Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Kerbala

College of Pharmacy

Department of Pharmacology and Toxicology

An Investigation of the Effect of Genetic Polymorphisms in the Insulin Receptor (*INSR*) Gene on Response to Exogenous Insulin Therapy in a Sample of Iraqi Patients with Type 1 Diabetes

A Thesis

Submitted to the Council of the College of Pharmacy / University of Kerbala as Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacology and Toxicology

By

Farah Ali Al-Quraishi

B.Sc. in Pharmacy (Baghdad College of Medical Sciences, 2014)

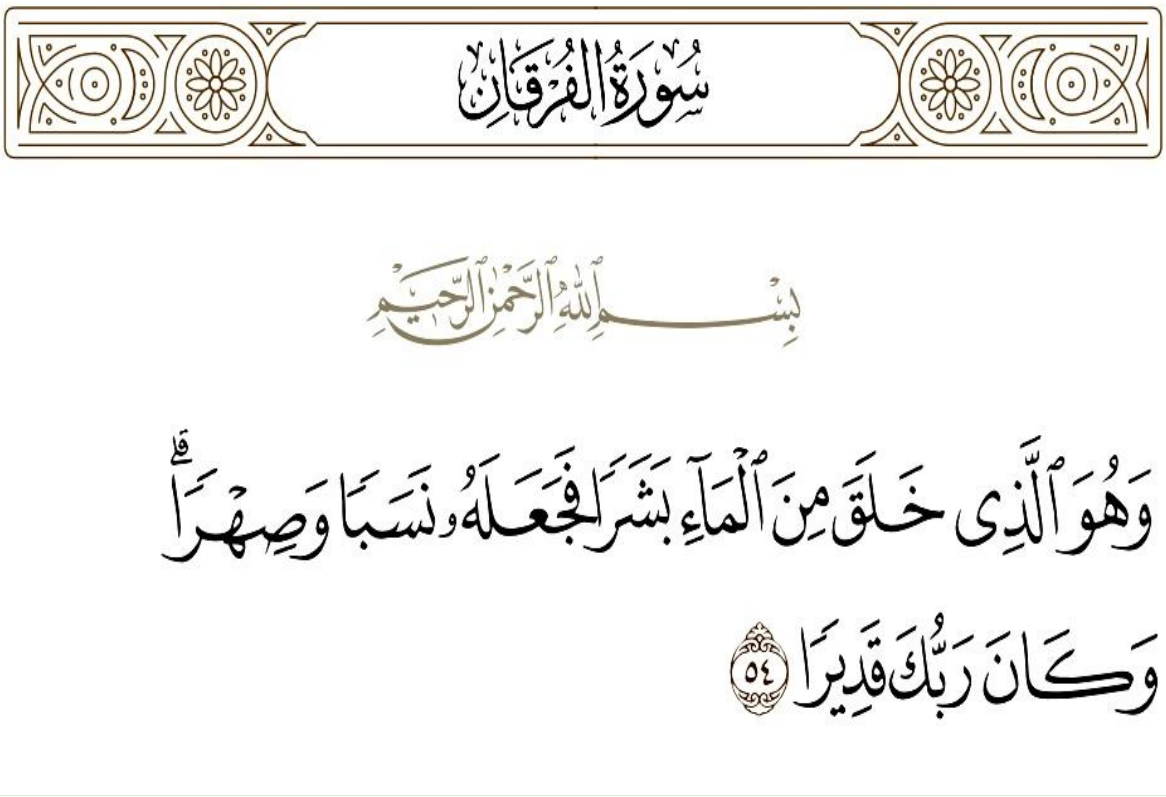
Supervised By

Asst. Prof. Dr. Mohammed Ibrahim Rasool

PhD. in Pharmacology and Therapeutics

2025 A.D.

1447 A.H.



صدق الله العلي العظيم

"And it is Him, who created from water a human being and made him a creature of lineage and marriage, and your Lord is ever Powerful"(surat al-Forqan-54).

Allah, the Most High, the Most Great, has spoken the Truth.

Supervisor Certification

I certify this thesis (**An Investigation of the Effect of Genetic Polymorphisms in the Insulin Receptor *(INSR)* Gene on Response to Exogenous Insulin Therapy in a Sample of Iraqi Patients with Type 1 Diabetes)** was prepared by (**Farah Ali Al-Quraishi**) under my supervision at the University of Kerbala, College of Pharmacy, Department of Pharmacology and Toxicology as a partial requirement for the Degree of Master of Science in Pharmacology and Toxicology.

Supervisor

Asst. Prof. Dr. Mohammed Ibrahim Rasool

College of Pharmacy/ University of Kerbala

Date: 8/ 08 / 2025

In the view of the available recommendation, I forward this M.Sc. thesis for debate by the examining committee.

Prof. Dr. Shatha Hussein Kadhim

Chairwoman of Pharmacology and Toxicology Department

University of Kerbala/ College of Pharmacy

II

Committee Certification

We, the examining committee, certify that we have read the thesis entitled (**An Investigation of the Effect of Genetic Polymorphisms in the Insulin Receptor *(INSR)* Gene on Response to Exogenous Insulin Therapy in a Sample of Iraqi Patients with Type 1 Diabetes)**, and have examined the student (**Farah Ali Al-Quraishi**) in its contents, and found it adequate withstanding as a thesis for the degree of Master of Science in Pharmacology and Toxicology.

