Arab American University
Faculty of Graduate Studies
Department of Health Sciences
Master Program in Molecular Genetics and Genetic Toxicology

The Role of Two Protein Tyrosine Phosphatase, Non-Receptor Type 22(PTPN22) Gene Variants (rs2476601 and rs1310182) in the Development of Type 1 Diabetes

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Declaration

I declare that, except where explicit reference is made to the contribution of others, this thesis is substantially my own work and has not been submitted for any other degree at the Arab American University or any other institution.

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Dedication

I would like to dedicate this thesis to my beloved family, my number one source of support and encouragement throughout my entire educational journey. To my parents, who never lost faith in me, who are my inspiration and my source of love, and who believed in my ability to reach this level of success. I also dedicate this work to my beloved husband, who continued to support and encourage me, and has always believed in me. Thank you for your patience and understanding.

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The Role of Two Protein Tyrosine Phosphatase, Non-Receptor Type 22(PTPN22) Gene Variants (rs2476601 and rs1310182) in the Development of Type 1 Diabetes.

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Abstract

Background: Type 1 diabetes (T1D) is a chronic autoimmune disorder characterized by the destruction of pancreatic beta cells, which results in persistent hyperglycemia and requires lifelong insulin therapy. T1D results from complex genetic, epigenetic, and environmental interactions. Genetic factors account for up to 80% of hereditary T1D risk, while environmental and epigenetic influences also play important roles in disease development. Among the genetic factors identified, the protein tyrosine phosphatase non-receptor type 22 (PTPN22) gene has been implicated in T1D susceptibility. PTPN22 encodes the lymphoid-specific phosphatase (LYP), which is a key negative regulator of T cell receptor signaling, thereby influencing immune tolerance and autoimmunity. Notably, two single nucleotide polymorphisms (SNPs) within PTPN22—rs2476601 and rs1310182—have been associated with variable risk for T1D, with their prevalence and impact differing across populations and geographic regions.

Objective: The objective of this study is to examine the molecular genetic basis of T1D in a Palestinian cohort by screening for the rs2476601 and rs1310182 polymorphisms in the PTPN22 gene, aiming to clarify their potential association with T1D risk in this population.

Methods: The study recruited a total of 205 individuals, comprising 100 confirmed T1D cases and 105 healthy controls, from the cities of Ramallah and Jenin. Genomic DNA was extracted from all participants and Sanger sequencing was utilized to identify the presence of the specified PTPN22 variants. Genotypic and allelic frequencies were compared between cases and controls to assess association with T1D.

Results: Analysis of the genotypic and allelic distributions for both rs2476601 and rs1310182 polymorphisms revealed no statistically significant association with T1D in the studied cohort. These findings suggest that, within the Palestinian population, these specified PTPN22 variants do not confer an increased susceptibility to T1D.

Conclusion: This study contributes to the growing body of evidence regarding the geographic variability of PTPN22 polymorphisms and their role in T1D. The lack of association observed emphasizes the need for larger sample size and the evaluation of additional genetic markers to comprehensively understand the genetic basis of T1D.
Keywords: Type 1 diabetes, PTPN22, rs2476601 polymorphism, rs1310182 polymorphism, SNP

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List of Definitions of Abbreviations:

Abbreviations	Title
Abl	Abelson murine leukemia
AD	Addison's Disease
AIC	Akaike Information Criterion
AIDS	Acquired Immunodeficiency Syndrome
AKT	Protein kinase B
AP4B1	Adaptor Related Protein Complex 4 Subunit Beta 1
AP-4	Transcription factor activator protein4
BCR	B cell receptor
Bcr	breakpoint cluster region
BD	Bechet's Disease
BIC	Bayesian information criterion
Bp	Base Pair
BMI	Body Mass Index
CBP	Csk Binding Protein
CD	Cluster of Differentiation
CI	Confidence Intervals
CLL	Chronic Lymphocytic Leukemia
CML	Chronic Myeloid Leukemia
CSK	C-terminal Src Kinase
CTLA4	Cytotoxic T Lymphocyte-Associated Protein 4
DKA	Diabetic Ketoacidosis
DNA	Deoxyribonucleic acid
HLA-DQ	Human leukocyte Antigen (heterodimer of type MHC class II)
HLA-DR	Human Leukocyte Antigen – DR isotype
dsDNA	double stranded Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FOXP3	Forkhead box protein P3
GAD	Glutamic Acid Decarboxylase
GDM	Gestational Diabetes
GEMS	Gem- associated protein
GWAS	Genome-Wide Association Studies
HAART	Highly Active Antiretroviral Therapy
HbA1c	Glycated hemoglobin
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HWE	Hardy-Weinberg Equilibrium
IBD	Inflammatory Bowel Disease
IDF	International Diabetes Federation
IFIH1	Interferon-Induced with Helicase C Domain 1

IFN	Interferon
IGF1R	Insulin Like Growth Factor 1 Receptor
IL2	Interleukin 2
IL2RA	Interleukin 2 Receptor Alpha
INS	Insulin Gene
IRF3	Interferon Regulatory Factor 3
IRF7	Interferon Regulatory Factor 7
ITAM	Immunoreceptor Tyrosine-Based Activation Motif
JAK	Janus Kinase
JIA	Juvenile Idiopathic Arthritis
JNK	C-Jun N-terminal kinases
Kg	Kilogram
LCK	lymphocyte-specific protein tyrosine kinase
LD	linkage disequilibrium
LDL	low-density lipoprotein
LYP	lymphoid-specific phosphatase
Lyn	Lck/Yes-related novel tyrosine kinase
M	Meter
MAF	Minor Allele Frequency
MAPK	Mitogen-Activated Protein Kinases
MDA5	Melanoma Differentiation-Associated Protein 5
MG	Myasthenia Gravis
MHC	Major Histocompatibility Complex
MODY	Maturity Onset Diabetes of the Young
MS	Multiple Sclerosis
N	Number
NEUROD	Neuronal Differentiation 1
NF-κB	Nuclear factor kappa B (NF-κB)
NLR	Nucleotide-binding oligomerization domain (NOD)-like receptors
NOD	Nucleotide-binding oligomerization domain
NOD2	Nucleotide-binding oligomerization domain type 2
NTC	No template control
OD	Optical Density
OGTT	Oral Glucose Tolerance Test
OR	Odd Ratio
P1	Proline-rich motif type 1
P4	Proline-rich motif type 4
PAG	Phosphoprotein Associated with Glycosphingolipid-Enriched Microdomains 1
PBAS	Population-Based Analysis Studies
PBMC	Peripheral Blood Mononuclear Cells

PCR	Polymerase Chain Reaction
PEP	PEST domain-enriched tyrosine phosphatase
PRR	Pattern Recognition Receptors
PTP	Protein Tyrosine Phosphatases
PTPN22	Protein Tyrosine Phosphatase, Non-receptor Type 22
RA	Rheumatoid Arthritis
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
Rpm	Round Per Minute
SD	Standard Deviation
SH2	Src Homology 2
SH3	Src Homology 3
SLE	Systemic Lupus Erythematosus
SNP	Single-Nucleotide Polymorphism
STAT	Signal Transducer and Activator of Transcription
SYK	Spleen Tyrosine Kinase
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
TAE	Tris Acetate EDTA
TCR	T Cell Receptor
Th	Helper T cell
TLR	Toll Like Receptor
TRAF3	Tumor necrosis factor receptor associated factor 3
Treg	Regulatory T cell
UC	Ulcerative Colitis
UTR	Untranslated Region
UV	Ultraviolet
VNTR	Variable Number Tandem Repeats
WBC	White Blood Cells
ZAP70	Zeta chain-Associated Protein of 70kDa
β-cells	Beta Cells

Chapter One: Introduction

1.1. Overview of Diabetes Mellitus and its Types

Diabetes is characterized by elevated levels of glucose in the bloodstream, known as hyperglycemia. Severe hyperglycemia can lead to classic symptoms such as polyuria, polydipsia, fatigue, loss of performance, unexplained weight loss, visual disturbances and increased susceptibility to infection. These conditions can progress to more severe complications like ketoacidosis or non-ketotic hyperosmolar syndrome, with the risk of coma. Chronic hyperglycemia also disturbs the secretion and/or action of insulin which can lead to long term damage or dysfunction of various tissues and organs (including eyes, kidneys, nerves, heart and blood vessels), and it increases the chance of cancer (Harreiter & Roden, 2023; Kharroubi, 2015).

Diabetes mellitus is becoming increasingly prevalent globally. It is anticipated that by 2045, this disease will impact approximately 693 million adults, representing 50% growth compared to 2017 (Tremblay & Hamet, 2019).

The disease is classified into four types: type 1 diabetes, type 2 diabetes, gestational diabetes, and other specific types of diabetes ("2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2022," 2022; Harreiter & Roden, 2023).

1.2. Types of Diabetes Mellitus

1.2.1. Type 1 Diabetes

Type 1 Diabetes (T1D) is primarily characterized by the immune-mediated destruction of pancreatic β-cells, leading to marked reduction in insulin secretion that results in absolute insulin deficiency. Consequently, individuals with T1D depend on lifelong administration of exogenous insulin. T1D is the predominant form of diabetes in children, accounting for about 80% of all childhood diabetes cases in the United States (Redondo et al., 2017). While the etiology of T1D is still not well understood, it is influenced by a combination of genetic, epigenetic, and environmental factors. For example, certain genetic markers increase susceptibility to T1D, while environmental

triggers may initiate or accelerate the autoimmune process that leads to the destruction of β-cells. Factors like infections, alterations in gut microbiota, vaccines, dietary exposures, weight, and β-cell stress have all been implicated as potential contributors to the onset of islet autoimmunity and, ultimately, T1D (Rewers & Ludvigsson, 2016).

1.2.2. Type 2 Diabetes Mellitus

Described as adult-onset diabetes or non-insulin-dependent diabetes, Type 2 Diabetes Mellitus (T2D) accounts for 90-95% of all diabetes cases (Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2022," 2022). T2D develops due to reduction in insulin action (insulin resistance) accompanied by a progressive loss of beta cell function, initially often resulting in relative insulin deficiency and typically a disruption of glucose-dependent insulin secretion. These functional disorders are present at varying degrees long before the clinical manifestation of diabetes, either alone or as part of a metabolic syndrome, associated with increased risk of macrovascular consequences (Harreiter & Roden, 2023). In contrast to T1D, in T2D, autoimmune destruction of beta cells does not occur where patients do not depend on insulin treatment and ketoacidosis seldom occurs spontaneously. Many factors can lead to T2D, such as obesity, overweight, hyperlipidemia (high triglycerides and LDL levels) (Erion et al., 2016), smoking (Chang, 2012), physical inactivity, vitamin D deficiency (Lips et al., 2017), aging (Kirkman et al., 2012), genetics, and family history.

1.2.3. Other Specific Types of Diabetes

There are many other factors that could lead to other types of diabetes. For example, genetic defects of the β-Cell, genetic defects in insulin secretion (e.g. forms of Maturity Onset Diabetes of the Young [MODY]), insulin action (e.g. lipoatrophic diabetes), other genetic syndromes (e.g. Down syndrome, Klinefelter syndrome, Turner syndrome), diseases of the exocrine pancreas (e.g. pancreatitis, trauma, operations, tumors, hemochromatosis, cystic fibrosis), endocrine organs (e.g. Cushing's syndrome, acromegaly), drug-chemical (e.g. glucocorticoids, α-interferon , post-transplant diabetes, Highly Active Antiretroviral Therapy (HAART in HIV/AIDS), infections (e.g. congenital rubella) and rare forms of autoimmune-mediated diabetes (e.g. "stiff-man" syndrome. ("2.

Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2022," 2022; "Diagnosis and Classification of Diabetes Mellitus," 2014; Harreiter & Roden, 2023).

1.2.4. Gestational Diabetes (GDM)

This type of diabetes develops during the second or third trimester of pregnancy in those without previous diabetes ("Diagnosis and Classification of Diabetes Mellitus," 2014), arising from the body's inability to compensate for increased insulin resistance caused by pregnancy hormones (Johns et al., 2018). Affecting up to 45% of pregnancies worldwide depending on region (Lawrence et al., 2019), it poses several risks including high birth weight, preeclampsia, and complications for both parent and child. Major risk factors include obesity, inactivity, advanced age, and a family history of diabetes (Johns et al., 2018; Sweeting et al., 2022).

1.3. Diagnostic Criteria for Diabetes Mellitus

Diagnosing diabetes mellitus involves measuring fasting plasma glucose, oral glucose tolerance, and Hemoglobin A1c. A diagnosis is made if fasting plasma glucose is at least 126 mg/dL, the 2-hour plasma glucose during an OGTT is 200 mg/dL or more, or the HbA1c is 6.5% or higher. These benchmarks are based on their strong link to diabetes-related complications ("2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2022," 2022; "Diagnosis and Classification of Diabetes Mellitus," 2014; Harreiter & Roden, 2023).

1.4. Diagnosis of T1D

Classically, type 1 diabetes develops over the course of days or weeks in children and adolescents, manifesting with symptoms such as polyuria, polydipsia, and weight loss resulting from glycosuria. Alongside clinical features, laboratory results typically reveal profound hyperglycemia—often exceeding 300 mg/dL—accompanied by ketonuria, with or without ketoacidemia. In addition to these clinical and laboratory features, most patients have more than one diabetes-related autoantibody present at diagnosis including: Insulin autoantibody, Glutamic acid decarboxylase autoantibody, Islet antigen 2 autoantibody, and Zinc transporter 8 autoantibody (Subramanian et al., 2024).

1.5. Epidemiology of T1D

Albeit the fact that T1D represents only 10% of diabetes cases worldwide, its incidence rate is rising globally (Mobasseri et al., 2020). In 2021, the approximate number of individuals with T1D was 8.4 million worldwide. The prevalence of T1D has been predicted to increase to 13.5-17.4 million in 2040, which is 60%-107% higher than in 2021(Gregory et al., 2022). According to The International Diabetes Federation (IDF), the estimated number of new T1D cases among children aged zero to 14 years increased from 98,200 to 108,300, and among those aged zero to 19 years from 128,900 to 149,500 over a-three years. Increases were most pronounced in African, Middle East and North Africa Regions (Ogle et al., 2022). In the Middle East, the Arab populations represent only 5.4% of the total world population yet they contribute remarkably to the increasing global burden of T1D, with 60,000 cases reported in children with age < 14 years (Zayed, 2016). Four countries from the Arab region are among the top 10 countries globally with the highest incidence rates of T1D are Kuwait (41.7%), Qatar (38.1%), Saudi Arabia (31.4%), and Algeria (34.8%) (Ogle et al., 2022).

In Palestine, according to the latest annual health report in 2023 (Ministry of Health Annual Report Palestine2023 State of Palestine PHIC, 2024), diabetes mellitus was ranked number three for cause of death in the West Bank, representing 16.26% of deaths in 2023. Based on the latest statistics from the West Bank diabetic clinics, additional 5,466 new cases of diabetes mellitus were reported, with an incidence rate of 186 per 100,000 population. These new cases are divided into 2,427 males (162.2 cases per 100,000 population) and 3,039 females (210.6 cases per 100,000 population). It was determined that 4.92% of these diabetic patients have type 1 diabetes.

1.6. Genetics of T1D

T1D has a strong genetic predisposition, with genetic factors contributing to about 80% of the overall familial risk, underscoring the hereditary nature of the disease. Furthermore, twin studies, linkage studies, and genome wide association studies (GWAS) consistently demonstrate that a substantial portion of T1D risk is both heritable and measurable at the genetic level. To elucidate the relative risks: while individuals in the

general population face a 0.4% risk of developing T1D, siblings of patients have a significantly higher risk of 6–7% (Mrena et al., 2006). Additionally, monozygotic twins, who share most of their genes, have more than a 70% long-term risk of both developing the disease, compared to only 6% for dizygotic twins, who share about half their genes (Redonclo et al., 1999). The occurrence of autoimmune diabetes in association with genetic mutations further supports the concept that genetic factors are fundamental to the etiology of T1D.

