3 Recommendations

Based on the findings of our study, future research should involve larger and more geographically diverse studies across the West Bank to validate these associations. Measuring vitamin D levels, along with diet and lifestyle factors, is recommended to better understand gene–environment interactions that may influence T2DM risk. Functional analyses using bioinformatics tools should be conducted to predict the impact of VDR variants on protein function, gene expression, and related metabolic pathways. Additionally, exploring other VDR SNPs and haplotypes may help identify population-specific genetic markers. Longitudinal studies could further clarify the predictive value of these polymorphisms for T2DM and its complications.

Chapter Five
Conclusion

In conclusion, this study identified a significant association between rs731236 (TaqI) and T2DM risk in the West Bank Palestinian population, whereas FokI, ApaI, and BsmI variants showed no significant effects. These results highlight the potential role of TaqI as a genetic marker for T2DM susceptibility in this population and underscore the importance of considering population-specific genetic and environmental factors when evaluating VDR polymorphisms. Further research in larger and more diverse cohorts is necessary to confirm these associations and to explore their relevance for disease prevention and management.

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Appendices

Appendix 1. Research approval by research ethics subcommittee of faculty of medicine at Al-Quds University

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

جامعة القدس
كلية الطب
أبوديس – القدس

Research Ethics subcommittee of Faculty of medicine

Letter of Ethical approval

Date:21/11/2024

Ref#: Re7-21-24

Dear Applicants: Dr. Abdelmajeed Nasereddin and Miss. Marah Atrash

Biochemistry and Molecular Biology master program

The Research Ethics subcommittee of faculty of medicine has recently reviewed your proposal entitled "Association of Vitamin D Receptor gene Polymorphisms with Type 2 Diabetes 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

P.O Box 20002
Tel 02-2799203, Fax 02-2796110

ص. ب 20002
هاتف 022799203 فاكس 022796110

Appendix 2. Consent form for research participation

Consent Form for Research Participation

Study Title: Association of Vitamin D Receptor gene Polymorphisms with Type 2 Diabetes in Palestine

Researcher: Marah Imad Ali Al-Atrash
Institution: Al-Quds University | College of Graduate Studies

Dear participants you are invited to participate in a research study. This study aims to investigate the association between specific variations of Vitamin D Receptor (VDR) gene and the occurrence of type 2 diabetes mellitus (T2DM) in Palestine. Your participation will help us to understand these genetic factors and may contribute to improve early detection and prevention strategies for T2DM.

Procedures

If you agree to participate, you will be asked to

- Allow us to collect certain information from your medical records.
- Provide a blood sample for DNA extraction to analyze gene polymorphisms, your samples may be used in other genetic study related to T2DM

Risks and Discomforts

There are minimal risks associated with participating in this study, including discomfort or bruising during blood sample collection.

Benefits

While there may be no direct benefit to you, your participation will contribute to scientific knowledge about genetic factors associated with T2DM, benefiting futures patients.

Confidentiality:

Your confidentiality will be maintained throughout the study and your personal information will only be accessible to the research team.

Results of this study will be published, but they will not include any identifying information.

Voluntary Participation and Right to withdraw:

Participation in this study is entirely voluntary, also you have the right to refuse to participate.

Consent:

I have read and understood the information provided above. I voluntarily consent to participate in this research study.

Participant's Name:_____ Participant's Signature: _____
Researcher's Name:_____ Researcher's Signature: _____
Date: _____

نموذج موافقة على المشاركة في البحث

عنوان الدراسة: دراسة المتغيرات الجينية لمستقبل فيتامين د وارتباطها بمرض السكري من النوع الثاني في فلسطين

الباحثة: مرح عماد علي الأطرش

المؤسسة : جامعة القدس | كلية الدراسات العليا

عزيزي المشارك/ة,

ندعوك للمشاركة في دراسة بحثية تهدف إلى دراسة العلاقة بين أنواع معينة من التغيرات الجينية لمستقبل فيتامين د (VDR) وظهور مرض السكري من النوع الثاني في فلسطين. ستساعدنا مشاركتك في فهم هذه العوامل الوراثية وقد تساهم في تحسين طرق الكشف المبكر واستراتيجيات الوقاية من مرض السكري من النوع الثاني.