Thesis Committee Names :

Chairman

Dr Fadhil A. Rizij

Member

Member

Amal Umran

Supervisor

Asst. Prof. Dr. Mohammed Ibrahim Rasool

III

Approved By

College of Pharmacy /University of Kerbala

As a thesis for the degree of Master of Science

in Pharmacology and Toxicology

Asst. Prof.

Dr. Mohammed Ibrahim Rasool

Dean

College of Pharmacy /University of Kerbala

Seal

Higher Studies Registration

College of Pharmacy/ University of Kerbala

IV

Dedication

With a heart full of gratitude, All praise and gratitude are due to Allah the Almighty, whose divine wisdom and mercy have guided every step of this work. It is through His boundless grace, strength, and patience that I have been able to endure, persevere, and complete this journey. His guidance has been my anchor in moments of doubt and my strength in times of weariness.

To my beloved father, his belief in me has been my guiding light, inspiring me to push beyond my comfort zone and strive for excellence. This degree is not mine alone; it belongs to him, for all he has have done and given.

To my dear friends, I am deeply thankful to all the wonderful individuals, mentors, colleagues, and every pure soul who has inspired me to grow, to learn, and to rise. They have embraced me with their unconditional love, support, and their unwavering encouragement, and loyality has been a source of strength that carried me through the most difficult moments.

Farah

Acknowledgements

I would like to extend my sincere appreciation to the College of Pharmacy at the University of Kerbala, particularly the Departments of Pharmacology and Toxicology, and Clinical Laboratory Science, for their significant contributions to the realization of this work.

My profound gratitude goes to the Dean of the College of Pharmacy and my esteemed supervisor, Asst. Prof. Dr. Mohammed Ibrahim. I am truly and forever grateful for his belief in my potential, and his thoughtful guidance at every step. His leadership has greatly enriched my academic journey.

To every child with a chronic disease, this work is dedicated to your strength, your courage, and your journey. May you grow up to lead bright, healthy, and fulfilling lives. You are the heroes of your own stories, and your resilience fuels the future of science and compassion. This thesis is not only the result of academic effort but also a humble contribution to the greater mission of improving human health. May this work serve as a step toward that future, where even the smallest discoveries can spark progress and help improve lives.

Table of Contents

List of Tables

List of Figures

List of Abbreviations	
Abbreviations	**Full-Text**
ADA	American Diabetes Association
ADP	adenosine diphosphate
ANOVA	Analysis of Variance
APCs	The Antigen-Presenting cells
AS-PCR	Allele-Specific Polymerase Chain Reaction
ATP	Adenosine triphosphate
BMI	Body Mass Index
bp	basepair
CDA	Canadian Diabetes Association
CHH	Congenital Hyperinsulinemic Hypoglycemia
CI	Confidence Interval
DD	Double Diabetes
DKA	Diabetic Ketoacidosis
dL	deciliter
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra Acetate
ELISA	Enzyme-Linked Immunosorbent Assay
ERK	Extracellular signal regulated kinase
FBS	Fasting Blood Sugar
g	gram
GAD65	Glutamic Acid Decarboxylase 65-kilodalton
GDM	Gestational Diabetes Mellitus
gDNA	Genomic Deoxyribonucleic Acid
GLUT-4	Glucose Transporter Type 4
GRB2	Growth Factor Receptor-Bound Protein 2
HbA1c	Glycated Hemoglobin
HLA	Human Leukocyte Antigen
HPLC	High-Performance Liquid Chromatography
HRP	Horseradish Peroxidase
HWE	Hardy-Weinberg Equilibrium

IA2A	Insulinoma-Associated Autoantigen 2
IAA	Insulin-Reactive Autoantibodies
IDF	International Diabetes Federation
INSR	Insulin Receptor Gene
IR	Insulin Receptor
IRS	Insulin Receptor Substrate
kg	kilogram
LDL	Low- Density Lipoprotein
m^2	Square meter
MAF	Minor Allele Frequency
MAPK	Mitogen-Activated Protein Kinase
mg	milligram
MHC	Major Histocompatibility Complex
mL	milliLiter
MODY	Maturity-Onset Diabetes Of The Young
MRDM	Malnutrition Related Diabetes Mellutis
n	Numbers of Patients
NADP+	Nicotinamide Adenine Dinucleotide Phosphate
NDM	Neonatal Diabetes Mellitus
nm	Nanometer
NPH	Neutral Protamine Hagedorn
OD	Optical Density
OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio
PCOS	Polycystic Ovarian Syndrome
PCR	Polymerase chain reaction
PDK1	Phosphoinositide-dependent kinase-1
PI3K	Phosphoinositide 3-kinase
PI3K/AKT	Phosphoinositide-3- kinase/protein kinase B
PIP2	Phosphatidylinositol 4,5-bisphosphate
PIP3	Phosphatidylinositol-3,4,5-Trisphosphate
PK	Proteinase K
RAF	Rapidly Accelerated Fibrosarcoma Kinase
RAS	Rat Sarcoma Protein
RBS	Random Blood Sugar

RNA	Ribonucleic Acid
RPM	Round per minute
Ser	Serine
SH2	Src Homology 2 Domain
sIR	Soluble Insulin Receptor
SNPs	Single Nucleotide Polymorphisms
SoS	Son of Sevenless
SPSS	Statistical Package for Social Science
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
Taq Polymerase	Thermus Aquaticus
TBE	Triesborate EDTA
Tyr	Tyrosine
UV-VIS	Ultraviolet Visible
vs.	versus
WHO	World Health Organization
WHO Diamond	World Health Organization Diabetes Mondiale
ZnT8a	Zinc Transporter 8
α-subunit	Alpha-Subunit
β-cells	Beta-cells
β-subunit	Beta-subunit
µL	microliter
χ^2	Chi-square test