The most influential genetic component in T1D susceptibility is the Human Leukocyte Antigen (HLA) region on chromosome 6p21, which accounts for 40% to 50% of familial T1D risk. This region is a critical part of the major histocompatibility complex (MHC), responsible for presenting peptide antigens on cell surfaces so they can be recognized by T cells. HLA is generally classified into three classes: class 1, class 2, and class 3 (Choo, 2007; Mutar Mahdi, 2019) . While class 1 is expressed by most of the cells, class 2 is expressed only by immune cells. Certain variations within the HLA genes—particularly at the HLA-DQA1, HLA-DQB1, and HLA-DRB1 loci—significantly increase the likelihood of developing T1D. These HLA variants can alter the way antigens are presented to T cells, potentially leading to an autoimmune response against insulin-producing cells in the pancreas. As a result, the immune system may mistakenly target and destroy these cells, ultimately causing the onset of T1D. Notably, HLA genotype and haplotype frequencies, as well as their effects on T1D, can vary among populations (Noble & Valdes, 2011). For example, the DR3 haplotype is associated with increased risk in European populations but is protective in African Americans, while the DR7 haplotype is protective in Europe and a risk factor among African Americans.

Candidate gene approaches and GWAS have mapped over 50 additional loci associated with T1D. These crucial genes associated with T1D include as shown on Table (1.1): the insulin gene (*INS*), cytotoxic T lymphocyte-associated protein 4 (*CTLA4*) gene (Ueda et al., 2003), protein tyrosine phosphatase, non-receptor type 22 (*PTPN22*) gene (Bottini et al., 2004), interleukin 2 receptor alpha (*IL2RA*) gene (Vella et al., 2005), and interferon-induced with helicase C domain 1 (*IFIH1*) gene (Smyth et al., 2006). These factors may either increase susceptibility to T1D or confer some degree of protection.

Table 1.1: Candidate Genes associated with T1D pathology and their function (Paschou et al., 2018; Redondo et al., 2018)

Gene	Chromosomal Location	Function and role in T1D Pathogenesis
INS	11p15	Plays a pivotal role in insulin production and glucose regulation in the body, with its expression primarily in pancreatic beta cells. Polymorphisms near the insulin gene promoter, specifically variable number tandem repeats (VNTRs), contribute about 10% to the genetic risk for T1D. These VNTRs exist in two main forms: Type I (small class): 26–63 repeats, and Type III (large class): 140–243 repeats.
CTLA4	2q33	Acts as an immune checkpoint that suppresses T cell activation. It sends inhibitory signals to activated T lymphocytes, inducing anergy (a non-responsive state). Polymorphisms in the CTLA4 gene region are associated with T1D: A49G SNP at exon 1, C-318T at the promoter region, and the (AT)n repeat at the 3′UTR
PTPN22	1p13	Potent inhibitor of the activation of naive T lymphocytes, prevents spontaneous T-cell activation by dephosphorylating and inactivating a kinase enzyme. C1858T (Arg620Trp) PTPN22 Polymorphism has shown strong association with T1D and it's the most known SNP, however many other less known SNPs have been associated with T1D.
IL2RA	10p15	Also known as CD25, encodes a component of the interleukin-2 receptor involved in T-cell regulation; its expression on regulatory T cells is important for

		development, homeostasis, and suppressing T-cell immune responses. Among IL2RA polymorphisms, two (rs706778 and rs3118470) had a significant association with T1D.
IFIH1	2q24	Encodes for melanoma differentiation-associated protein 5 (MDA5), which binds to double stranded RNA viruses and thus mediates the innate immune system's interferon response to certain viruses. Polymorphisms in IFIH1 can impair antiviral responses, leading to increased inflammation and beta cell death, which may contribute to the development of T1D.

1.7. PTPN22 Gene in the Development of T1D

The protein tyrosine phosphatase, non-receptor type 22 (*PTPN22)* gene, also known as PEP and LYP, encodes a member of the non-receptor subfamily of the protein tyrosine phosphatase family. Protein tyrosine phosphates play major role in the balance of the immune system as a negative regulator of effector/ memory T-cell pool (Bottini et al., 2006). The PTPN22 gene is widely acknowledged as the third major genetic loci confronting with the risk of T1D (Bottini et al., 2004). PTPN22 gene is known for its functional polymorphism rs2476601 (c.1858C>T, p.620R>W) and the less understood polymorphism rs1310182 (c.2054 852T>C). These variants are associated with many autoimmune diseases, including T1D. The prevalence of these risk variants alleles is subject to enormous geographic variability. The main goal of this project is to investigate the role of the two specified variants in the development of type 1 diabetes among Palestinian patients.

1.8. Significance of Study

This is the first study on the association of the PTPN22 gene polymorphisms (rs2476601 and rs1310182) in T1D patients in Palestine. Given that T1D constitutes 4.92% of Diabetes Mellitus cases in Palestine, further research in this area is justified. The significance of this study lies in its potential to clarify the specific role of PTPN22 gene polymorphisms (rs2476601 and rs1310182) in the development of T1D among Palestinian patients. The expected outcomes include the development of early genetic screening protocols for T1D risk in Palestinian patients, identification of potential carriers, and foundational data for future therapeutic interventions targeting PTPN22-related pathways. By generating population-specific genetic knowledge, this research will bridge the gap between genetic discoveries and clinical applications, ultimately guiding the translation of genetic research into effective screening and treatment protocols. Furthermore, the findings may help inflected families' members to have healthy children free of the disease. By expanding the understanding of genetic contributors to T1D within the Palestinian population, this study will contribute both to improved patient care at the local level and to the broader field of global T1D research.

1.9. Research Problem

Genetic factors influence the development of T1D during fetal life and several studies have linked specific genetic variants to disease susceptibility in different populations. Notably, certain variants in PTPN22 have been associated with altered immune responses, which may trigger the autoimmune destruction of pancreatic beta cells. While these associations have been extensively explored in various populations, their relevance to Palestinian patients remains uninvestigated, creating a significant knowledge gap. Addressing this gap is not only vital for advancing population-specific genetic knowledge but could also shape future strategies in disease management, early detection, and therapeutic interventions, ultimately improving patient care and outcomes.

1.10. Objectives of the Study

Population-based genetic association studies (PBASs) examining the possible associations between various SNPs of the PTPN22 gene and susceptibility to T1D have

been frequently conducted in American and European populations, and to a lesser extent in Asia and the Middle East. The goal of this study is to investigate whether two SNPs (rs2476601 and rs1310182) of the PTPN22 gene are associated with increased risk of developing T1D among Palestinian patients, given their reported relevance in previous studies. These specified SNPs were selected due to their established links to immune regulation and autoimmune disease susceptibility, making them important candidates for understanding population-specific genetic risk factors in the development of T1D.

1.11. Study Questions

The study addresses three main questions. First, what are the allele and genotype frequencies of rs2476601 and rs1310182 in T1D patients and healthy controls? Second, do these variants have a significant association with the elevated risk of T1D? Third, can these genetic variations serve as prognostic indicators for T1D in the Palestinian population?

1.12. Hypothesis

The main hypothesis predicts the specified indicated variants are positively associated with an elevated risk of developing T1D in Palestinian patients. These variants, rs2476601 and rs1310182, were selected based on previous studies indicating their involvement in immune regulation and susceptibility to autoimmune diseases. The prevalence of these variants is expected to differ between T1D patients and healthy controls within the Palestinian population.

1.13. Limitation

The major limitation encountered in this study arises from restricted movement between cities in Palestine, coupled with limited public awareness of genetic studies and occasional refusal to participate. These challenges may result in a smaller and potentially biased sample size, which in turn can affect the reliability and generalizability of the findings. Another potential limitation is the focus on only two polymorphisms, which may not fully capture the complex genetic and environmental factors influencing T1D. Evidently, since the study is conducted exclusively on Palestinian T1D patients in the West

bank region of Palestine, the findings may not be representative of other ethnic or geographical populations.

1.14. Terminological and Keywords Definitions:

- Type 1 diabetes: A condition resulting from the autoimmune destruction of beta cells—these are insulin-producing cells located in the endocrine pancreas, which is the hormone-producing part of the pancreas. The loss of beta cells leads to insulin deficiency. This autoimmune process typically develops over several months or years in genetically susceptible individuals, often triggered by one or more environmental factors.
- PTPN22 gene: A gene that encodes a protein tyrosine phosphatase, an enzyme crucial for the immune system by regulating the inhibition of T cell activation and down-regulation of immune response.
- Single-nucleotide polymorphisms (SNP): These are variations at a single position in the DNA sequence among individuals. SNPs can influence how people develop diseases, as well as how they respond to pathogens, chemicals, drugs, vaccines, and other environmental agents. Understanding SNPs helps researchers identify genetic factors that contribute to disease susceptibility and variation in treatment responses.

Chapter Two: Literature Review

2.1. Type 1 Diabetes Pathophysiology

T1D represents one of more than 80 diseases considered to have an autoimmune etiology. In this case, the immune system targets and destroys the insulin-producing beta-cells (β-cell) located in clusters called islets of Langerhans within the pancreas. These β-cells are much more than just factories for insulin; they act as sophisticated "glucose thermostats," constantly sensing blood sugar levels and releasing insulin to maintain them within a narrow, healthy range.

The destruction of β-cells disrupts this delicate balance, causing patients with T1D to lose control over their blood glucose regulation. As a result, people with the disease can experience acute conditions such as diabetic ketoacidosis and severe hypoglycemia. Over time, the inability to properly regulate glucose can also lead to serious long-term complications, including heart disease, vision loss, and kidney failure, even with access to current insulin replacement therapies.

The exact etiology of T1D is still unknown. However, it is widely accepted that multiple factors can influence an individual's susceptibility to T1D before the onset of β-cell-directed autoimmunity. These factors include developmental differences in establishing functional β-cell mass and genetic risk. Both environmental and intrinsic factors within the pancreas and β-cells can also play a role. The autoimmune destruction of β-cells is believed to be triggered by certain events in susceptible individuals. These events lead to the development of islet autoantibodies and eventually cause a loss of functional β-cell mass. Importantly, the process of β-cell destruction typically lasts months or years before clinical symptoms become apparent.

The development of T1D can be divided into well-defined stages (Figure 2.1). Stage 1, also known as the "asymptomatic phase," is characterized by the presence of at least two islet autoantibodies. During this period, blood glucose levels remain normal and there are no symptoms. Following stage 1, the disease progresses to stage 2, described as the "early metabolic alterations with asymptomatic state." At this point, individuals continue to show positivity for two or more autoantibodies. However, they begin to experience changes in

glucose metabolism that are not yet diagnostic for diabetes. Clinical symptoms are still absent.

As the disease advances to stage 3, known as “clinical diabetes,” symptoms of hyperglycemia become evident. These may include polyuria, polydipsia, enuresis, weight loss, and blurred vision. In some cases, diabetic ketoacidosis (DKA) or diabetic hyperosmolar syndrome can occur (Insel et al., 2015; Paschou et al., 2018; Zajec et al., 2022).

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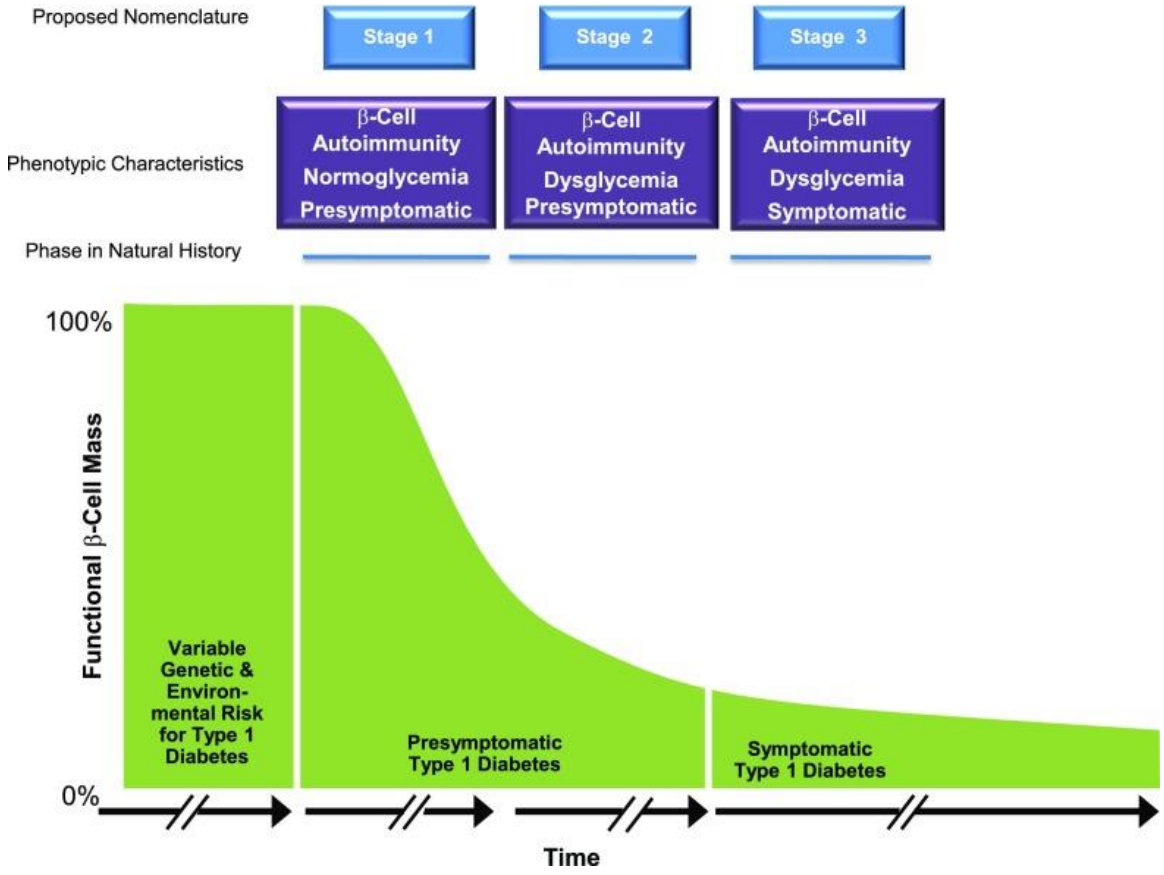

Figure 2.1 :Early stages of type 1 diabetes (Insel et al., 2015).

The pathogenesis of T1D begins with an innate immune response. This includes the activation of pattern recognition receptors on β-cells by endogenous “danger signals” or by exogenous ligands produced during viral infections. This process may link environmental risk factors to the development of T1D.

During this immune activation, β-cells and other islet cells produce cytokines. This cytokine production attracts immune cells to the islets. As a result, macrophages respond first, followed by other immune cells such as naïve and activated T cells. The ongoing inflammation increases vascular permeability, allowing further infiltration of immune cells. The infiltrates are predominantly composed of CD8+ T cells, but also include CD20+ B cells, CD4+ T cells, and CD68+ macrophages.

In response to cytokines, β-cells undergo several changes. Their metabolic and electrical activity becomes disrupted, insulin granule synthesis and content are impaired, and gap junction coupling is compromised. Additionally, excessive generation of reactive oxygen species (ROS) and activation of caspases further contribute to β-cell dysfunction and loss (Paschou et al., 2018; Zajec et al., 2022).