الإجراءات :

إذا وافقت على المشاركة، ستطلب منك الإجراءات التالية:

• السماح بجمع بيانات معينة من سجلاتك الطبية.

• تقديم عينة دم لاستخلاص الحمض النووي وتحليل تعدد الأشكال الجيني, قد تستخدم هذه العينة في أبحاث جينية أخرى متعلقة بمرض السكري.

المخاطر والإزعاجات:

توجد مخاطر بسيطة مرتبطة بالمشاركة في هذه الدراسة، تشمل إزعاجًا أو كدمة أثناء سحب عينة الدم.

الفوائد:

على الرغم من أنه قد لا يكون هناك فائدة مباشرة لك، فإن مشاركتك ستساهم في تعزيز المعرفة العلمية حول العوامل الوراثية المرتبطة بمرض السكري، مما قد يفيد المرضى في المستقبل.

السرية:

ستحافظ الدراسة على سرية معلوماتك الشخصية وستكون معلوماتك متاحة فقط لفريق البحث. إذا شاء الله سيتم نشر نتائج هذه الدراسة، لكنها لن تتضمن أي معلومات تعريفية عنك. المشاركة في هذه الدراسة طوعية تمامًا، ويحق لك رفض المشاركة في أي وقت.

الموافقة:

لقد قرأت وفهمت المعلومات المقدمة أعلاه. أوافق طوعًا على المشاركة في هذه الدراسة البحثية.

اسم المشارك/ة: _____ توقيع المشارك/ة: _____

اسم الباحثة: _____ توقيع الباحثة: _____

التاريخ: _____

Appendix 3. Genomic DNA extraction manual fromWizard® Genomic DNA Purification Kit for blood by Promega

https://ita.promega.com/-/media/files/resources/protocols/technical-manuals/0/wizard-genomic-dna-purification-kit-protocol.pdf

Promega

3. Protocols for Genomic DNA Isolation

We tested the purification of genomic DNA from fresh whole blood collected in EDTA, heparin and citrate anticoagulant tubes and detected no adverse effects upon subsequent manipulations of the DNA, including PCR (2). Anticoagulant blood samples may be stored at 2–8°C for up to two months, but DNA yield will be reduced with increasing length of storage.

The protocol in Section 3.A has been designed and tested for blood samples up to 3ml in volume. The protocol in Section 3.B has been designed and tested for blood samples up to 10ml in volume. The yield of genomic DNA will vary depending on the quantity of white blood cells present. Frozen blood may be used in the following protocols, but yield may be lower than that obtained using fresh blood, and additional Cell Lysis Solution may be required.

Caution: When handling blood samples (Sections 3.A, B and C), follow recommended procedures at your institution for biohazardous materials or see reference 3.

3.A. Isolating Genomic DNA from Whole Blood (300µl or 3ml Sample Volume)

Materials to Be Supplied by the User

- sterile 1.5ml microcentrifuge tubes (for 300µl blood samples)
- sterile 15ml centrifuge tubes (for 3ml blood samples)
- water bath, 37°C
- isopropanol, room temperature
- 70% ethanol, room temperature
- water bath, 65°C (optional; for rapid DNA rehydration)

1. For 300µl Sample Volume: Add 900µl of Cell Lysis Solution to a sterile 1.5ml microcentrifuge tube.

   For 3ml Sample Volume: Add 9.0ml of Cell Lysis Solution to a sterile 15ml centrifuge tube.

   Important: Blood must be collected in EDTA, heparin or citrate anticoagulant tubes to prevent clotting.

2. Gently rock the tube of blood until thoroughly mixed; then transfer blood to the tube containing the Cell Lysis Solution. Invert the tube 5–6 times to mix.

3. Incubate the mixture for 10 minutes at room temperature (invert 2–3 times once during the incubation) to lyse the red blood cells. Centrifuge at 13,000–16,000 × *g* for 20 seconds at room temperature for 300µl sample. Centrifuge at 2,000 × *g* for 10 minutes at room temperature for 3ml sample.

4. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Approximately 10–20µl of residual liquid will remain in the 1.5ml tube (300µl sample). Approximately 50–100µl of residual liquid will remain in the 15ml tube (3ml sample).