Abstract

Background: Lifelong administration of exogenous insulin remains the cornerstone of treatment for type 1 diabetes mellitus. The efficacy of insulin in achieving optimal glycemic control is largely dependent on its binding affinity to the insulin receptor. Single nucleotide polymorphisms (SNPs) in the insulin receptor (INSR) gene may influence this efficacy by altering receptor function, potentially contributing to insulin resistance in individuals treated with exogenous insulin. This cross-sectional pharmacogenetic study is particularly significant as it introduces innovative approaches within precision medicine and identifies specific genetic loci associated with reduced sensitivity to exogenous insulin. Moreover, it provides valuable insights into the development of chronic hyperglycemia despite strict adherence to exogenous insulin regimens. The findings related to the examined SNPs may facilitate the development of novel genome engineering applications aimed at precise gene modifications or modulation of gene expression.

Aim: This study investigates the impact of two single nucleotide polymorphisms (SNPs), (rs2245649) T>C and (rs2229429) G>A, in the *INSR* gene on the therapeutic response to exogenous insulin in a cohort of Iraqi individuals with type 1 diabetes mellitus.

Method: The impact of SNPs in the *INSR* gene (rs2245649) T>C and (rs2229429) G>A have been examined in 99 T1D individuals with poor glycemic control treated with a monotherapy basal-bolus exogenous insulin regimen. Moreover 30 healthy individuals were typed as healthy control, with both groups having a mean age of 12.3 years old. Genotyping was performed using an allele-specific polymerase chain reaction technique and the data was analyzed statistically.

Results: Type 1 diabetic individuals which are homozygous for the mutant alleles of INSR SNPs (rs2245649) T>C and (rs2229429) G>A, exhibited significantly poorer glycemic control compared to those homozygous for the wild-type alleles. The minor allele frequencies of (rs2245649) T>C and (rs2229429) G>A were 26% and 12%, respectively, within the Iraqi sample population. Patients with type 1 diabetes carrying the minor allele of (rs2245649) had a 2.523-fold increased risk of developing insulin resistance ($p = 0.04$). Furthermore, homozygous mutant carriers of (rs2229429) demonstrated a 10.479-fold higher risk of developing severe hyperglycemia compared to wild-type genotype carriers ($p = 0.008$). Notably, non-responders to exogenous insulin harboring the (rs2229429) G>A SNP exhibited significantly elevated serum levels of insulin receptors ($p<0.001$). Similarly, individuals possessing the C allele of (rs2245649) showed higher concentrations of soluble insulin receptors compared to those with the T allele ($p = 0.02$).

Conclusion: The minor alleles of the investigated SNPs, INSR (rs2245649) T>C and (rs2229429) G>A, were identified in the Iraqi population and found to be significantly associated with severe hyperglycemia and elevated levels of soluble insulin receptor. These findings suggest a potential link between these genetic variants and the development of insulin resistance.

Chapter One

Introduction

1.Introduction

1.1. Definition and Classifications of Diabetes Mellitus

Diabetes Mellitus (DM) is a wide range of chronic metabolic disorders marked by hyperglycemia, caused by either a lack of insulin production, impaired insulin action, or both. Resulting in metabolic dysfunction affecting carbohydrate, lipid, and protein metabolism (Pleus et al., 2024).

Hyperglycemia is a condition in which blood glucose levels are elevated above the normal physiologic range, whereby fasting glucose levels are greater than 126 mg/dL (on two occasions) or random glucose levels are greater than 200 mg/dL in the presence of symptoms (Antar et al., 2023).

Uncontrolled diabetes impairs the body's ability to transport glucose into cells, resulting in cellular deprivation, leading to chronic hyperglycemia. This triggers an increased breakdown of protein and fat to generate cell energy. Diabetes can progress gradually and is associated with a wide range of symptoms. It is a significant contributor to conditions such as stroke, heart attack, infections, kidney disease, nerve damage, and vision loss (Villegas-Valverde et al., 2018).

Throughout history, mankind has encountered numerous outbreaks of infectious diseases. After overcoming these diseases, new threats have emerged in the form of non-communicable illnesses, which could be considered an epidemic, if not a pandemic. Currently, non-communicable diseases pose a significant risk to global health. The United Nations has recognized DM as the first non-communicable disease in the 21st-century pandemic. The World Health Organization (WHO) estimates that diabetes will be the 7th primary cause of fatality by 2030 (Wolosowicz et al., 2020; Alam et al., 2021).

Diabetes mellitus is classified into four categories:

A) Type 1 diabetes mellitus (T1D): typically manifests during childhood or adolescence and arises when the immune system destroys pancreatic β-cells, leading to an absolute insulin deficiency (Wolosowicz et al., 2020).

B) Type 2 diabetes mellitus (T2D): encompasses a heterogeneous group of disorders involving insulin resistance and reduced insulin production by the pancreas. T2D is most common in adults, but can occur in children due to obesity (Alam et al., 2021).

C) Gestational diabetes mellitus (GDM): a frequent pregnancy problem that mainly affects women in the second or third trimester when spontaneous hyperglycemia arises (Farrukh et al., 2022).

D) There are other specific types of DM: resulting from a mutation in a single gene abnormality in pancreatic β-cell function, such as maturity-onset diabetes of the young (MODY), neonatal diabetes mellitus (NDM), and congenital hyperinsulinemic hypoglycemia (CHH) (Alam et al., 2021; Goyal et al., 2023). Moreover, Type 3c diabetes mellitus (T3cDM), also referred to as pancreatogenic diabetes, is a subtype of diabetes that develops secondary to pancreatic disorders or injury (Ewald et al., 2013).