It is well established that T cells of both CD4+ and CD8+ subpopulations play crucial roles in the autoimmune destruction of pancreatic beta cells in T1D (Roep, 2003). The development, differentiation, and function of T cells are regulated by intracellular signaling pathways that are activated through the T cell receptor (TCR) and co-stimulatory receptors. (Nel, 2002).

Aberrant TCR signaling can cause autoimmunity by impacting several critical cellular processes, such as proliferation, apoptosis, cytoskeletal dynamics, cytokine production, and anergy. Therefore, TCR signaling is the object of many studies in T1D and other autoimmune diseases (Nel & Slaughter, 2002).

Importantly, genetic factors also influence TCR signaling. The protein tyrosine phosphatase non-receptor type 22 gene (PTPN22) encode the lymphoid tyrosine phosphatase (LYP), which plays a critical role in negatively regulating T cell activation. By modulating signals from antigen and co-stimulatory receptors, PTPN22 helps maintain immune tolerance and prevents excessive T cell responses. Variants in the PTPN22 gene can alter lymphocyte signaling, potentially leading to the survival and activation of self-reactive T cells, and thereby increasing susceptibility to autoimmune diseases such as T1D.

2.2. Protein Tyrosine Phosphatase Non-Receptor Type 22 Gene (PTPN22)

The *PTPN22* gene (protein tyrosine phosphatase, non-receptor type 22) is located on chromosome1p13.3–13.1 and encodes an 807-amino acid residue protein referred to as the lymphoid tyrosine phosphatase (LYP) (Cohen et al., 1999). The mouse homolog of LYP is PEP (PEST domain-enriched tyrosine phosphatase). Protein tyrosine phosphatases (PTPs) regulate immune responses by maintaining lymphocyte resting states and modulating signals from antigen, costimulatory, and cytokine receptors(Mustelin et al., 2005). Because LYP is expressed in lymphocytes and T1D is caused by β-cell destruction due to cytotoxic T lymphocytes and CD4+ T cells (Vang et al., 2005), disease predisposition could be caused by altered T lymphocyte function. This protein plays a critical role in negatively regulating T cell activation, which explains the association of PTPN22 with autoimmune disorders other than T1D, including Graves' disease (Velaga et al., 2004), rheumatoid arthritis (RA) (Begovich et al., 2004), and systemic lupus erythematosus (SLE) (Kyogoku et al., 2004).

2.3. Structure and Biological Functions of LYP Protein Encoded by *PTPN22* Gene

The N-terminus of LYP protein has a classical and strict phosphotyrosine-specific protein tyrosine phosphatase (PTP) domain, followed by a linker region, a long C-terminus and a non-catalytic portion. LYP has proline-rich motifs (P1-P4) in the C-terminus and putative phosphorylation sites in the inter-domain region (Figure2.2), which plays an important role in the regulation of catalytic activity of the protein. P1 has high affinity to the SH3 (Src homology 3) domain of C-terminal Src Kinase (CSK); a negative regulator of Src family kinases such as lymphocyte-specific protein tyrosine kinase (LCK), Fyn, and other Src family kinases involved in TCR and B cell receptor (BCR) signaling. The location of Csk is the cytoplasm but can interact with plasma membrane proteins through SH2 domain. Such an interaction occurs in the lipid rafts termed Csk binding protein (CBP) or protein associated with GEMS (PAG). Lyp targets to the plasma membrane through binding to Csk which results in a Csk/Lyp complex anchored to the cell membrane by PAG. Consequently, Lyp and Csk near the cell membrane allows for access to their target substrates which are localised in the same region. Hence, the Csk phosphorylates the

negative regulatory tyrosine in Src family, while LYP dephosphorylates the positive tyrosine of the Src family kinases (Fousteri et al., 2013; Tizaoui et al., 2021).

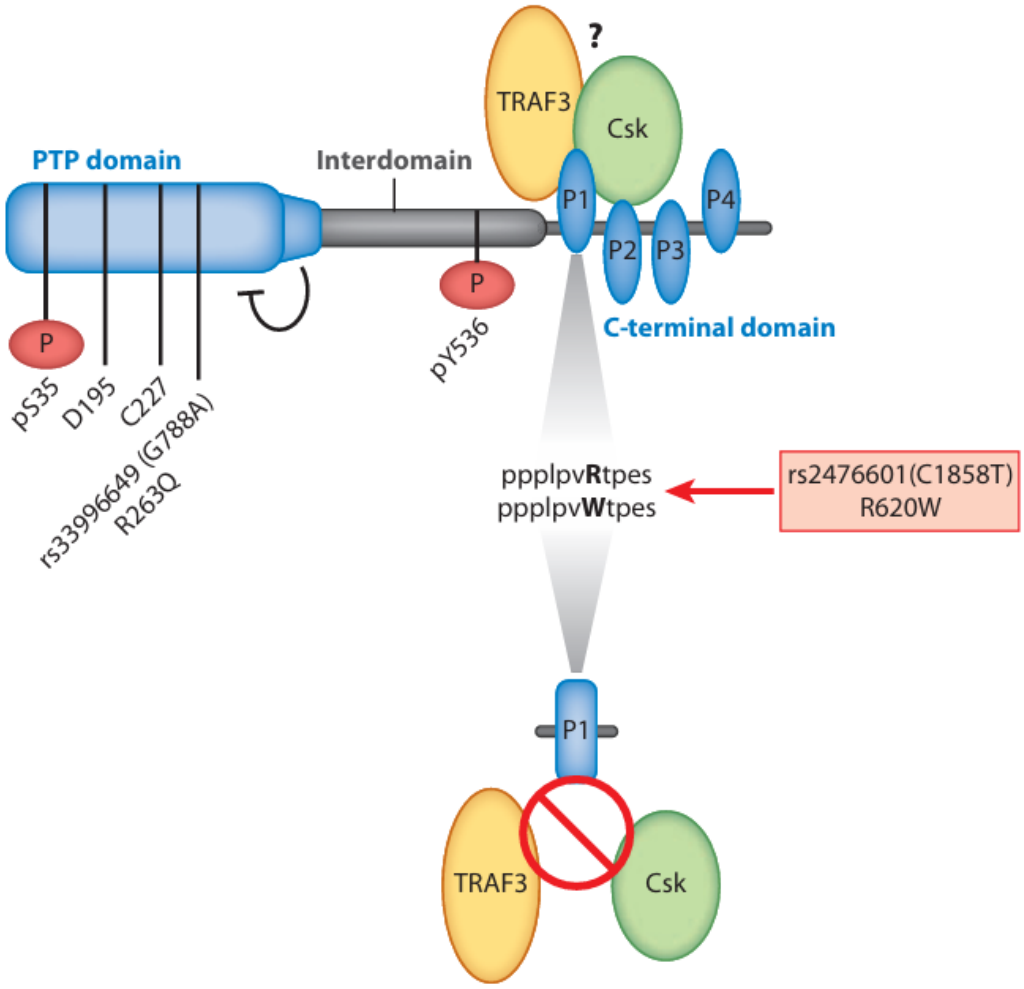

Figure 2.2: Schematic of the structure of PTPN22 *protein (Bottini & Peterson, 2014)*. PTPN22 consists of three main domains: the N-terminal phosphatase (PTP) domain, an interdomain region, and a C-terminal domain. The protein forms a high-affinity complex with Csk via the P1 motif in its C-terminal domain binding to the SH3 domain at the N-terminus of Csk. In myeloid cells, PTPN22 also directly associates with TRAF3, although it remains uncertain whether a three-part complex involving PTPN22, Csk, and TRAF3 exists. Phosphorylation sites S35 and Y536 (shown in red) are highlighted. A conserved segment within the interdomain, spanning amino acids 300 to 320 constitutively inhibits the phosphatase activity. The R263Q mutation in the PTP domain reduces enzymatic activity, while the R620W mutation in the P1 motif of the C-terminal domain lowers the binding strength between PTPN22 and Csk, as well as between PTPN22 and TRAF3.

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2.4. Lymphoid Tyrosine Phosphatase Isoforms

In humans, Lyp has three splice forms designated as Lyp1–3 (Cohen et al., 1999). Lyp1; full length protein of 807 amino acid, is the subject of all functional studies, and the most abundant of the 3 isoforms. The second isoform is Lyp2, which is the shortest splice variant that contains only the first P1 motif within the N-terminal. Whereas, Lyp3 has a 28 amino acid deletion between P1 and P2. The functions of Lyp2 and Lyp 3 are not identified. Lyp localize in cytosol but can be membrane bound through interactions with other proteins. However, the sub cellular location of the different isoforms has not yet been indicated.

PTPN22 gene expressed by both the innate and adaptive immune systems, explaining its impact over various cells of the immune system, such as B and T cells, myeloid cells, and natural killer cells (Fousteri et al., 2013). Gene expression studies show that immune cells express PTPN22 at differing levels. Of the highest levels are natural killer cells and neutrophils, followed by CD8+T cell but CD4+T cells and monocytes express the lowest levels of PTPN22.

2.5. Major Effectors Modulated by PTPN22 in Innate Immunity

PTPN22 plays a pivotal role in innate immune receptor signaling, resulting in proliferation and cytokine production. In myeloid cells (such as monocyte, macrophages, dendritic cell and neutrophils), PTPN22 enhanced Toll like receptor (TLR) signaling through its interaction with tumor necrosis factor receptor associated factor 3 (TRAF3). This interaction results in augment polyubiquitination of TRAF3, thus increasing the production of type 1 interferon (IFN): a mechanism crucial for controlling inflammation. Mutants in *PTPN22* gene show reduced interaction to TRAF3 which results in reduced IFN production levels (Figure 2.3a).

Detection of pathogens occurs via TLRs and pattern recognition receptors (PRRs), including nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). Upon recognition of invading bacteria, these receptors are activated. In this context, PTPN22

functions by inhibiting the phosphorylation of NF-κB-MAPK signaling proteins, which play a critical role in the activation and differentiation of monocytes and macrophages.

Additionally, in macrophages, PTPN22 adjusts inflammatory responses by suppressing M1 pro-inflammatory genes while promoting M2 anti-inflammatory differentiation and enhances phagocytosis and cytokine secretion. Loss of PTPN22 turns monocyte and macrophage more reactive towards bacterial antigen enhancing antigen-presentation and secretion of pro-inflammatory cytokines (Figure 2.3b) (Fousteri et al., 2013; Ivashkiv, 2013; Tizaoui et al., 2021).

In dendritic cells, PTPN22 increases antigen presentation efficiency and cytokine secretion which help in the activation of adaptive immunity. Furthermore, PTPN22 effects extend to neutrophils, where it enhances reaction oxygen species (ROS) production and NETosis (special form of cell death used by neutrophils to trap and kill pathogens) processes linked to tissue damage in autoimmune conditions.

Interestingly, the effects of PTPN22 are highly context-dependent, varying across cell types, stimuli, and genetic backgrounds. This multi-role can lead to protective effects in certain autoimmune diseases, such as Crohn's disease, while increasing susceptibility in others such as SLE. These dynamic effects underscore PTPN22's importance in maintaining immune balance and its potential as a target for therapeutic strategies (Fousteri et al., 2013; Ivashkiv, 2013; Tizaoui et al., 2021).

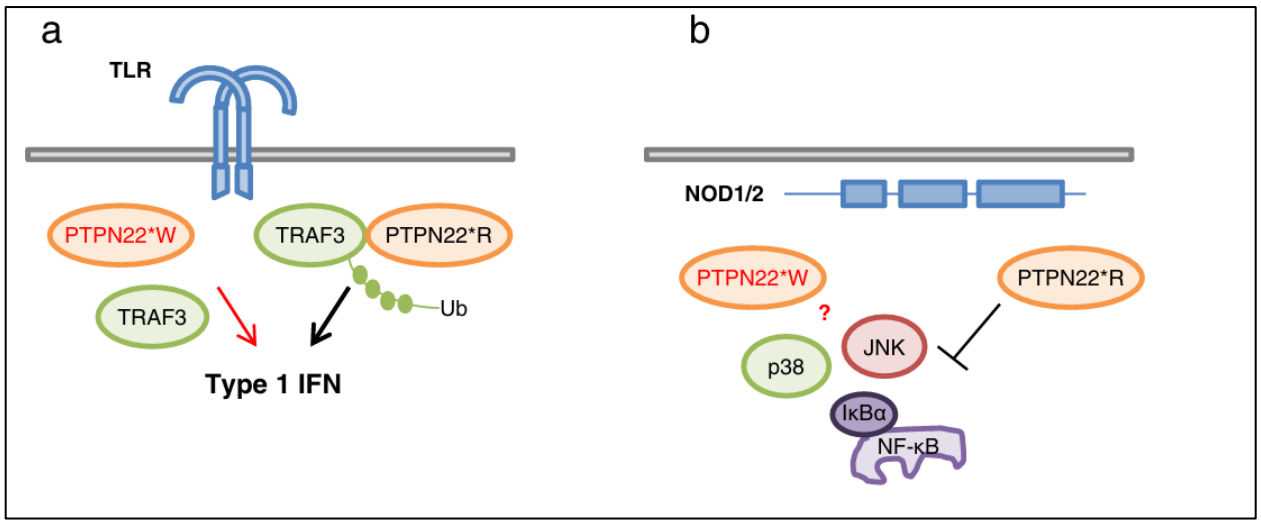

Figure 2.3: Central role of PTPN22 in innate immune receptor signaling. a. In myeloid cells, PTPN22 promotes TLR signaling through its association with TRAF3. PTPN22 and TRAF3 interaction augments the latter's polyubiquitination (Ub) and thus enhancing IRF3 and IRF7 activation (not shown) resulting in type I IFN production. The PTPN22*W variant demonstrates decreased interaction with TRAF3 and acts as a hypomorph of TRAF3 ubiquitination, leading to lower levels of type I IFN production. b. Monocytes detect bacteria through nucleotide binding and oligomerization domain-containing type 1 or 2 (NOD1 and NOD2) cytosolic receptors. Activation of NOD2 leads PTPN22 to inhibit phosphorylation of proteins in the NF-κB and MAPK (JNK, p38) signaling pathways, which play roles in the activation and differentiation of monocytes and macrophages. Loss of PTPN22 increases monocyte and macrophage reactivity to NOD2 stimulation, leading to higher pro-inflammatory cytokine levels. The effect of the PTPN22*W variant on human innate immune response remains unclear (Fousteri et al., 2013).

2.6. PTPN22 Regulates the Function of Adaptive Immunity Cells

In T cells, the interaction between LYP and the SH3 domain of tyrosine-protein kinase CSK (Begovich et al., 2004; Bottini et al., 2004) acts to suppress kinases involved in T cell and B cell activation. LYP, which is specifically expressed in lymphocytes, inhibits T cell activation by dephosphorylating and inactivating the TCR-associated kinases Lck, Fyn, and zeta chain-associated protein of 70kDa (ZAP-70) (Figure 2.4).

PTPN22 has been found to adjust the number and function of regulatory T cells as well as helper T cell in mice (Brownlie et al., 2012),(Maine et al., 2012). Experimental models in mice showed that PTPN22 deficiency leads to increased regulatory T (Treg) and helper T (Th) cells (Maine et al., 2012), while knock-in mice with R619W variant (the mimicry of human autoimmune-risk allele) showed elevated effector/memory T cell population and augment TCR signaling (Dai et al., 2013). Furthermore, PTPN22 impacts the balance between Treg and Th1 cells, and its absence increases FOXP3+ Treg frequency which results in protection from T1D in some mice lines (Zheng & Kissler, 2013).

Regarding B cells, studies showed no effect on B cell receptor (BCR) signaling. Nevertheless, later studies revealed that the R619W mutation elevates transitional B cells and promotes autoimmunity; characterized by circulation autoantibodies, and immune cell infiltration in multiple tissues and hyper-responsiveness (Dai et al., 2013).