   If blood sample has been frozen, repeat Steps 1–4 until pellet is white. There may be some loss of DNA from frozen samples.

   Note: Some red blood cells or cell debris may be visible along with the white blood cells. If the pellet appears to contain only red blood cells, add an additional aliquot of Cell Lysis Solution after removing the supernatant above the cell pellet, and then repeat **Steps 3–4.**

5. Vortex the tube vigorously until the white blood cells are resuspended (10–15 seconds).

Completely resuspend the white blood cells to obtain efficient cell lysis.

6. Add Nuclei Lysis Solution (300µl for 300µl sample volume; 3.0ml for 3ml sample volume) to the tube containing the resuspended cells. Pipet the solution 5–6 times to lyse the white blood cells. The solution should become very viscous. If clumps of cells are visible after mixing, incubate the solution at 37°C until the clumps are disrupted. If the clumps are still visible after 1 hour, add additional Nuclei Lysis Solution (100µl for 300µl sample volume; 1.0ml for 3ml sample volume) and repeat the incubation.

7. **Optional:** Add RNase Solution (1.5µl for 300µl sample volume; 15µl for 3ml sample volume) to the nuclear lysate, and mix the sample by inverting the tube 2–5 times. Incubate the mixture at 37°C for 15 minutes, and then cool to room temperature.

8. Add Protein Precipitation Solution (100µl for 300µl sample volume; 1.0ml for 3ml sample volume) to the nuclear lysate, and vortex vigorously for 10–20 seconds. Small protein clumps may be visible after vortexing.

   Note: If additional Nuclei Lysis Solution was added in **Step 6**, add a total of 130µl Protein Precipitation Solution for 300µl sample volume and 1.3ml Protein Precipitation Solution for 3ml sample volume.

9. Centrifuge at 13,000–16,000 × *g* for 3 minutes at room temperature for 300µl sample volume. Centrifuge at 2,000 × *g* for 10 minutes at room temperature for 3ml sample volume.

   A dark brown protein pellet should be visible. If no pellet is observed, refer to Section 4.

10. For 300µl sample volume, transfer the supernatant to a clean 1.5ml microcentrifuge tube containing 300µl of room-temperature isopropanol. For 3ml sample volume, transfer the supernatant to a 15ml centrifuge tube containing 3ml room-temperature isopropanol.

    Note: Some supernatant may remain in the original tube containing the protein pellet. Leave this residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.

11. Gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.

12. Centrifuge at 13,000–16,000 × *g* for 1 minute at room temperature for 300µl sample. Centrifuge at 2,000 × *g* for 1 minute at room temperature for 3ml sample. The DNA will be visible as a small white pellet.

13. Decant the supernatant, and add one sample volume of room temperature 70% ethanol to the DNA. Gently invert the tube several times to wash the DNA pellet and the sides of the microcentrifuge tube. Centrifuge as in Step 12.

14. Carefully aspirate the ethanol using either a drawn Pasteur pipette or a sequencing pipette tip. The DNA pellet is very loose at this point and care must be used to avoid aspirating the pellet into the pipette. Invert the tube on clean absorbent paper and air-dry the pellet for 10–15 minutes.

15. Add DNA Rehydration Solution (100µl for 300µl sample volume; 250µl for 3ml sample volume) to the tube and rehydrate the DNA by incubating at 65°C for 1 hour. Periodically mix the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature or at 4°C.

16. Store the DNA at 2–8°C.

دراسة المتغيرات الجينية لمستقبل فيتامين د وارتباطها بمرض السكري من النوع الثاني في محافظة الخليل في فلسطين