Type 5 diabetes, also termed malnutrition-related diabetes mellitus (MRDM), it is neither autoimmune nor associated with insulin resistance. It has been officially recognized by the International Diabetes Federation (IDF) in April 2025. Type 5 Diabetes according to the IDF, refers to a distinct form of diabetes caused by chronic malnutrition, particularly during early life stages such as childhood and adolescence (Misra et al., 2025).

1.1.1. Type 1 Diabetes Mellitus

Insulin is a vital anabolic hormone that regulates growth and the metabolism of carbohydrates, fats, proteins, and minerals. In T1D, a deficiency or absence of insulin secretion leads to impaired glucose uptake by cells, resulting in systemic hyperglycemia, which is the hallmark of the disease. T1D makes up only about 5-10% of diabetes cases worldwide (Paschou et al., 2018). While in Iraq, the prevalence of T1D is approximately 5% among Iraqi children (Hussein et al., 2023). Poor glycemic control was reported recently to be 23% among Iraqi children (Hadi et al., 2018).

Several investigations have shown that genetic and autoimmune factors play a major role in the initial development of T1D. The scientific consensus holds that the β-cell is the primary source of the illness and that autoreactive T cells destroy healthy β-cells mistakenly. β-cells have weak defense systems and are vulnerable to biosynthetic stress due to their nature. Pancreatic-specific β-cell auto-immunity can develop gradually at late onset or swiftly at juvenile onset. Furthermore, the primary susceptibility gene is found on chromosome 6 in the Human Leukocyte Antigen (HLA) region. The HLA complex is linked to a 40–50% increased likelihood of acquiring T1D (Popoviciu et al., 2023).

Under some conditions, the many signs and symptoms of β-cell deficiency and malfunction in young people might lead to diabetic ketoacidosis (DKA). Some patients may experience a delayed onset of acute hyperglycemia, while others may experience a sluggish advancement of the condition (Roep et al., 2021).

While onset can happen at any age, incidence rises in puberty and the early years of adulthood. Psychosocial problems and other autoimmune illnesses are particularly prevalent in patients with T1D. To minimize both short- and long-term consequences, management should concentrate on improving glucose control (Katsarou et al., 2017).

The most common form of T1D is type 1A diabetes is (90% of global T1D cases), which is caused by the autoimmune destruction of β-cells in the endocrine pancreas due to a combination of genetic and environmental factors. Both of which play a role in the pathogenesis of the disease, leading to insulin depletion and elevated blood sugar levels. Therefore, replacing insulin is crucial for managing the condition (Antar et al., 2023).

While idiopathic diabetes, or Type 1B (less than 10% of global T1D cases) is characterized by an unexplained cause without evidence of autoimmunity and variable insulin deficiency, absence of autoantibodies, low or absent C-peptide. It is commonly present in African, Hispanic and Asian ethnics. Another rare form of T1D is fulminant diabetes (less than 1% globally) is distinguished by its abrupt onset within days, severe insulin shortage, and increased risk of Diabetic Ketoacidosis (DKA). Mainly occurs in Japanese and East Asian ethnics (Syed et al., 2022; Antar et al., 2023).

Other form of T1D is Latent autoimmune diabetes in adults (LADA). It is a form of diabetes that arises due to an autoimmune response targeting pancreatic beta cells, similar to type 1 diabetes. However, unlike classical type 1A diabetes, LADA presents later in life, commonly in individuals over 30 years of age, and progresses more gradually. At diagnosis, patients typically do not require insulin and may be mistakenly treated as having type 2 diabetes. The presence of diabetes-specific autoantibodies, such as GAD65, confirms its autoimmune nature (Quattrin et al., 2023).

Double diabetes (DD), alternatively termed hybrid diabetes, describes a clinical condition in which individuals exhibits characteristics of both T1D and T2D (Bielka et al., 2024). It is defined by the coexistence of autoimmune-mediated beta-cell destruction, typical of T1D, alongside insulin resistance or other metabolic disturbances associated with T2D. This overlap may arise when a person with T1D

develops insulin resistance, often influenced by obesity or sedentary lifestyle or family history of T2D (Gagnon et al., 2024).

Individuals with T1D exhibiting obesity or possess a genetic predisposition to T2D, may most likely develop insulin resistance, where peripheral tissues respond inadequately to exogenous insulin (Ferreira-hermosillo et al., 2020).

Previous studies have identified obesity in patients with T1D as a significant contributor to the development of insulin resistance. Another important key factor for developing DD is the influence of genetic polymorphisms on various genes affecting insulin sensitivity. This phenomenon can worsen hyperglycemia and significantly complicate the clinical management of diabetes (Chaudhary et al., 2025).

Insulin resistance is a condition where insulin-mediated glucose uptake is impared, especially in the skeletal muscle, liver, and adipose tissue. Thereby, exhibiting a reduced biological response to circulating insulin, leading to impaired glucose uptake. In insulin resistance, the insulin signaling pathway might be disrupted at three levels. At pre-receptor-level due to insulin abnormailities, receptor-level, or at receptor level which might be caused by insulin receptor gene mutation leading to decreased activity or number of the insulin receptors, or at post-receptor level due to insulin receptor substrate (IRS) protein dysfunction leading to impaired signaling pathway (Sesti et al., 2001; Renna et al., 2017; Chaudhary et al., 2025).