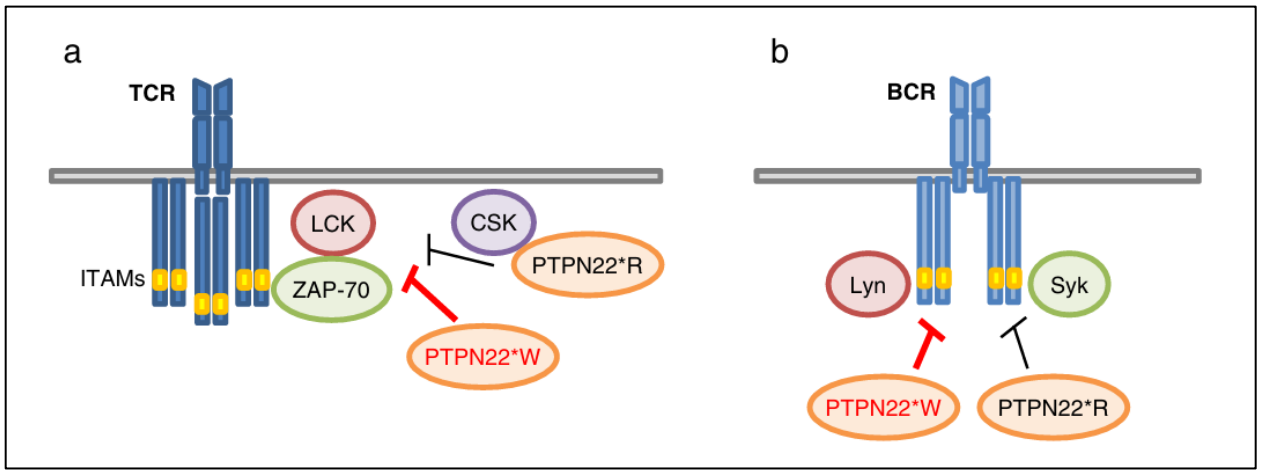

Figure 2.4: Modulation of the TCR and BCR signaling by PTPN22. a. PTPN22 inhibits TCR signaling by dephosphorylating key tyrosine residues on Src family kinases like ZAP-70 and LCK, as well as immunoreceptor tyrosine-based activation motifs ITAMs, via CSK interaction. The disease-linked PTPN22*W variant interacts less with CSK but has increased inhibitory activity. b. PTPN22 inhibits BCR signaling, and human studies indicate that PTPN22*W further diminish B cell activation. However, recent mouse studies suggest that polymorphism makes B cells hyper-responsive (Fousteri et al., 2013).

2.7. Genetic Variants in the PTPN22 Gene as a Candidate Gene in Human Autoimmune Diseases

2.7.1. Polymorphism rs2476601 (c.1858C>T, p.620R>W)

This autoimmunity-predisposing allele in the *PTPN22* gene is a missense mutation that changes cytosine to thymidine at position 1858, which changes amino acid residue 620 from Arginine (R) to Tryptophan (W) in exon 14 of the encoded LYP protein. The uniqueness of this SNP is that R620 is a critical residue in the Proline-rich motif in LYP that binds the SH3 domain of Csk to form the Csk-LYP complex and therefore LYP regulates TCR signaling (Gregorieff et al., 1998). This R620W mutation disrupts the binding between LYP and CSK, resulting in altered T cell signaling and increased susceptibility to autoimmune diseases. Large number of studies demonstrated that this polymorphism is an allelic variant associated with susceptibility to a growing number of autoimmune diseases. Remarkably, the association is highly reproducible, found in many different populations and is restricted to disorders that usually have strong autoantibody component (Burn et al., 2011).

2.7.1.1. Geographical Distribution of R620W

The prevalence of the PTPN22 rs2476601 (c.1858C>T, p.620R>W) allele varies widely across human populations. Its frequency reaches about 15% in Scandinavia, the northernmost region of Europe, while in Spain and France, the frequency declines to approximately 7%. In Italy and Sardinia, the allele occurs at an even lower rate, around 2–3% (Burn et al., 2011; Totaro et al., 2011). This allele is quite rare in Africa and South Asia and is nearly absent in East Asia. Larger, ongoing studies in African American, Hispanic, Asian, and Native American populations aim to clarify the distribution and significance of the PTPN22 R620W allele. These studies may help explain why certain groups—including African American, Hispanic, Asian, and Native American women—are more susceptible to specific autoimmune diseases (Shapira et al., 2010). These variations in allele frequency may partly explain the differing rates of autoimmune diseases observed among these populations.

Recent studies have begun to investigate the presence of the R620W allele in Middle Eastern and Arab populations. Research in specific groups such as Kuwaiti Arabs, Iranians, and Emiratis has identified a significant association between the R620W variant and increased risk of T1D. For example, allele frequency in Kuwaiti T1D patients is 22% and 5% in controls (Haider et al., 2018). While the frequency of R620W in Emiratis T1D patients is 5.4% and 6.1% in Iranian T1D patients (Abbasi et al., 2017; C. Sharma et al., 2021). This indicates that this allele contributes to T1D susceptibility in these populations. However, more detailed studies are needed to accurately determine the prevalence and impact of the allele across various Arab countries. By consistently comparing the frequency of the PTPN22 R620W allele across ethnic groups and geographic regions, researchers can gain new insights into how genetic and environmental factors contribute to T1D.

2.7.1.2. Functional Consequences of the R620W Variant

Bottini et al. first reported a significant association between the PTPN22*W polymorphism and T1D onset in two different populations. The study showed that unlike the variant encoded by the more common allele 1858C, the T1D-associated variant does not bind the negative regulatory kinase Csk (Bottini et al., 2004). The amino acid substitution causes changes in the binding site of LYP to the SH3 domain of Csk, as the side chain of Arginine (Arg620) fits into the binding cleft of SH3 domain. While tryptophan substitution (Trp620) disrupts this interaction due to its large size that wouldn't fit into the binding pocket. The disruption of LYP-Csk binding caused by the tryptophan substitution may lead to altered TCR signaling, which in turn contributes to increased susceptibility to T1D. Subsequent studies confirmed that the R620W variant is a remarkable risk factor for T1D, enhancing the risk of developing the disorder by 2-4 folds (Bottini & Peterson, 2014; Tizaoui et al., 2021). However, The PTPN22*W variant is significantly associated with other autoimmune diseases (in addition to T1D, RA, and SLE) including thyroid disease, myasthenia gravis (MG), juvenile rheumatoid arthritis, Addison's disease (AD), autoimmune thrombocytopenia, idiopathic inflammatory myopathies, inflammatory bowel disease (IBD), and vitiligo. While, other diseases include multiple sclerosis (MS), Behcet's disease (BD), pemphigus vulgaris, ulcerative colitis (UC) showed no association with the PTPN22*W variant (Tizaoui et al., 2021).

Experiments showed that this SNP is a gain of function mutation (Vang et al., 2005). Vang et al. showed that IL-2 production (a cytokine crucial for activating and proliferating T cells) decreased in T cell populations isolated from heterozygous T1D patients (PTPN22 1858C>T) compared to homozygous genotyped T1D patients (PTPN22 1858C/C). Furthermore, the disease-predisposing PTPN22*W variant was more efficient inhibitor of T cell activation as it encodes for a significantly more active phosphatase, which suppresses TCR signaling better. However, there is contrast between several experimental studies that generated confusion regarding the function of the PTPN22*W variant in immunity and autoimmunity. Most studies indicate this variant acts as a gain-of-function allele while others support the notion that it has reduced activity (loss-of function). Zhang et al. reported that primary T cells from PTPN22*W healthy donors and patients with RA exhibited increased TCR-mediated proliferation (J. Zhang et al., 2011).

Possible mechanisms on how PTPN22*W may produce autoimmunity based on studies of human T cell lines provided evidence that LYP binds and dephosphorylates several compounds involved in TCR signaling (Wu et al., 2006). PTPN22*W variant likely causes disease by suppressing TCR signaling more effectively during thymic development, affecting negative selection. Consequently, autoreactive T cells that would have been deleted by negative selection would survive (Maine et al., 2012). The threshold for TCR

signaling during thymic development in individuals expressing the mutant LYP protein is elevated, potentially allowing autoreactive T cells to survive (Figure 2.5).

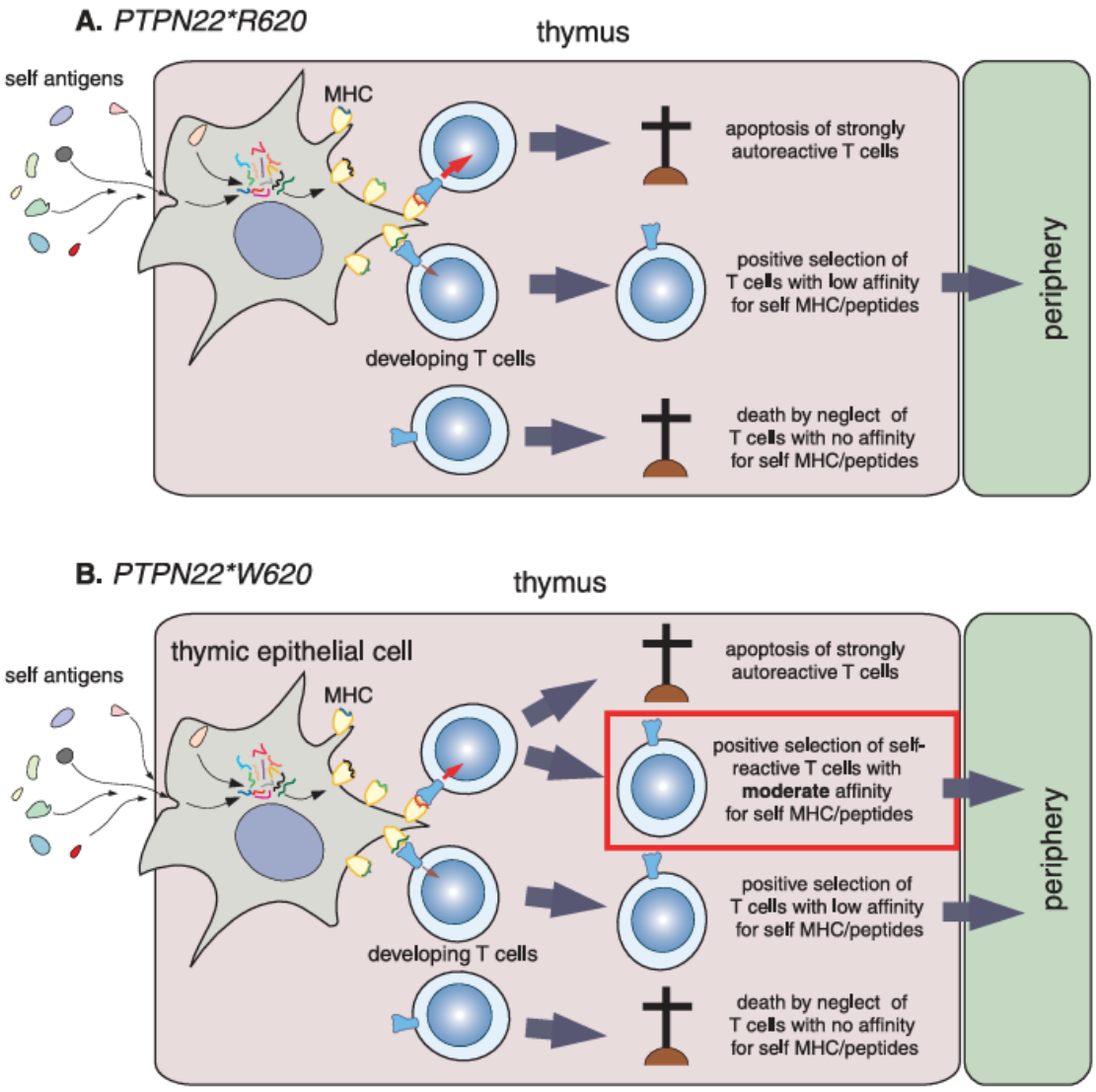

Figure 2.5: Model illustrates the effects of disease-associated LYP variant on thymic selection, which may allow for more autoreactive T cells to survive and escape into circulation. The size of the red arrow inside the thymocytes show the magnitude ("strength") of TCR signaling (Bottini et al., 2006).

Evidently, PTPN22*W variant is commonly associated with autoimmune diseases characterized by autoantibodies, such as RA, SLE, and T1D. This variant may contribute to autoimmunity by disrupting B cell clonal deletion and receptor editing (Cambier, 2013).

Moreover, PTPN22*W variant alters B cell function through; survival of autoreactive B cells that normally should be deleted, reduced BCR signaling and increased the production of autoantibodies (Habib et al., 2012; Metzler et al., 2017; Rieck et al., 2007).

Myeloid cells are emerging as key players in many autoimmune conditions. PTPN22 is highly expressed in these cells, where it regulates the pattern of recognition receptors (PRRs) and promotes type I IFN production through enhanced downstream signaling. It also facilitates IFN-γ-dependent JAK-STAT signaling. The PTPN22*W variant enhances myeloid phagocytosis and modulates macrophage behavior and morphology (Li et al., 2017). Furthermore, the PTPN22*W variant acts as a hypomorph in myeloid cells which can protect against Crohn's disease but may exacerbate others like SLE driven by excessive signaling by TLRs and type I IFN production (Lichtman et al., 2012). In addition, this variant elevates reactive oxygen species and Ca^{2+} levels in neutrophils, which is particularly relevant in RA and SLE (Bayley et al., 2015).

Intriguingly, the PTPN22*W is linked to lymphoid and myeloid leukemias (Bottini & Peterson, 2014). Compared to normal levels in normal B cells, PTPN22 is markedly overexpressed in chronic lymphocytic leukemia (CLL); a malignancy involving autoreactive B cells that show altered BCR signaling (Negro et al., 2012). Hebbring et al. reported increased frequency of PTPN22-1858 C>T allele in CLL cohorts (Hebbring et al., 2013). Overexpression of PTPN22 leads to inhibition of apoptosis following BCR stimulation, allowing CLL cells to survive longer. Altered PTPN22 levels may inhibit and promote different pathways downstream of the BCR in CLL. PTPN22 selectively enhances anti-apoptotic AKT signaling while blocking pro-apoptotic signals from other BCR pathways (Negro et al., 2012). Because PTPN22 can inhibit certain subsets of BCR signals while enhancing others in CLL cell (Negro et al., 2012), this suggests the impact of PTPN22-R620W on BCR signaling may not be fully explained by simple gain-of-function or loss-of-function models.

PTPN22 might also play a key role in myeloid leukemia. PTPN22 is highly expressed in chronic myeloid leukemia (CML); a malignant transformation is mediated by a constitutively active fusion protein (tyrosine kinase Bcr-Abl). Overexpression of LYP protein caused reduction in the phosphorylation levels of multiple proteins in KCL22 CML

cells. Of particular interest, Bcr-Abl levels and phosphorylation considerably decreased in these cells. This suggests that LYP may play an antagonistic role in signaling by the Bcr-Abl fusion protein (Chien et al., 2003). However, PTPN22 levels in CML positively correlate with resistance to imatinib (Villuendas et al., 2006). Similarly, Guillem et al. (Guillem et al., 2012)found that CML patients with PTPN22-1858 C>T have a higher risk of poor treatment response.