إعداد: مرح عماد علي الأطرش

إشراف: د. عبد المجيد ناصر الدين

المشرف الثاني: د. سهير عريقات

الملخص

داء السكري من النوع الثاني هو اضطراب أيضي مزمن يتميز بارتفاع مستويات السكر في الدم نتيجة مقاومة الأنسولين وضعف إفرازه. يلعب فيتامين د دورًا مهمًا في استقلاب الجلوكوز، وتنظيم إفراز الأنسولين، والسيطرة على الالتهاب، ويتم ذلك عبر مستقبل فيتامين د (VDR) قد تؤثر الاختلافات الوراثية في جين VDR ، بما في ذلك التغيرات FokI وApaI وTaqI وBsmI، على وظيفة المستقبل وكمية البروتين المنتج، وه ذه التغيرات قد تزيد احتمالية خطر الإصابة بمرض السكري من النوع الثاني . ومع ذلك، فإن البيانات حول العلاقة بين هذه المتغيرات وداء السكري من النوع الثاني لدى السكان الفلسطينيين لا تزال محدودة. هدفت هذه الدراسة إلى تقييم ارتباط هذه التغيرات الجينية بخطر الإصابة بالنوع الثاني من داء السكري لدى السكان الفلسطينيين من محافظة الخليل في الضفة الغربية.

شملت الدراسة 300 مشارك فلسطيني من محافظة الخليل، منهم 200 مريض مصاب بالسكري من النوع الثاني و 100 شخص غير مصابين، جميعهم بعمر 40 عامًا فأكثر، من مستشفى عالية الحكومي والمعهد الفلسطيني للسكري، خلال الفترة من كانون الثاني إلى نيسان 2025. تم جمع البيانات من السجلات الطبية، وأُخذت عينات دم لاستخلاص الحمض النووي وتحديد التغيرات الجينية في جين VDR.

تشير نتائج هذه الدراسة إلى أن الوسيط لكل من العمر، والوزن، ومؤشر كتلة الجسم كان أعلى لدى مرضى داء السكري مقارنةً بالأشخاص غير المصابين بالمرض. وكما هو متوقع، فقد سُجِّلت قيم وسيطية أعلى لكل من الهيموغلوبين السكري (HbA1c) ، وسكر الدم الصائم، ومستويات الدهون الثلاثية لدى مرضى السكري مقارنةً بالمجموعة الضابطة . تم التحليل الجيني باستخدام تقنيات التسلسل الحديثة ، من بين المتغيرات الجينية الأربعة التي شملتها الدراسة (FokI, BsmI, TaqI, and ApaI)، أظهر متغير TaqI فقط ارتباطًا ذا دلالة إحصائية بداء السكري من النوع الثاني وفق النموذج الجيني المتنحي، حيث ارتبط النمط الجيني GG بزيادة خطر الإصابة بالمرض (OR = 1.5، p = 0.016) كما لوحظ ارتفاع في مستوى الهيموغلوبين السكري لدى مرضى السكري، إضافةً إلى ارتفاع مستوى الدهون الثلاثية

لدى الأفراد غير المصابين بالسكري من حاملي النمط الجيني GGمقارنةً بحاملي النمطين الجينيين (AA,AGحاملي الجين السائد).

لم تُلاحظ أي ارتباطات ذات دلالة إحصائية مع تعدد أشكال FokIأوApaIأوBsmI. وتشير هذه النتائج إلى أن متغير TaqIقد يُعد علامة وراثية محتملة لاحتمالية الإصابة بداء السكري من النوع الثاني لدى سكان الخليل في الضفة الغربية، كما تُبرز هذه النتائج الدور المحتمل لمسار إشارات فيتامين د في تطور المرض.وقد تُسهم هذه النتائج مستقبلًا في توجيه الاستراتيجيات العلاجية أو تطوير مقاربات علاجية مخصّصة قائمة على الخصائص الوراثية للأفراد.

وفي الختام، توضح هذه النتائج أن التنوعات والتغيراتالجينية لجين مستقبل فيتامين د تؤثر على قابلية الإصابة بداء السكري من النوع الثاني يتباين بين المجتمعات المختلفة.كما تبيّن أن أحد هذه المتغيرات الجينية (TaqI) أظهر ارتباطًا ذا دلالة إحصائية بالمرض في العينة المدروسة من محافظة الخليل ، مما يدعم فرضية مشاركة المسارات الحيوية لفيتامين د في آليات نشوء المرض لدى الفلسطينيين.

الكلمات المفتاحية:داء السكري من النوع الثاني،جين مستقبل فيتامين د،التغيرات الجينية, تقنيات التسلسل الحديثة،المجتمع الفلسطيني.