Insulin resistance is strongly linked with an elevated risk of cardiovascular morbidity and diabetic nephropathy, underscoring the importance of prompt recognition and early therapeutic intervention (Cleland, 2013).

1.1.2. Epidemiology of Type 1 Diabetes Mellitus

Globally, 8.4 million people were predicted to have T1D in 2021, with 500,000 new cases being reported. The number of people with T1D is expected to increase to 13.5–17.4 million by 2040. According to registry data, the incidence of T1D has increased by 3-4% worldwide during the previous three decades, supporting the influence of environmental factors (Norris et al., 2020).

The environmental factors are important in the autoimmune process that results in the breakdown of β-cells. The occurrence of T1D has been related to perinatal variables such as mode of delivery, mother and child nutrition, and environmental factors like air pollution and climate. Furthermore, it has been suggested that the obesity epidemic, which causes lipid disorders and insulin resistance, may hasten the onset of T1D in people who are genetically prone to the disease (Ogrotis et al., 2023).

According to data from 193 publications published between 1990 and 2019, the incidence of type 1 diabetes was found to be 15 per 100,000 in Asia, 8 per 100,000 in Africa, 15 per 100,000 in Europe, and 20 per 100,000 in America, correspondingly (Mobasseri et al., 2020).

In a study conducted in 2016, the prevalence of T1D in Basra, Iraq, was 87 per 100,000 people. Iraq's average annual incidence rate of type 1 diabetes for those aged 0 to 40 was 7.4 per 100,000 between 2012 and 2016. This places Iraq in the intermediate group of the WHO Diabetes Mondiale (WHO DiaMond) project classification **(**Almahfoodh et al., 2017). In another study in Karbala city, the incidence rate (12.11/100,000), while the prevalence (44.65/100,000) in 2023 (Oleiwi et al., 2023).

1.1.3. Clinical Presentation and Complications of Type 1 Diabetes Mellitus

Classic presentation occurs as a gradual onset of the classic triad (polyuria, polydipsia, polyphagia) along with weight loss, fatigue, weakness, and blurred vision. Silent Presentation occurs in some individuals who may have minimal or no symptoms, leading to delayed diagnosis and potential for complications. Diabetic Ketoacidosis is a life-threatening complication characterized by severe hyperglycemia, dehydration, and the buildup of ketones in the blood, often requiring immediate medical attention (Chiang et al., 2018).

T1D chronic complications are divided into two categories: macrovascular and microvascular. Microvascular complications occur in prepubertal adolescents with diabetes who have had the condition for one to two years, diabetic retinopathy, neuropathy, and nephropathy are uncommon. While nerve damage is less common in those with diabetes who have had the disease for 1-2 years, these problems may begin to manifest throughout adolescence or after 5–10 years of living with the disease (Marcovecchio, 2020).

While macrovascular complications remain a primary source of morbidity and mortality in T1D patients. Dyslipidemia, cardiovascular diseases and hypertension are also key risk aspects. Other long-term complications are diabetic foot, gastroparesis, skin disorders, increased risk of autoimmune diseases (Maahs et al., 2010).

1.1.4. Diagnosis of Type 1 Diabetes Mellitus

T1D is confirmed using laboratory testing that assesses blood glucose levels. The consensus statement from the ADA and the standards from the Canadian Diabetes Association (CDA) align with the 2006 diabetes diagnostic criteria published by the WHO. These include ≥126 mg/dL (7.0 mmol/L) of plasma glucose measured during fasting on two or more occasions, or ≥200 mg/dL (11.1 mmol/L) of random plasma glucose. The ADA has added Hemoglobin A1c, (HbA1c), as a diagnostic criterion for T1D and as a measure of how well treatments are working and how hyperglycemia is being managed. The certified and standardized value is 48 mmol/mol or ≥ 6.5% (Chiang et al., 2018).

Capillary Whole Blood Glucose Monitoring Technological advancements have enabled the measurement of blood glucose levels with a drop of capillary blood. Additional Examinations might involve checking urine for ketones and running antibody tests to identify any reactions, against cells and C-peptide (Atkinson et al., 2013).

The existence of autoantibodies against β-cell autoantigens distinguishes T1D from T2D. Elevated islets cell antibodies like Insulin-reactive autoantibodies (IAA), insulinoma-associated autoantigen 2 (IA2A), glutamic acid decarboxylase 65 (GAD65) and zinc transporter 8 (ZnT8A) are a marker to autoimmunity and thus support a diagnosis of autoimmune diabetes. Genetic testing for HLA class II detection is crucial for accurate diagnosis (Kumar et al., 2020).

1.1.5. Pathophysiology of Type 1 Diabetes Mellitus

There is a complex interplay between genetic, epigenetic, and environmental factors in the development of T1D. The body's immune system mistakenly identifies and attacks the insulin-producing pancreatic β-cells perceiving them as harmful. This involves two key players of the immune system: humoral immunity and cell-mediated immunity (Del Chierico et al., 2022).

Humoral immunity produce antibodies attacking β-cells. In T1D, these autoantibodies are hallmarks of T1D and target Insulin, GAD65, IA2A, and ZnT8A, marking them for destruction by immune cells like natural killer cells. These will in turn trigger the complement system, a cascade of proteins that can damage cell membranes, leading to β-cell death. Their presence, often detectable years before symptoms arise, can predict the development of T1D **(**Quattrin et al., 2023).