2.7.2. Polymorphism rs1310182 (c.2054 852T>C)

The rs1310182 polymorphism (c.2054 852T>C) is a point mutation in an intronic region of the PTPN22 gene that carries a putative transcription factor binding site. This SNP is located in a region that overlaps or is adjacent to the AP4B1 antisense RNA 1gene: which is a non-coding RNA transcribed from the opposite strand of the AP4B1 gene. This means that rs1310182 may influence transcriptional regulation, not only for PTPN22 but potentially of nearby or overlapping genes involved in immune or cellular processes (Carlton et al., 2005; Heneberg et al., 2018; Taniyama et al., 2010). Carlton et al. first described rs1310182 associated with RA independent of rs2476601 (R620W)(Carlton et al., 2005). Unlike rs2476601, the rs1310182 is less understood. The functional reflection of rs1310182 variant on the activity of LYP remains to be elucidated. Evidently, rs1310182 was not associated with T1D in the Sardinian population (Zoledziewska et al., 2008). Nevertheless, this SNP was found to be associated with many autoimmune diseases. Later studies revealed different associations, with no consistent identification of a specific allele related to the disease. The frequency of the C allele of rs1310182 differed significantly between Japanese T1D patients and controls (Taniyama et al., 2010), while the risk allele in Caucasian RA subjects was T (Carlton et al., 2005). A limited number of studies have investigated the association between rs1310182 and T1D across various populations. In the U.K. population, the correlation between this SNP and T1D was significant, however, it was dependent on rs2476601 (Smyth et al., 2008). Likewise, in Armenia and Pakistan populations, there is a close association of rs1310182 and T1D patients with genotype CT and TT genotypes respectively (Žak et al., 2023)(Rafaqat et al., 2023). the Middle East, a study in Arab population was conducted in the Emirates, a significant correlation was found between rs1310182 and T1D, with the G allele in GG genotype was associated with

increased risk for T1D (C. Sharma et al., 2021). In contrast, Iranian T1D and control groups did not differ on genotype distribution or allele frequency for rs1310182. However, in Iranian population, rs1310182 has been found to be associated with other autoimmune diseases rather than T1D (Aflatounian et al., 2017)(Bahrami et al., 2020). It should be noted that rs1310182 has been described using different allele notations (G/A or T/C) across studies.

2.8. Other Polymorphisms in the PTPN22 Gene

2.8.1. Polymorphism rs2488457 (G1123C)

The rs2488457 (G1123C) polymorphism is located in the 5′ promoter region of PTPN22 and its function has not yet been identified. The impact of this non-coding SNP on transcription and stability remains to be fully explained. However, examination of the DNA sequence around rs2488457 site showed that this SNP perfectly matched with the binding site for transcription factor activator protein4(AP-4); a member of the basic helix-loop-zipper family of transcription factor on the antisense strand (Kawasaki et al., 2006). A study conducted in the Tunisian population found that the rs2488457 (-1123G/C) SNP is associated with T1D (Ferjeni et al., 2015). Similarly, this SNP was shown to be associated with T1D in the Japanese population (Kawasaki et al., 2006). In contrast, the rs2488457 (-1123G/C) SNP was not associated with T1D in two Caucasian populations (Cinek et al., 2007). Beyond its association with T1D, this SNP has also been linked to other autoimmune diseases such as rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA), latent autoimmune diabetes, and ulcerative colitis (UC) (Chen et al., 2013; Fan et al., 2015; Feng et al., 2010; F. Liu et al., 2012)

2.8.2. Polymorphism rs33996649 (G788A)

The rs33996649 polymorphism reveals missense G788A mutation, which maps to exon 10 within the catalytic domain of LYP. It is classified as a loss-of-function variant that leads to substitution of arginine (R) to glutamine (Q) at codon 263 (R263Q), which results in reduced phosphatase activity due to changes in the conformation of the active site. This SNP is not associated with PTPN22 R620W variant (Orrú et al., 2008). Unlike the R620W variant, which increases autoimmune risk, R263Q appears to restrain T-cell receptor

signaling, potentially lowering autoimmunity risk. Meta-analysis showed that the A allele of PTPN22 G788A variant is associated with decreased risk of autoimmune diseases in all subjects ($p < 0.001$). However, ethnic stratification demonstrated significant association of PTPN22 788A allele with autoimmune diseases in Europeans ($p < 0.001$) but not in Latin Americans (Bae & Lee, 2018). Moreover, other studies showed no associations with systemic sclerosis, giant cell arteritis, Henoch-schonlein purpura, uveitis, and Grave's disease. In contrast to 1858T, the 788A allele protects against both SLE and RA. Abbasi et al. investigated the association between the rs33996649 in the PTPN22 gene and T1D for the first time and demonstrated no significant difference between T1D patients and controls in the Iranian population (Abbasi et al., 2017).

2.8.3. Polymorphism rs56048322 (C2250G)

SNP rs56048322 is a rare missense variant in exon 18 of the PTPN22 gene, resulting in an amino acid change from Lysine to Asparagine at position 750. This change affects RNA splicing, as previously observed in PBMCs from a heterozygous individual, where exon 17 was joined to exon 19, creating a premature stop codon at the junction (Ge et al., 2016; Onengut-Gumuscu et al., 2006). As a result, two alternative PTPN22 transcripts and a novel isoform of its encoded protein, LYP, are produced. This rare missense alternate allele (G) at rs56048322 in exon 18 is associated with the expression of these novel transcripts. Genotyping studies have demonstrated that rs56048322 is significantly associated with T1D risk. For instance, Ge et al. identified several rare deleterious variants in PTPN22, including rs56048322, by sequencing the coding regions of 301 genes in 49 loci linked to T1D in 70 cases of European ancestry (Ge et al., 2016). Subsequent analysis in 3,609 families with T1D confirmed that rs56048322 (MAF = 0.87%) is associated with T1D independently of the common variant rs2476601. This reinforces the role of PTPN22 as a candidate gene for T1D susceptibility. Functional analysis has shown that the novel LYP isoform resulting from altered splicing at rs56048322 causes hypo-responsiveness of CD4+ T cells to antigen stimulation in T1D patients. This suggests that altered LYP isoforms may contribute to immune dysregulation in T1D patients, highlighting a potential mechanism for disease susceptibility.

Chapter Three: Methodology

3.1. Study Subject

The study included 100 patients with T1D of both sexes from Jenin and Ramallah health directorate aged from 18-30 years. Only T1D patients with no other known chronic or acute diseases were included, while overweight and obese patients, or patients suffering from chronic and acute diseases were excluded. The control group included 105 healthy subjects recruited from the same directorates with age and other health markers match without a history of diabetes type 1 or type 2 or any other known chronic or acute diseases as described in Table (3.1). All participants (patients and control subjects) enrolled in the study filled in a written informed consent indicating their agreement to participate in the study.

Table 3.1: Descriptions of patients and control subjects. Values are mean ± SD, t-test was used to compare means between control and patients, P<0.05 is significant. M: Mean, SD: Standard deviation, N: Number of subjects.

Character	Patients	Controls	P-value
Subjects Number	100	105	
Age (year)	23.44±4.08	22.79±2.92	0.21
Height (cm)	164.68±10.40	167.93±9.97	0.03
Weight(kg)	64.66±13.56	68.06±18.80	0.18
BMI	23.93±4.39	23.79±4.92	0.84
Glucose level (mg/dl)	450.44±50.3	95.67±4.08	0.00
C-peptide (ng/ml)	0.1±0.32	2.85±0.7	0.00
Hemoglobin A1c (HbA1c) (%)	10.5±0.55	5.71±0.64	0.00
Autoantibody (Anti-GAD)	Positive	Negative	

3.2. Genomic DNA Preparation

Whole blood samples (3-5 mL) were collected in EDTA tubes from patients and control groups and transported to the Genetics lab on ice at AAUP. Samples were centrifuged for 15 minutes at 2000 rpm. After centrifuging, DNA was extracted from the buffy coat according to the manufacturer guidelines of (Master PureTM Genomic DNA Purification Kit, Epicenter Technology Co. Cat.No.MG71100). The buffy coat was separated into two Eppendorf tubes each with 150 µl and one tube was frozen as a backup and the other processed for DNA extraction. First, the buffy coat was mixed with 600 µl Cell Lysing solution, tubes were inverted three times then the bottom was flicked. Then, tubes were incubated at room temperature (RT) for 5 minutes, mixed again followed by incubation for additional 5 minutes at RT. Then, the samples were mixed and centrifuged at high speed (14000 rpm) for 30 seconds to collect intact white cell pellet. After discarding the supernatant (which contains lysed RBC), 50 µl of lysate was kept, and the remaining white pellet (containing WBC) was vigorously vortexed and resuspended and 300 µl of lysis buffer 2 was added then mixing was done by pipetting up and down to induce lysis of the WBCs. Next, 250 µl of precipitation solution was added to the WBCs suspension which masked all components in the lysate except DNA. The mixture was centrifuged at 10,000 RCF for 10 minutes to get rid of cells components. The supernatant which contained the genomic DNA was transferred to a new Eppendorf tube, followed by the addition of 700 µl of ice-cold isopropanol. The tube was inverted several times until a string-like DNA precipitate became visible. By centrifugation for 10 minutes at 10,000 RCF at 4Cº, the DNA was pelleted, and the supernatant was carefully discarded without dislodging the pellet. After rinsing the DNA pellet with 500 µl of 75% cold ethanol, the samples were centrifuged for one minute to aspirate the ethanol keeping the pellet contact. After that, the tube was inverted upside down on tissue paper for air dry. Lastly, by adding 100µl of ultra-pure distilled water, the DNA pellet was dissolved, and the samples were stored frozen at-30C until use.

3.3. DNA Quantification

Purity and concentration of DNA samples were checked by Nanodrop spectrophotometer. Ultra-pure water free of nucleic acids was used as blank. 1µl of DNA samples were used to assess the quantification and purity of the DNA. Absorbance was measured at 260 nm for nucleic acids, 280 nm for proteins, and 230 nm for salts. To check the purity from protein debris, the ratio of 260/280 more than 1.6 is considered satisfactory. All prepared DNA samples from the study subjects had ratio more than 1.8. DNA concentration (µg/mL) was calculated as: absorbance at 260 nm × dilution factor × 50 µg/mL (using 1 OD = 50 µg/mL for dsDNA).

3.4. DNA Qualification:

Gel electrophoresis was used to check the extracted genomic DNA integrity. Also, to determine if there is an intact high molecular band and smaller DNA fragmentation.

3.5. Agarose Gel Preparation.

1 g agarose (GIBCO, Invitrogen, Cat No. 15510-019) was dissolved in 100 ml 1x Tris Acetate EDTA (TAE) by boiling and having clear solution. After cooling to ~60ºC, one drop of ethidium bromide was added, mixed thoroughly, and poured into the gel cast. The tray was placed in a Bio-Rad sub-cell®GT electrophoresis tank filled with 1x TAE buffer. Each DNA sample (2 µl) was combined with 3 µl Blue/Orange Loading dye (6X, Promega, Cat No. G1881: 0.03% bromophenol blue,0.03% Xylene cyanol FF, 0.4% orange G,15% Ficoll 400,10mM Tris-HCl (pH8)) and loaded into wells. Electrophoresis was run at 100 V for 30 minutes.

3.6. SNP Selection and Genotyping

Two SNPs were selected after extensive literature review of studies on T1D from other populations. The most studied SNPs were Investigated; rs2476601 (Abbasi et al., 2017; Haider et al., 2018; C. Sharma et al., 2021; Žak et al., 2023), and the less understandable one; rs1310182 (Taniyama et al., 2010; Žak et al., 2023). In the present study, specific DNA fragments with the indicated SNPs of the PTPN22 gene were PCR amplified from the extracted DNA.

The amplification of DNA fragments with rs1310182 SNP was amplified by direct PCR. PCR amplification of the indicated DNA fragments using the extracted DNA was done as follows: 10 µL PCR master mix (2X), one µL of extracted genomic DNA, 0.5 µl forward primer (10 picomole), 0.5 µl reverse primer (10 picomole), and 8 µl of Nuclease free H2O. The total volume was 20 µL. PCR was carried out for 31 cycles with 2 minutes of initial denaturation at 94˚C, 30 sec annealing at 55˚C, followed by 30 sec extension at 72˚C. Cycling started by 30 sec cycle denaturation at 95˚C and terminated by 5 minutes incubation at 72˚C. The expected fragment size for the direct PCR amplification of rs1310182 was 285 bp.

The amplification of DNA fragments containing rs2476601 SNP was performed by nested PCR. The first round of PCR, employing the first primer pair, was conducted with same reaction setup and cycling conditions as described above. After completion, 1 µL of the first-round PCR product was used as template for the second (nested) PCR by the second primer pair designed to target a region within the first amplicon. The nested PCR reaction was carried out as the previous PCR reactions in volumes and cycling conditions. The expected fragment size for the nested PCR product targeting rs2476601 was 215 bp.

Primer sequences for both PCR protocols were designed using Primer 3 web (https://primer3.ut.ee/) and are listed in Table (3.2). PCR products were observed by 1.5% agarose gel electrophoresis (1.5g of agarose with 100 ml TAE buffer). NTC and DNA ladder (50 bp) were included in the run. A picture was taken after visualization with a UV transilluminator.

Table 3.2: The primer sequences used for the amplification of PTPN22 polymorphisms.

SNP	Forward primer	Reverse primer
First pair rs2476601	CCTCCTGGGTTTGTACCTTAAGAG	CTGGAATTAAAGGCATGAGCCACCATG
Second pair rs2476601	TCACCAGCTTCCTCAACCACA	GATAATGTTGCTTCAACGGAATTT
rs1310182	AATGGACATATTTTCCCATGATGT	TGCCTACTGTATGCCAGTTATTTT

3.7. Sanger Sequencing

Sanger sequencing was performed using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Cat# 4336774) according to the manufacturer's instructions. First, 5 µL of PCR product was cleaned up by 1 µL of EPPIC-FAST reagent (Catalog #1021-100F A&A Biotechnology); a mixture of two enzymes thermolabile nucleotide hydrolase and recombinant exonuclease I with increased efficiency. The mixture was placed on thermocycler at 37°C for 10 minutes followed by 1 minute at 80°C. Thereafter, sequencing was started by adding 18 µL of Big Dye Terminator mix that contains Sequencing buffer (5x), Sequencing primers identical to the forward primers used in the second PCR round for rs2476601 and the PCR amplification of rs1310182, Big Dye Terminator ready reaction Mix, and H2O and 2µL of cleaned PCR as described in Table (3.3). The mixture was placed in thermocycler for 5 minutes using the program shown in Table (3.4). To clean the sequenced samples, EDTA Ethanol precipitation was used, by adding 2.5 µL of EDTA (0.125M) and 30 µL of cold 100% Ethanol and mixed for about 3 minutes. Next, centrifugation for 30 minutes at 2200g was done. After discarding the supernatant, 30 µL of 80% ethanol was added to the pellet and centrifuged for 15 minutes at 1600g. Next, the supernatant was discarded, and the pellet was air dried for 15 minutes. Finally, after adding High dye, the sample was put on a hot plate at 95°C for 5 minutes followed directly by ice incubation for another 5 minutes. Capillary electrophoresis was done using 3500 genetic analyzer (Applied Biosystems). The sequences were visualized using Finch TV version 1.4.0.

Table 3.3: Preparation for Big Dye Terminator mix

Component	Volume
BigDye™ Terminator v1.1 Ready Reaction Mix	4 µL
BigDye™ Terminator v1.1 & v3.1 5X Sequencing Buffer	2 µL
Deionized water (RNase/DNase-free)	10 µL

Sequencing primer	2 µL
Template (cleaned PCR)	2 µL
Total volume	20 µL

Table 3.4: Cycle sequencing

Step	Temperature °C	Time	Cycle numbers
Incubation	96 °C	20 seconds	1 cycle
Denaturation	96 °C	10 seconds	25 cycles
Annealing	50 °C	5 seconds	
Extending	60 °C	4 minutes	
Hold	4°C	∞	∞

3.8. Statistical Analysis

SNPStats (https://www.snpstats.net/) program was used for analyzing the associations between PTPN22 SNPs with T1D using a logistic regression model. SNPStats, is a web-based application designed for the analysis of genetic-epidemiology association studies utilizing Single Nucleotide Polymorphisms (SNPs) (Solé et al., 2006). It can provide all data analysis, from descriptive statistics to SNP and haplotypes analyses. Each SNP is described as allele and genotype frequencies. Furthermore, it supports various inheritance models and facilitates interaction studies. The logistic regression analysis is summarized with genotype frequencies, proportions, odds ratios (OR) and 95% confidence intervals (CI). For more than one SNP, SNPStats also allows linkage disequilibrium (LD) statistics analysis. In all statistical assessments, a P-value below 0.05 (with a 95% confidence interval) was considered statistically significant. The Chi Square test was employed to determine whether observed allele and genotype frequencies differed significantly between T1D patients and

control subjects using online Chi Square Calculator tool (www.standarddeviationcalculator.io).

3.9. Allele and Genotype Frequencies

The observed frequencies of alleles and genotypes both for T1D patients and controls were calculated and tabulated, providing fundamental descriptive statistics.

3.10. Association Analysis with a Response Variable

The statistical association between the selected SNPs and T1D status was determined using appropriate regression models.

3.11. Multiple Inheritance Models

SNPStats analyze associations under five distinct genetic inheritance models: co-dominant, dominant, recessive, over-dominant, and additive. This ability provides understanding the etiology of complex diseases through exploring various genetic patterns. In the present case-control study, determining the inheritance models is powerful means to understand if SNPs in PTPN22 gene might exert their effects through distinct biological mechanism that line up with specific genetic models and how a genetic variant influences disease risk. By using statistical criteria like the Akaike Information Criterion (AIC), the best-fitting model can be identified. Lower values of AIC generally indicate a better fit.

3.12. Linkage Disequilibrium Analysis

To quantify the non-random association of alleles between the specified SNPs located within the same gene, linkage disequilibrium (LD) was used. Measurements such as D, D', and r^2 statistics are commonly used to quantify the LD between SNPs.

D statistic: the deviation between observed and anticipated haplotype frequencies is measured by this metric. It assumes that the two loci have independent inheritance and that the alleles are in complete linkage equilibrium. There is no correlation when the D value is 0, but a greater association is indicated by values that are further from 0 (either positive or negative).

D' statistic: D' is a normalized measure of D, which has the same maximal value irrespective of the allele frequencies at the constituent loci. It ranges from -1 to 1. A D' value of 1 (or -1) indicates complete LD.

r^2 statistic: It represents the squared correlation coefficient between the alleles at two loci. Sensitive to minor allele frequencies; tends to be low when either allele is rare. An r^2 value of 1 indicates perfect correlation, meaning the alleles at the two SNPs are always inherited together. An r^2 value of 0 indicates that the alleles are in complete linkage equilibrium, meaning they are inherited entirely independently of each other. Values between 0 and 1 indicate varying degrees of correlation.

3.13. Haplotype Analysis.

The combinations of alleles were analyzed across rs1310182 and rs2476601 polymorphisms to better understand disease risk and provide a more comprehensive genetic profile. Haplotype analysis reveals stronger associations than analyzing individual SNPs alone. This is particularly relevant for genes like PTPN*22* where multiple SNPs might contribute to disease risk. SNPStats measured the frequencies of different haplotypes formed by alleles of the two specified SNPs. Furthermore, association analysis is performed to evaluate the relationship between haplotypes and a response variable; T1D risk in the present case-control study.

Chapter Four: Results

4.1. Region Detection of PTPN22 Polymorphisms (rs1310182 and rs2476601)

Polymerase Chain Reaction (PCR) was performed to amplify regions containing rs2476601 and rs1310182 single-nucleotide polymorphism (SNP) in the *PTPN22* gene from 205 subjects DNA samples. The rs2476601 variant was amplified by nested PCR with two sets of primers and the rs1310182 variant was amplified with one set of primers. The PCR products were visualized on a 1.5% agarose gel stained with an intercalating dye (ethidium bromide). As shown in Figures (4.1 and 4.2), representative gels show the amplification of the target regions was successful for all subject samples. A DNA ladder was used in the first lane to determine the size of the amplified products. All subjects' samples displayed single, distinct bands with 215 bp and 285 bp DNA fragment sizes with rs2476601and rs1310182 respectively, which is consistent with the expected amplicon size for the rs2476601 and rs1310182 regions. No detection for non-specific amplification bands or primer dimers. The presence of a single, clean band in each lane indicates successful and specific amplification of the target DNA sequence from all subject samples. These results demonstrated that the targeted DNA region was successfully amplified. Sanger sequencing was performed to determine the specific genotype for each sample in the study cohort.

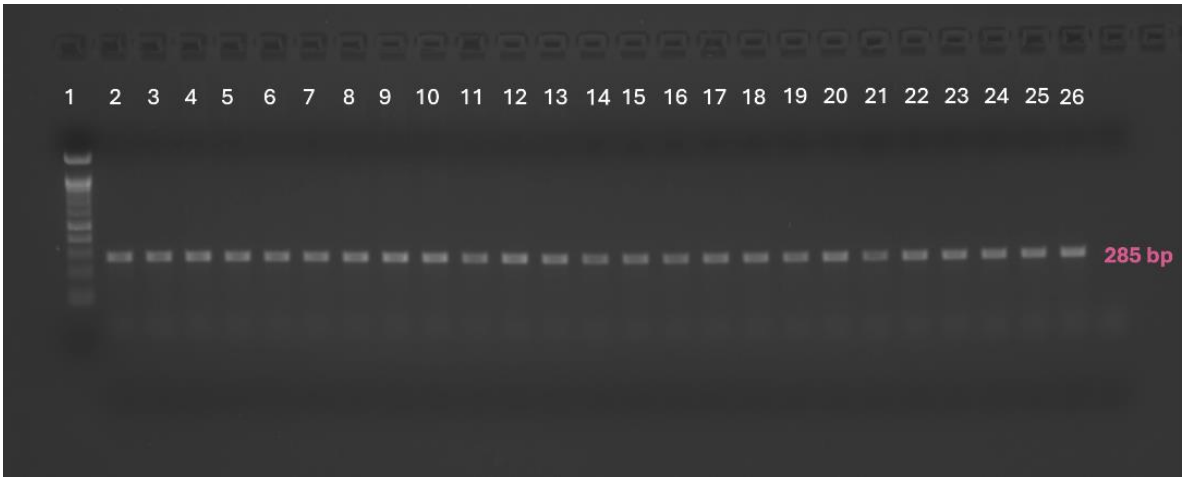

Figure 4.1: PCR amplification of the PTPN22 gene fragment containing the rs1310182 polymorphism, analyzed by agarose gel electrophoresis. Lane 1: 50bp DNA ladder; lanes (2-26) amplified PCR products. The bands are perfect with 285 bp size.

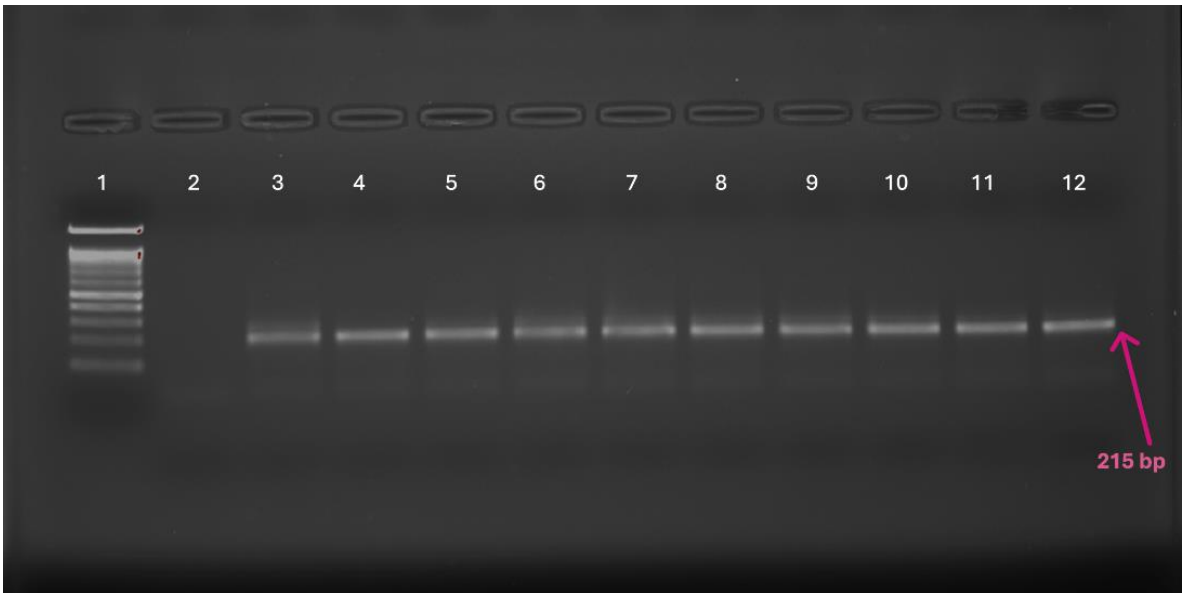

Figure 4.2: PCR amplification of the PTPN22 gene fragment containing the rs2476601 polymorphism, analyzed by agarose gel electrophoresis. Lane 1: 50bp DNA ladder; lanes (3-12) amplified PCR products. The bands are perfect with 215 bp size.

4.2. Genotyping of PTPN22 SNPs in T1D Patients and Controls

To determine the genotype of rs2476601and rs1310182 polymorphisms in patients and control samples, the amplified DNA products were subjected to Sanger sequencing. The resulting sequence chromatograms were analyzed using Finch TV software. Representative chromatograms for samples are shown in Figures (4.3 and 4.4). The sequencing data displayed high-quality read with clear, well-resolved peaks. The sequencing results revealed variation of the specified SNPs with heterozygous and homozygous genotypes. For example, at the position corresponding to the rs2476601 SNP, a clear double peak was observed (Figure 4.4), there were two overlapping peaks of approximately equal height, one corresponding to Cytosine (C, blue peak) and the other to Thymine (T, red peak). This result indicates that the subject is heterozygous for the rs2476601 SNP. Therefore, the genotype for this individual is confirmed to be C/T. For reference, one blue peak of Cytosine indicates homozygous genotype as shown in Figure (4.4). On the other hand, the rs1310182 SNP was analyzed using the G/A allele notation, double peak of Adenine and Guanine indicates heterozygous genotype (Figure 4.3). The findings of one single peak of either Adenine or Guanine are consistent with a homozygous genotype of A/A or G/G at this locus. The total number of genotyping samples was 199 and 195 for rs2476601 and rs1310182, respectively.

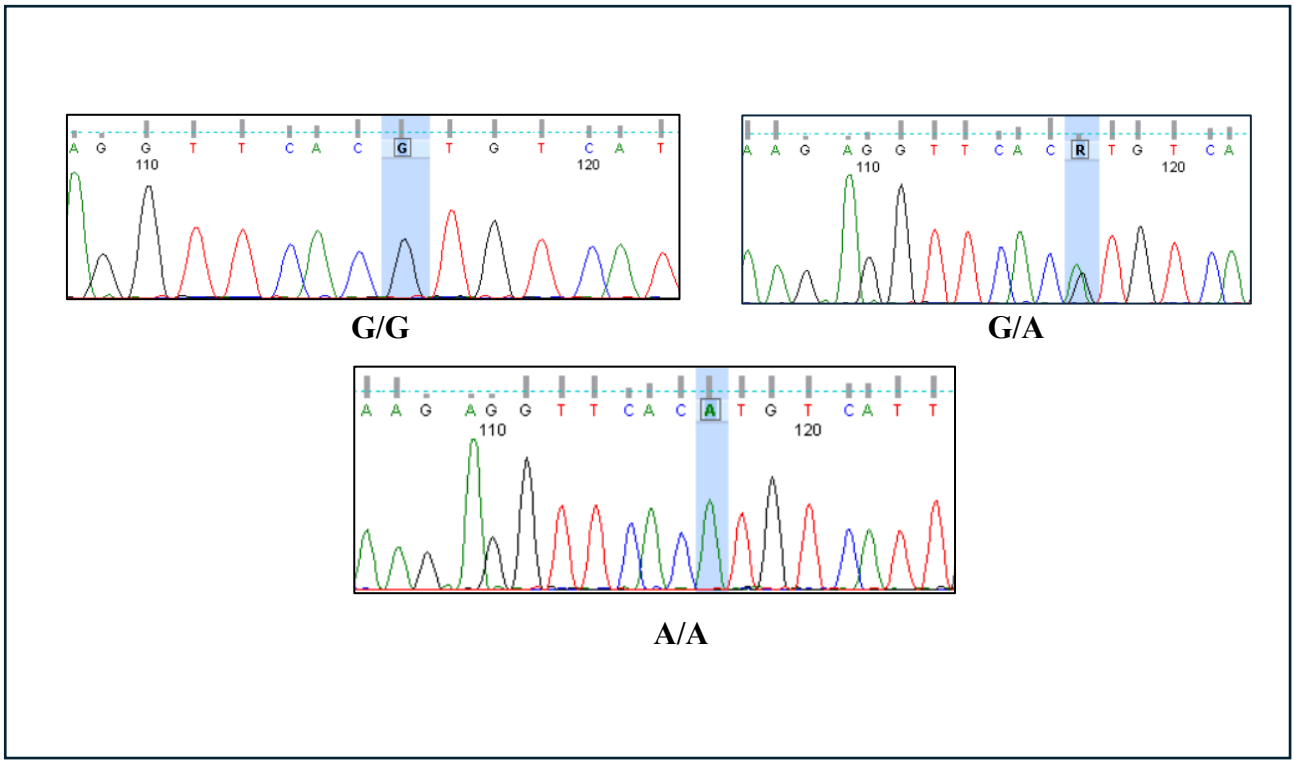

Figure 4.3: Sanger Sequencing Chromatogram for samples showing different genotypes for PTPN22 rs1310182 polymorphism

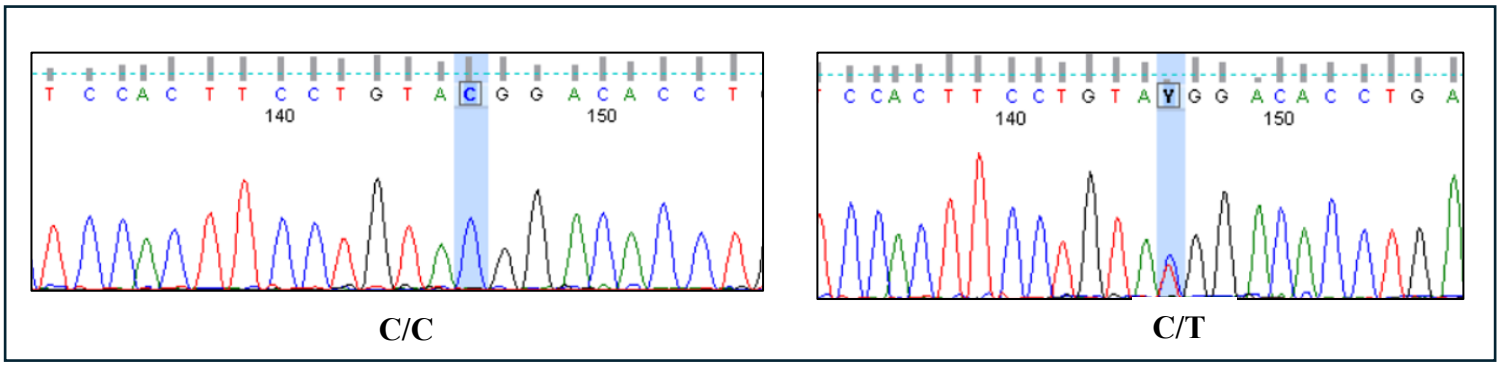

Figure 4.4: Sanger Sequencing Chromatogram for samples showing different genotypes for PTPN22 rs2476601 polymorphism.