The cell-mediated immunity involves T-cell activation. In T1D the antigen-presenting cells (APCs) may present beta cell antigens in a manner triggering an immune response by activating Cytotoxic T cells (Ardestani, 2018).

Once activated, these T cells release pro-inflammatory cytokines (Interferon-gamma, Tumor Necrosis Factor-alpha, Interleukin-1 beta) that amplify the immune response and directly attack the β-cells, injecting them with toxic substances that lead to their demise (James et al., 2023).

The risk of developing T1D is heavily influenced by the genetic factors that are located within the HLA region on chromosome 6 (6p21.3). HLA gene regions are divided into two categories based on the functions of their products. Class I HLA molecules are associated with antigens found on cells, recognized by T lymphocyte receptors (CD8+) which target and eliminate the antigen. On the other hand Class II HLA molecules are only found on cells and play a crucial role, in aiding T helper lymphocytes (CD4+) in identifying antigens (Paschou et al., 2018).

The Major histocompatibility Complex (MHC) on chromosome 6, which includes the protective DR2-DQ6 allele and the risk-raising DR3-DQ2 and DR4-DQ8 alleles, is the main genetic component influencing the risk of T1D. Despite this family risk, merely 10% to 20% of those with type 1 diabetes have a relative who also has the disease (Pociot et al., 2002; Zaccardi et al., 2015).

1.1.6. Risk Factors of Chronic Hyperglycemia in Type 1 Diabetes Mellitus

Numerous factors, including as autoimmune diseases, viral infections, HLA gene variations, and family history, can affect the development of T1D. With peak periods in early childhood and adolescence, the disease is most frequently diagnosed in children, adolescents, and young adults. The risk is also influenced by factors such as gut microbiota, stress and psychiatric issues, seasonality, ethnicity, geography, and characteristics related to pregnancy and motherhood (Paschou et al., 2018).

In people with T1D, chronic hyperglycemia can result from a variety of factors, including missed or inadequate insulin doses, dietary miscalculations, illness, physical inactivity, lipodystrophy, sex and growth hormones, or genetic polymorphisms in the insulin receptor (Paschou et al., 2018; Masserenti et al., 2022).

1.1.7. Management of Type 1 Diabetes Mellitus

A multidisciplinary team of physicians, diabetes educators, nurses, dieticians, social workers, psychologists, the patient's family and support systems, must work closely together to manage T1D. To prevent severe hyperglycemia, ketoacidosis and to preserve normal growth. The goal is to encourage healthy living, glycemic control, and near-normalization of blood glucose levels and HbA1c readings (Katsarou et al., 2017).

1.1.7.1. Non-Pharmacological Management of Type 1 Diabetes Mellitus

One essential component of managing T1D is self-monitoring of blood glucose. Many glucose meters contain memory storage that may hold several hundred readings, making them accurate, compact, and portable devices. Many people check in eight or ten times a day, often more frequently during athletic events, illnesses, or times of metabolic instability. The diabetes team should see patients and their families approximately every three months. HbA1c is measured typically every three months, and is an objective measure of the average blood glucose concentration (Holt et al., 2021).

Children should be specifically encouraged to exercise for 30 to 60 minutes, at least five times a week. A fundamental component of diabetes education for all children with diabetes should include comprehensive teaching in carbohydrate counting. Assessment and periodic monitoring of emotional health, on an annual basis, is recommended. Diabetes-specific emotional distress affects 20–40% of people with T1D (Cooke et al., 2008; Blonde et al., 2022).

Whole organ pancreas transplantation is performed simultaneously with a kidney transplant. This is the "gold-standard" therapy for people with T1D and pre-final or end-stage renal disease if no contraindications are present (Holt et al., 2021).

1.1.7.2. Pharmacological Management of Type 1 Diabetes Mellitus

Numerous daily injections of recombinant human insulin formulations is mandatori in T1D. Many Insulin analogs have largely been used currently as a combination of long-acting or intermediate-acting (basal) and short-acting (bolus) insulin (Katsarou et al., 2017).

Amylin, a hormone typically secreted alongside insulin and known to reduce glucagon secretion, which has shown potential as an adjunct treatment for T2D by enhancing insulin response, are currently under investigation as potential treatments for individuals with T1D (Haller et al., 2005; Blonde et al., 2022). Pramlintide is the only non-insulin medication that has been approved for improving glycemic control in T1D patients (DiMeglio et al., 2018).

1.2. Exogenous Insulin Therapy

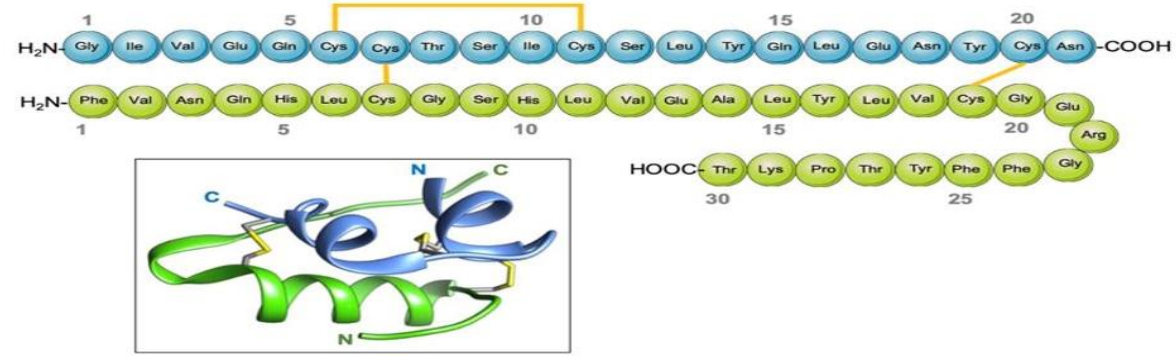
In 1921, Frederick Banting and Charles Best isolated insulin from a dog's pancreas, proving it could lower blood sugar. In 1922, Leonard Thompson became the first human successfully treated with insulin. By 1923, Eli Lilly & Co. partnered with the University of Toronto to produce insulin globally (Gerstein et al., 2021). Exogenous insulin has the same basic protein structure as endogenous insulin and is chemically equivalent to endogenous insulin. The endogenous insulin molecule consists of 51 amino acids arranged in two chains, an A chain (21 amino acids) and a B chain (30 amino acids) that are linked by disulfide bonds as shown in Figure (1-1A) (Alyas et al., 2021).