4.3. Hardy-Weinberg Equilibrium (HWE)

The distribution of rs2476601 SNP genotyping was assessed by testing for Hardy-Weinberg equilibrium (HWE) using the exact test for both control group and the overall sample (Table 4.1). The rs2476601 polymorphism showed no significant deviation from HWE in the combined cohort, with genotype distributions of 193 (C/C), 5 (C/T), and 1 (T/T) out of a total 199 subjects, with P-value 0.052. In the control group, genotype frequencies were fully consistent with HWE (P-value = 1.0), suggesting that the control sample is representative of the general population and that there are no major genotyping errors or selection biases. In contrast, a borderline deviation from HWE was observed in the patient group (P-value = 0.05). This borderline deviation may suggest a potential association between rs2476601 and disease status, warranting further investigation. Alternatively, such a result could be due to sampling variation or may indicate a biological effect related to the patient group.

Table 4.1: Analysis of HWE parameters for rs2476601 polymorphism

rs2476601 exact test for Hardy-Weinberg equilibrium (n=199)						
	N11 C/C	N12 C/T	N22 T/T	N1 C	N2 T	P-value
All subjects	193	5	1	391	7	0.052
Patients group	95	3	1	193	5	0.05
Control group	98	2	0	198	2	1

The rs1310182 SNP genotypes showed a statistically significant deviation from HWE (Table 4.2) in the overall sample (n=195), with genotype counts 63 (GG), 112 (GA), and 20 (AA) out of a total of 195 subjects with P-value =0.0044. In the patient group, there is a borderline significant deviation from HWE (P-value=0.044). For the control group, the P-

value of 0.09 indicates that the genotype distribution is generally consistent with HWE, suggesting that genotyping for this SNP is likely reliable in this cohort and that the controls indicate unbiased representation of the general population. The deviation for HWE in all subjects could be a sign of population stratification, non-random mating (like inbreeding or assortative mating), or natural selection.

Table 4.2: Analysis of HWE parameters for rs1310182 polymorphism

rs1310182 exact test for Hardy-Weinberg equilibrium (n=195)						
	N11 G/G	N12 G/A	N22 A/A	N1 G	N2 A	P-value
All subjects	63	112	20	238	152	**0.0044**
Patients group	31	53	8	115	69	**0.044**
Control group	31	56	11	118	78	0.09

4.4. Allelic and Genotypic Frequencies of PTPN22 Polymorphisms

To identify the association between PTPN22 single nucleotide polymorphisms and T1D in the studied cohort, the frequency of alleles and genotypes was compared in each of the indicated polymorphisms between those with T1D and the healthy control subjects using Chi-square test. As summarized in Table (4.3), the rs2476601 SNP shows remarkably high frequency of the C allele (98%) and low frequency of the T allele (2%) across all subjects. The distribution of these alleles is almost equal in both T1D patients (C: 97%, T: 3%) and the control group (C: 99%, T: 1%), with no significant difference observed between the subject groups (P-value=0.3124). The genotype frequencies of rs2476601 also indicate no significant association with the disease status (P-value=0.2692). The CC genotype is overrepresented, found in 97% of all subjects, while the CT genotype is rare (3%). This pattern is consistent in both T1D patients (CC: 96%, CT: 3%) and controls (CC: 98%, CT: 2%), (Table 4.4). However, the TT genotype shows low frequency (1%) in T1D patients, while it is absent in the control group.

For rs1310182, the G allele is more common with a prevalence of 61%, while the A allele has a prevalence of 39% across all subjects. The allele frequencies are comparable between T1D patients (G: 62%, A: 38%) and controls (G: 60%, A: 40%). There is no significant difference in allele distribution between the patients with T1D and the controls (P-value=0.5619) as shown in Table (4.3). The genotype frequencies for rs1310182 are, as expected, not significantly different between T1D patients and controls (P-value=0.3922). The most frequent genotype is GA (57%), followed by GG (32%) and AA (1%). This distribution is consistent across both the T1D patient and control groups as shown in Table (4.4).

Table 4.3: Allele frequencies of PTPN22 polymorphisms in Type1 diabetes patients and controls.

Position	Allele	T1D patient	control	All subjects	P-value
rs2476601	C	97%	99%	98%	0.3124
	T	3%	1%	2%	
rs1310182	G	62%	60%	61%	0.5619
	A	38%	40%	39%	

Table 4.4: Genotype distribution of PTPN22 polymorphisms in Type1 diabetes patients and controls

Position	Genotype	T1D patient	Control	All subjects	P-value
rs2476601	CC	96%	98%	97%	0.2692
	CT	3%	2%	3%	
	TT	1%	0%	1%	
rs1310182	GG	34%	32%	32%	0.3922

	GA	58%	57%	57%	
	AA	9%	11%	10%	

4.5. SNP rs2476601 Does Not Associate with Type 1 Diabetes

Statistical analysis of the various rs2476601 genotypes showed no significant association with T1D since the P-values from all genetic models are greater than 0.05. The comparison was made between all respected genotypes including C/C, C/T, and T/T as shown in Table (4.5), which include the Codominant (P=0.44), Dominant (P =0.4), Recessive (P =0.24), Overdominant (P =0.64), and Log-additive (P =0.29) models. The odds ratio (OR) of 1 means no association and the odds of the event are the same in both groups. The odds ratio for C/T genotype compared to the C/C or T/T genotypes, is 0.65, however this might mean a protective effect. Based on the confidence interval that ranges from 0.10 to 3.70 with P= 0.44, the odd ratio of this model is not significant. In addition, the combined C/T and T/T genotypes in the dominant model has an odd ratio of 0.48, which indicates that individuals with at least one T allele are less likely to have T1D compared to those with the C/C genotype, suggesting a protective effect. Though, the wide confidence interval (0.09-2.71) with P=0.4 confound this suggestion. The model fit statistics (AIC and BIC) have high values supporting the conclusion that there is no significant difference in the distribution of rs2476601 genotypes between T1D patients and controls. In summary, the lack of significant differences suggests that the rs2476601 genotype does not play a significant role in susceptibility to T1D in this population, indicating limited biological or clinical relevance for this genetic marker associated withT1D risk.

Table 4.5: Association of rs2476601 SNP with T1D

Model	Genotype	Patient group	Control group	OR (95% CI)	P-value	AIC	BIC
Codominant	C/C	95 (96%)	98(98%)	1.00	0.44	280.2	290.1

	C/T	3 (3%)	2 (2%)	0.65 (0.11-3.95)			
	T/T	1 (1%)	0 (0%)	0.00			
Dominant	C/C	95 (96%)	98 (98%)	1.00	0.4	279.1	285.7
	C/T-T/T	4 (4%)	2 (2%)	0.48 (0.09-2.71)			
Recessive	C/C-C/T	98 (99%)	100 (100%)	1.00	0.24	278.5	285.1
	T/T	1 (1%)	0 (0%)	0.00			
Overdominant	C/C-T/T	96 (97%)	98 (98%)	1.00	0.64	297.7	286.2
	C/T	3 (3%)	2 (2%)	0.65 (0.11-3.99)			
Log-additive	---	---	---	0.47 (0.10-2.14)	0.29	278.8	285.4

4.6. SNP rs1310182 Does Not Associate with Type 1 Diabetes

Statistical analysis of rs1310182 SNP variant genotypes showed no statistically significant association with T1D in the studied population. The analysis of all genetic models revealed that the P-value from all five genetic models evaluated was greater than 0.05 as shown in Table (4.6). Under the Codominant model, the odd ratios (OR) for the A/G (0.95) and A/A (0.73) genotypes, compared to the G/G reference, are not significant (P =0.83). For the Dominant model, following comparing the G/G genotype to the combined

A/G-A/A genotypes, the OR was 0.91 (P=0.76), again indicating no significant association. Similarly, the Recessive model (A/A vs. G/G-A/G) has OR of 0.75 , however, the association was not statistically significant (P=0.56). Similarly, in the Overdominant model, the OR for the A/G genotype compared to the combined G/G-A/A genotypes is 1.02 , which is also not significant (P=0.95) and finally, the Log-additive model shoed OR of 0.88 , with a no-significant P= 0.61 . The consistent lack of significance,where the Confidence Intervals for all ORs cross 1.0 , strongly supports the conclusion that there is no significant difference in the distribution of the indicated genotypes between T1D patients and controls. The high values of the model fit statistics (AIC and BIC) further reinforce this finding. In summary, the lack of significant differences suggests that the rs1310182 genotype does not play significant role in susceptibility to T1D in this population, indicating limited biological or clinical relevance for this genetic marker in the context of T1D risk.

Table 4.6: Association of rs1310182 SNP with T1D

Model	Genotype	Patient group	Control group	OR (95% CI)	P-value	AIC	BIC
Codominant	G/G	31 (33.7%)	31(31.6%)	1.00	0.83	268.8	278.6
	A/G	53(57.6%)	56 (57.1%)	0.95 (0.51-1.77)			
	A/A	8 (8.7%)	11 (11.2%)	0.73 (0.26-2.05)			
Dominant	G/G	31 (33.7%)	31 (31.6%)	1.00	0.76	267.1	273.6
	A/G-A/A	61 (66.3%)	67 (68.4%)	0.91 (0.50-1.67)			
Recessive	G/G-A/G	84 (91.3%)	87 (88.8%)	1.00	0.95	266.9	273.4

	A/A	8 (8.7%)	11 (11.2%)	0.75 (0.29-1.96)			
Overdominant	G/G-A/A	39 (42.4%)	42 (42.9%)	1.00	0.95	267.2	273.7
	A/G	53 (57.6%)	56 (57.1%)	1.02 (0.57-1.81)			
Log-additive	---	---	---	0.88 (0.56-1.41)	0.61	266.9	273.4

4.7. Linkage Disequilibrium (LD) Analysis

To analyze the genetic linkage between alleles at different locations, the LD test was performed for the rs1310182 and rs2476601 variants (Table 4.7). This analysis helps determine if alleles at these two loci are inherited together more often than expected by chance. The D, D', r-statistic were utilized, and the corresponding P-value to assess LD. The D statistic measures the absolute difference between the observed and expected frequencies of a specific alleles at different locations on the same chromosome. D = 0 means perfect linkage equilibrium; values significantly above or below zero indicate linkage disequilibrium. The small value of D = 0.0073 in the present study suggests a slight deviation from equilibrium. The D' value of 0.6769 indicates a moderate to strong linkage disequilibrium between these two SNPs. This suggests that the alleles of these SNPs are not inherited independently. The r-statistic, or correlation coefficient, has a value of 0.1133, which suggests that the SNPs are not highly correlated. Finally, the P-value of 0.0276 indicates that the observed linkage disequilibrium is statistically significant, meaning the non-random association of alleles is unlikely to be due to chance. Despite the absence of direct association with Type 1 diabetes, multiple-SNP analysis revealed statistically significant linkage disequilibrium between the two SNPs.

Table 4.7: Linkage disequilibrium analysis for PTPN22 SNPs

Measure	rs1310182 vs. rs2476601
D statistic	0.0073
D ′ statistic	0.6769
r statistic	0.1133
P-value	**0.0276**

4.8. Haplotype Analysis

To further investigate if the tested SNPs are associated with T1D, haplotype analysis was performed using SNPStats. The software generated four possible haplotypes from rs1310182 and rs2476601SNP variants (Table 4.8). The most frequent haplotype is G-C, with a frequency of 0.61 across all subjects. The next most common haplotype is A-C, with a frequency of 0.3723. A rare haplotype, A-T, was also observed with a frequency of 0.0141. The fourth possible haplotype, G-T with a frequency of 0.0035, was also rare and found in a small number of individuals. None of the observed haplotypes were significantly associated with the patients group (T1D patient vs. control). The global haplotype association P-value was 0.55, indicating no overall significant association. Haplotype 1 (G-C) was used as the reference, with odd ratio (OR) of 1.00. Haplotype 2 (A-C) had an OR of 0.86, with a non-significant P-value of 0.53. Haplotype 3 (A-T) had an OR of 1.28, which also was not statistically significant with P-value 0.81 (Table 4.9). In conclusion, the haplotype analysis confirms that neither of these SNPs nor their combined haplotypes are significantly associated with the disease status in the studied population.

Table 4.8: PTPN22 gene SNPs Haplotype frequencies for rs1310182, and rs2476601, in type 1 diabetes patients and controls. The table shows the frequency of each haplotype for the study cohort and shows the cumulative frequencies that should be 1 to indicate that all possible haplotypes are calculated.

Haplotype	rs1310182	rs2476601	Total	T1D patients	Controls	Cumulative Frequencies
1	G	C	0.61	0.619	0.6022	0.61
2	A	C	0.3723	0.3557	0.3878	0.9824
3	A	T	0.0141	0.0185	0.01	0.9965
4	G	T	0.0035	0.0068	0	1

Table 4.9: Haplotype frequencies response

Haplotype	Frequency	Odd ratio (95% CI)	P-value
1	0.6105	1	----
2	0.3718	0.86 (0.53-1.38)	0.35
3	0.014	1.28 (0.18-8.92)	0.87
4	0.0036	0	1
Global haplotype association P-value: 0.55			

Chapter Five: Discussion

Autoimmunity is defined as a lack or low levels of tolerance to self-antigens that makes T cells reactive and contribute to the immune assault on self-tissues. It plays significant role in tumor immune escape and transplant rejection (Sakaguchi et al., 2001). The thymus plays crucial role in developing CD25+ CD4+ regulatory T cells, which are essential to help the body tolerate its own tissues and prevent immune reactions against itself. T1D or insulin-dependent diabetes mellitus is a chronic autoimmune disease caused by the destruction of pancreatic beta cells. The main immune cells that are responsible for this destruction are type 1 T-helper cells (IFN-gamma) (Wilson et al., 1998). Furthermore, research suggests that reduced tolerance in patients with T1D is partially due to the impaired capacity of CD25+ CD4+ regulatory T cells to suppress T-cell proliferation (Lindley et al., 2005). It is anticipated that primary factors (both genetic and environmental) and mechanisms (including genetic and epigenetic) are involved in the pathogenesis of T1D development at the balance of the immune system, particularly within T-cell receptor signaling ((Bottini et al., 2006), (Bluestone et al., 2010)).

T cell receptor (TCR) plays central role in autoimmunity by acting as the primary molecular gatekeeper for antigen recognition. When this regulatory mechanism is compromised, the immune system may erroneously target self-tissues. Through the lens of downstream signaling proteins that shape the threshold and fidelity of T cell activation including LYP, CSK, ZAP-70, Lck, and other proteins, dysregulation of these molecules can tip the immune system toward self-reactivity (Brand et al., 2005). *PTPN22* gene encodes LYP, a phosphatase that dampens TCR signaling by dephosphorylating kinases like Lck, Fyn, and ZAP-70. Although the Major Histocompatibility Complex (MHC) is closely connected to genetic susceptibility to T1D, non-HLA genes such as PTPN22 may in the events leading to the promotion of the disease. Recent studies identified PTPN22 gene as a crucial locus for multiple autoimmune diseases, and genetic variation in PTPN22 may contribute to T1D development in different populations (Blasetti et al., 2017). Bottini et al. suggested the risk of carrying the 1858T allele suppresses T-cell receptor signaling more efficiently during thymic development, which results in the survival of autoreactive T cells (Bottini et al., 2006). The association between PTPN22 C1858T polymorphism and type 1

diabetes was first identified by Bottini et al. (Bottini et al., 2004)) and subsequently supported by subsequent studies (Hermann et al., 2006; Petrone et al., 2008). Meta-analytic data suggest that this genetic variation may elevate the risk of T1D, especially among European and American populations (Su et al., 2025; Tang et al., 2012).