Currently, recombinant DNA technology is employed to produce human exogenous insulin and its analogs instead of relying on animal-derived insulin. This shift is primarily due to the enhanced immunogenicity and receptor affinity associated with recombinant insulin. Among animal-derived insulins, porcine insulin is recognized as the most structurally similar to human insulin and exhibits the lowest immunogenic response, as illustrated in Figure (1-1B) (Mo et al., 2014).

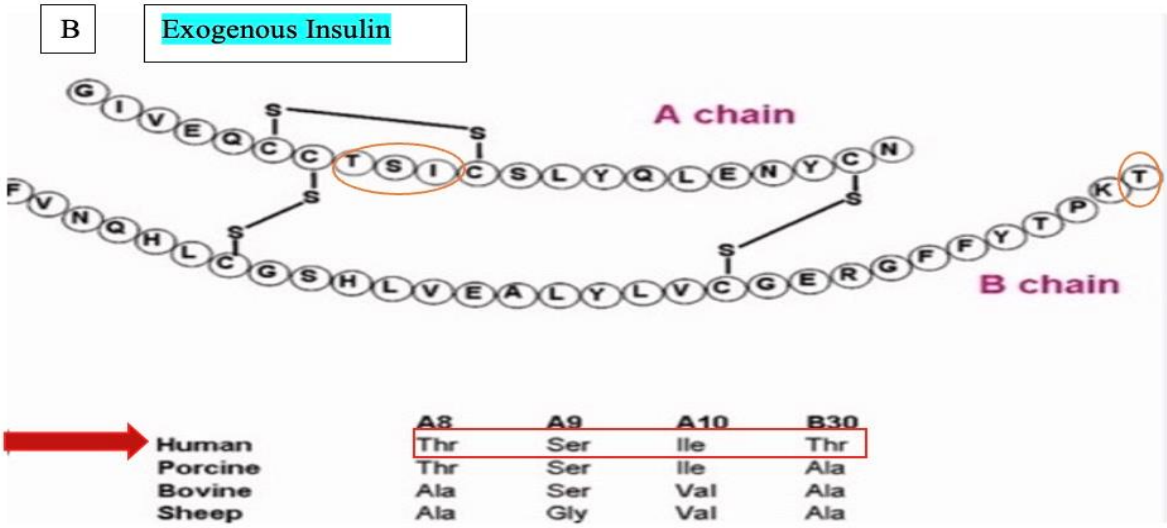
Insulin analogs are slightly modified forms of human insulin designed to alter their absorption rate and duration of action. Based on their onset, peak, and duration

of action, insulin analogs can be categorized into rapid-acting, short-acting, intermediate-acting, or long-acting types (Donner et al., 2023). In the current study, patients administered insulin via pens or vials through subcutaneous injection. Insulin initiates its action by binding to the insulin receptor at the cell membrane. This leads to a diverse range of effects in different target cells, primarily in muscle, liver, and fat. Since insulin is broken down in the gastrointestinal system, it is mainly administered via subcutaneous injection (Atkin et al., 2015).

A Endogenous Insulin



B Exogenous Insulin



	A8	A9	A10	B30
Human	Thr	Ser	Ile	Thr
Porcine	Thr	Ser	Ile	Ala
Bovine	Ala	Ser	Val	Ala
Sheep	Ala	Gly	Val	Ala

Figure (1-1): The Structure of Endogenous Insulin Compared to Exogenous Insulin. Shown in Figure (A) is the primary structure and the three-dimensional structure of endogenous insulin monomer molecule consisiting of A- and B-chains linked by disulfide bonds. The exogenous human insulin in Figure (B), which is structurally identical to endogenous insulin but produced with recombinant DNA technology. The amino acid differences in porcine, bovine and sheep insulin are highlighted relative to exogenous insulin. Porcine insulin differs by one amino acid position B30. Bovine insulin has three differences at A8, A10, and B30. Finally sheep insulin typically differs by all four residues (Mo et al., 2014; Alyas et al., 2021).

1.2.1. The Cellular Effects of Exogenous Insulin

The effects of exogenous Insulin can be categorized into four distinct groups:

- Transportation Process: stimulation of membrane transport processes (glucose, ions, amino acids) via receptors, transporters, or ion channels.
- Anabolic effects: such as stimulating protein synthesis glycogen synthesis, and lipogenesis.
- It has anticatabolic effects: preventing the breakdown of fats, proteins, and glycogen.
- Growth-promoting effects: Insulin's effects take place rapidly (within seconds to minutes), including the promotion of ion and glucose transport, generation of mediators, stimulation of phospholipid turnover, and regulation of enzymes like glycogen synthase, pyruvate dehydrogenase, triacylglycerol lipase, and phosphorylase. Additionally, insulin triggers slower processes (spanning minutes to hours) such as the activation of amino acid transport, and the synthesis of proteins, lipids, RNA, and DNA (DiMeglio et al., 2018; Santoro et al., 2021).