In the present study focused on investigating the risk of selected variants in the PTPN22 gene (rs1310182 and rs2476601) for T1D development among patients in Palestine comparable to its effect in other populations. The overall analysis of the individual SNPs revealed no statistically significant association with the disease in the studied population that is confirmed across multiple genetic models. This suggests that neither SNP individually serves as a reliable genetic marker for T1D in these patients. Several studies that investigated the association between PTPN22 rs2476601 and T1D risk demonstrated positive association in certain populations. The results evidently revealed that rs2476601 has no significant association with T1D with almost all the study subjects have C/C genotype (97%), and the frequency of the T allele was 3% in T1D patients, while the TT genotype was absent in control subject.

Population-based analysis studies (PBASs) not only support a strong association between the functional C1858T allele (R620W) of rs2476601 in the PTPN22 gene and T1D, but also suggest this variant as the chief variant in the PTPN22 gene encountered with the risk of T1D among white American and European populations, including Czech Republic (Cinek et al., 2007), United States (Steck et al., 2006), France (Chelala et al., 2007), Finland (Hermann et al., 2006) and Germany (Kahles et al., 2005). Additional reports showed the 1858T allele being linked to T1D in several Asian countries including Iran, North India, and Pakistan (Abbasi et al., 2017; Kumar et al., 2014; Rafaqat et al., 2023). However, in East Asian populations, rs2476601 was reported to be non-polymorphic Taniyama et al (Taniyama et al., 2010) reported no significant association between rs2476601 and Japanese T1D and, Pei et al. (Pei et al., 2014) reported there was no association between the single nucleotide polymorphism 1858C>T and Type 1 diabetes in their studied cohort. However, H.W. Liu et al. (H. W. Liu et al., 2015) reported that PTPN22 gene polymorphism 1858C>T may raise T1D risk in Chinese children and adolescents. This discrepancy might be an illustration of the genetic heterogeneity of T1D across different ethnic groups or specific sub-population. Nevertheless, recent meta-

analysis study confirmed that rs2476601 polymorphism was significantly associated with T1D risk among Caucasian and Asian populations (Su et al., 2025).

The results of the present study contradict the prevailing association of rs2476601 with T1D even in the Middle East countries and Arab population like, Kuwait, Emirati, and Egypt. A significant association was found with the T-allele of PTPN22 gene (C1858T, rs2476601) among T1D patients from these countries (Abdelrahman et al., 2016; Haider et al., 2018; A. Sharma et al., 1999). In contrast, a family-based study in Tunisia with T1D revealed other variants in the PTPN22 gene that increased the risk of T1D, while the rs2476601 polymorphism confer a protective effect against T1D (Ferjeni et al., 2015).

In agreement with this study, several investigations failed to demonstrate a significant association between PTPN22 (C1858T, rs2476601) polymorphism and T1D susceptibility. For instance, in Greece, although an increased 1858T allele frequency was observed in T1D patients, the result was not statistically significant which may reflect decreased frequency of the minor allele in the Greek population (Giza et al., 2013). Similarly, a study from Indonesia also failed to find significant associations, reinforcing the possibility that PTPN22's impact varies across populations. In the Indonesian study, a more frequent CC genotype (9.4%) and C allele (54.6%) were reported among T1D children, while the CT genotype (100%) and T allele (50%) were more frequent in the control group. However, the lack of statistical significance may be attributed to the small sample size or low minor allele frequency in this population (Rochmah et al., 2023).

Recent investigations continue to indicate the importance of PTPN22 in T1D susceptibility, yet inconsistences persist in the published findings regarding its association between the gene variants and T1D risk. The findings of the present study align with similar data from Egypt and India, which found no significant association between the T allele and T1D (Baniasadi & Das, 2008; Elsisi et al., 2015). Elsisi et al. reported 3% frequency of the T allele among Egyptian T1D patients, with its absence in controls and no significant association identified. Likewise, in Asian Indian cohorts, the distributions of wild-type homozygous C/C genotype, C allele, and mutant C/T genotype were nearly identical in both patients and normal subjects. The lack of significance in these studies may be partially explained by limited sample sizes or population-specific allele frequencies.

In the current study, the G-allele and GG-genotype of rs1310182 polymorphism were most prevalent in both T1D patients and controls (63% and 58%, respectively), while the AA genotype was rare (1% in both groups). However, these distributions did not differ significantly between the studied groups, indicating no association between rs1310182 and T1D risk in the studied cohort. Initially, rs1310182 (c.2054 852T>C) showed no correlation to T1D (Zoledziewska et al., 2008), contradicting the existence of its autoimmune association such as: celiac disease, pediatric systemic lupus erythematosus, allergic rhinitis, and others (Aflatounian et al., 2017; Bahrami et al., 2020; Ke et al., 2017). Subsequently, varying associations have been reported with inconsistent identification of the associated allele. These conflicting results across populations underscore the complexity of rs1310182 role in T1D susceptibility. The rs1310182 variants showed varying associations with T1D in different populations. Among the Armenian population, the data showed positive association between the T allele and T1D, while the CC genotype was negatively associated (Žak et al., 2023). Similarly, Japanese and Emirati populations have significant association between T1D and rs1310182, but different associated allele (C. Sharma et al., 2021; Taniyama et al., 2010). In contrast, studies from Iran and Tunisia failed to find significant association (Abbasi et al., 2017; Ferjeni et al., 2015). Consistent with some previous studies, the analysis did not reveal a significant association in Palestinian T1D patients. The present findings are consistent with recent meta-analyses concluding that rs1310182 polymorphism does not show significant association with T1D, in contrast to rs2476601 (Su et al., 2025). Furthermore, there are other studies that didn't find association between PTPN22 rs1310182 and certain autoimmune diseases including chronic urticaria and Behcet disease (Brzoza et al., 2012; Q. Zhang et al., 2012). Nonetheless, rs1310182 polymorphism remains of interest due to its putative transcription factor binding site, though functional studies are still lacking. Ongoing population-based research and new meta-analytic data will be essential for clarifying its potential role in T1D risk and for establishing these findings within the evolving global context.

The inconsistent findings regarding the correlation between PTPN22 variants and T1D indicate that the association may be population-specific or influenced by other genetic and environmental factors. This underscores the need for larger, multi-ethnic studies to clarify these relationships. Furthermore, the lack of association between PTPN22

polymorphisms and T1D in Palestinian patients remains valuable for research. Since PTPN22 may not act in isolation, its effects on T1D risk can be influenced by interactions with other genes, particularly those involved in immune regulation. For example, linkage disequilibrium between PTPN22 and loci within the HLA region or genes encoding other signaling proteins (e.g., CTLA4, INS, or IL2RA) could result in additive or synergistic effects on T1D predisposition, as these genes converge on pathways that shape central and peripheral immune tolerance (Bluestone et al., 2010). On the other hand, the PTPN22 gene might not harbor a risk allele shared by all autoimmune diseases, as the PTPN22 gene clearly participates in the development of various other autoimmune diseases. Thus, the negative results in this study may be attributed to insufficient sample size, ethnic variation such as the geographical variation in the frequency of the 1858T allele, and the multifactorial nature of T1D. Moreover, interpreting these results requires considering the different genetic backgrounds of populations, especially regarding HLA class II genes. Additionally, both known and unknown environmental factors may variably influence T1D susceptibility. For robust interpretation, it is important to examine how these factors interact with one another to affect disease risk.

Linkage disequilibrium (LD) refers to the non-random association of alleles at different loci, while D' and r-statistic are measures used to quantify this relationship. Hardy-Weinberg equilibrium describes the expected distribution of genotypes in a population under random mating. In the present study, a notable point from the individual SNP analysis is the deviation from HWE for rs1310182 SNP and a borderline deviation for rs2476601 SNP of patient group. LD analysis revealed a statistically significant non-random association between the two SNPs, rs1310182 and rs2476601, indicating that these genetic variants are often inherited together. Specifically, the D' value of 0.6769 points to moderate to strong LD, whereas the r-statistic of 0.1133 suggests a weak correlation between the SNPs, likely due to the low frequency of the minor T allele at rs2476601. Although LD analysis indicated a significant association, haplotype analysis did not support a connection with T1D. This discrepancy prompted further investigation into population genetic factors that might explain the observed patterns. The statistical significance of LD can originate from multiple factors, such as natural selection, genetic drift, population subdivision, bottlenecks, and inbreeding. As discussed by Slatkin (Slatkin,

2008), factors like population subdivision can artificially increase LD measures. In the present study, this may account for the deviation from Hardy-Weinberg equilibrium observed for rs1310182 patient group. After confirming the absence of genotyping errors, population stratification and extensive inbreeding remain plausible explanations for this deviation.

There is limited published studies on the incidence, prevalence, risk factors, and complications of T1D in Palestinian children (El Sharif & Imam, 2019). However, T1D in Palestine is believed to follow the global trend of rising incidence rates among children and adolescents (Ogle et al., 2022). In addition, the genetics of T1D in Palestinian patients is still understudied. This gap of research highlights the urgent need for comprehensive epidemiological and genetic studies in the region. Especially, the characterization of high rates of consanguinity, endogamy, and first-cousin marriage in Palestine and generally in Arab population. These types of marriages increase the probability of homozygosity of non-HLA genes associated with either protection or susceptibility to T1D (Zayed, 2016). Correspondingly, the significant LD between the PTPN22 variants in the current study could be due to the inbreeding status of the Palestinian society, which increases the chance of alleles being inherited together, augmenting LD across the genome. However, the non-correlation between the PTPN22 SNPs (rs1310182 and rs2476601) and T1D in the present study cohort could be authentic, otherwise, it is contradicting the prevailing significant association with T1D and the need for screening of other PTPN22 variants is crucial in unraveling the genetics of T1D in Palestine. Alternatively, these two SNPs could be associated with other autoimmune disease in Palestinian population. Only one study was conducted on two Palestinian families, each with members afflicted by T1D. Through whole exome sequencing of two probands from each family, researchers identified two genetic variants that segregated with the disease within these families. The identified variants were in the IGF1R and NEUROD genes, both of which are implicated in insulin signaling and pancreatic beta-cell function (Bawatneh et al., 2023). These novel findings contribute to the broader understanding of T1D genetics, and the variants discovered may be particularly significant for the Palestinian population, as they could be too rare to be detected in large-scale Genome-Wide Association Studies (GWAS) that primarily focus on Caucasian cohorts. Family-based genetic studies, such as this one, could be more efficient

in uncovering new genes and pathways important for T1D pathogenesis, potentially guiding future efforts in diagnosis, prevention, and treatment. As a result, these approaches may prove more effective than population-based studies in identifying rare variants and finding associations between genetic markers and T1D in underrepresented populations like Palestinian population.

5.1. Conclusion and Recommendations

This study represents the first genetic analysis of PTPN22 variants (rs1310182 and rs2476601) in Palestinian patients with T1D. The results indicate that these specific PTPN22 polymorphisms do not confer susceptibility to T1D in this population, a finding that contradicts with outcomes observed in several other populations. However, these findings should be interpreted with caution due to several key limitations. One option is to expand future studies and recruit larger, multicenter cohorts across diverse regions of Palestine. Moreover, the focus on only two polymorphisms means the study may not fully capture the complex genetic factors influencing T1D, suggesting that broader genetic screening—including additional PTPN22 variants and other immune-regulatory genes such as those in the HLA region—would be valuable for a more comprehensive understanding of T1D susceptibility. The potential impact of PTPN22 variants on diabetes-related antibodies and disease control parameters remains unexplored and should be addressed in future research. Functional studies are also recommended to clarify the biological mechanisms by which PTPN22 and related polymorphisms influence T1D development. Additionally, integrating environmental and lifestyle factors into future analyses will help elucidate gene-environmental interactions and their role in disease risk. Implementing longitudinal follow-up studies and ongoing collaboration between research centers will be essential for advancing the understanding of T1D genetics in Palestine and translating these findings into clinical practice. By linking these limitations to future research directions, this will help provide a cohesive and constructive path forward for the study of T1D genetics in the Palestinian population.

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دور متغيرات الجين بي تي بي ان 22(ار اس2476601 و ار اس1310182) في تطور حدوث مرض السكري من النوع الاول.

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ملخص:

مرض السكري النوع الأول هو اضطراب مناعي ذاتي مزمن يتميز بتدمير خلايا بيتا البنكرياسية، مما يؤدي إلى ارتفاع السكر في الدم ويتطلب العلاج بالأنسولين مدى الحياة. وتُعد مسببات مرض السكري النوع الأول معقدة ومتعددة العوامل، حيث تتداخل العوامل الوراثية والجينية والبيئية في حدوثه. تشير التقديرات إلى أن الاستعداد الوراثي يساهم بنسبة تصل إلى 80٪ من الخطر الإجمالي للإصابة بمرض السكري النوع الأول الوراثي، في حين أن التعرض البيئي والتعديلات اللاجينية تؤثر أيضاً بشكل كبير على ظهور المرض وتطوره. من بين هذه العوامل، برز جين PTPN22 كعامل وراثي مؤثر في القابلية للإصابة بالسكري النوع الأول، إذ يقوم بتشفير بروتين LYP الذي ينظم مستقبلات الخلايا المناعية ويؤثر بذلك على التحمل المناعي والمناعة الذاتية. وقد أظهرت الدراسات ارتباط اثنين من تعدد أشكال النوكليوتيدات المفردة (SNP) داخل PTPN22 (rs2476601 و rs1310182)
بخطر الإصابة بالسكري النوع الأول، مع اختلاف انتشارها وتأثيرها بين السكان والمناطق الجغرافية.

هدفت هذه الدراسة إلى فحص الأساس الجيني الجزيئي لمرض السكري النوع الأول في مجموعة فلسطينية من خلال تحليل تعدد الأشكال rs2476601 و rs1310182 في جين PTPN22، وذلك لتوضيح ارتباطها المحتمل بخطر السكري النوع الأول في هذه الفئة السكانية.

شملت الدراسة 205 من الأفراد، منهم 100 حالة مؤكدة من السكري النوع الأول و105 أشخاص أصحاء كعينة ضابطة، من مدينتي رام الله وجنين. تم استخراج الحمض النووي الجيني من جميع

المشاركين، واستخدم تسلسل سانجر لتحديد وجود متغيرات PTPN22 المحددة. وتمت مقارنة التحليلات الجينية والأليلية بين المرضى والأصحاء لتقييم الارتباط بالسكري النوع الأول.

كشف تحليل التوزيعات الجينية والأليلية لكل من تعدد الأشكال rs2476601 و rs1310182 عن عدم وجود ارتباط ذي دلالة إحصائية مع مرض السكري النوع الأول في المجموعة المدروسة.. تشير هذه النتائج إلى أن هذه الطفرات المحددة لجين PTPN22 بين السكان الفلسطينيين لا علاقة لها بزيادة قابلية الإصابة بمرض السكري النوع الأول.

تساهم هذه الدراسة في إضافة دليل جديد إلى الأدلة المتعلقة بالتباين الجغرافي لتعدد الأشكال لجين PTPN22 ودورها في السكري النوع الأول. ويؤكد عدم وجود ارتباط بين متغيرات هذا الجين ومرض السكري النوع الأول على الحاجة إلى حجم عينة أكبر وتقييم علامات وراثية إضافية لفهم الأساس الجيني للسكري النوع الأول بشكل شامل.

الكلمات المفتاحية: مرض السكري النوع الأول، تعدد الأشكال لجين PTPN22 (rs2476601 و rs1310182)، تعدد أشكال النوكليوتيدات المفردة (SNP)