1.2.2.Exogenous Insulin Side Effects

The primary side effect of insulin is hypoglycemia. A major barrier to the widespread use of rigorous diabetic control is fear of hypoglycemia. This is frequent and can result in abrupt cardiac death or brain damage if it gets too threatening. A sugary beverage or snack is recommended as a treatment for hypoglycemia (Atkinson et al., 2013; Roy et al., 2020).

Insulin-induced hypoglycemia can be followed by rebound hyperglycemia, due to the release of counter-regulatory hormones. Moreover, insulin can cause significant side effects such as weight gain, lipodystrophy, and even hypokalemia when taken in high doses (Donner et al., 2023).

1.2.3. Exogenous Insulin Regimen

For blood sugar levels to remain stable, insulin therapy regimens that closely resemble the body's normal insulin release pattern are essential. Split-mix, Basal-bolus and premixed insulin regimen are the main regimens used in human insulin therapy which are tailored to replicate natural insulin secretion and meet individual glycemic needs (Atkinson et al., 2013; DiMeglio et al., 2018).

In the current study, patients have been on a basal-bolus regimen. Typically, 40–50% of the daily dosage is given as basal insulin and 50–60% is given as bolus insulin, this regimen is referred to as a basal-bolus regimen. Using regular insulin or a rapid-acting analog three to four times a day (referred to as "bolus" insulin) and once or twice a day injections of a long-acting insulin known as "basal" insulin, which controls hepatic gluconeogenesis during fasting (blonde et al., 2022).

1.2.4. Exogenous Insulin Pharmacokinetics

Various subcutaneous insulin formulations are available on the market. These formulations vary in their rates of absorption and duration of action. Factors like physical activity, blood flow, temperature, lipodystrophy, and individual patient characteristics can also influence insulin absorption and action (Pey Wen et al.,2016; Pitt et al., 2020). Once absorbed insulin travels through the bloodstream and attaches to the insulin receptors on target like the liver, muscle, and adipose tissue (Mazucanti et al 2022).

The liver is the primary site of insulin metabolism. About 50–60% of Insulin is cleared by the liver. Insulinase enzymes (insulin-degrading enzymes) deactivate insulin by cleaving peptide bonds in the insulin molecule, reducing its bioactivity (Donner et al.,2023). The kidneys are the second major organ involved in insulin metabolism. They clear approximately 30–40% of the insulin (Jayakrishnapillai et al., 2017). A small amount may be eliminated in the feces (Liu et al., 2022).

Pre-prandial Insulin includes short-acting Insulin (Actrapid) with an onset of 30 minutes and peak of 2 to 4 hours and a duration of action of 6 to 8 hours, whereas rapid-acting (Aspart, glulisine, lispro) has an onset of 10 to 20 minutes and a peak of 1 to 2 hours and a duration of action of 3 to 5 hours **(**Pitt et al., 2020).

Intermediate-acting insulin Neutral Protamine Hagedorn (NPH) has an onset of 1 to 2 hours and a peak effect of 4 to 8 hours, with a duration of action of 12 to 18 hours. On the other hand, long-acting insulin (detemir, glargine), and ultra-long acting insulin (Degludec) are peakless and have an onset of 1 to 2 hours and a duration of action of 24 hours, and for ultra-long acting insulin up to 42 hours (Donner et al., 2023).

1.2.5. Exogenous Insulin Pharmacodynamics

1.2.5.1. Binding of Exogenous Insulin

The kinetics of insulin binding to its receptor has been thoroughly examined for more than 40 years using radioligands. Recent high-quality studies have suggested that insulin's high binding affinity to the receptor occurs through the interaction of its two potential insulin-binding sites, found on the two α-subunit of the receptor's dimers. Each α-subunit contains two distinct regions, called site 1 and site 2 that interact with insulin (Scapin et al., 2018).

For a proper approximation of sites 1 and 2' on protomer A and 1' and 2 on protomer B, the two α-subunits of the receptor need to exhibit antiparallel symmetry. In terms of structure, the INSR dimer has four potential binding regions due to its antiparralel symmetry. But in function, two binding sites exist at which only one insulin molecule binds per receptor dimer, bridging two domains to activate the receptor (Weis et al., 2018).

Sites 1 and 1’ are formed by the L1 domain of one INSR protomer, α-CT’and α-CT complex of the other promoter. Meanwhile, sites 2 and 2’ are located on the back of the FnIII-1 domain of each protomer at which both of them bind insulin with high affinity (Gutmann et al., 2019; Uchikawa et al., 2019).

The most remarkable result of such experiments was the finding that A-chain residues A12, A13, A15, A17 as well as B-chain residues B16 of insulin crosslinked to very distant epitopes of the FnIII-1’complex of each promoter on the receptor α-subunit, suggesting that the binding at the α-subunit creates high binding affinity to insulin and slows down the dissociation rate (De Meyts, 2015; De Meyts, 2016).

1.2.5.2. Mechanism of Action of Exogenous Insulin in Type 1 Diabetes Mellitus

Target cells have tyrosine kinase receptors on their surface that insulin binds to. Insulin binds to the insulin receptor in peripheral tissues, stimulating receptor activation followed by intracellular signaling cascades. Upon dimerization of two receptors, the initial step in signal transduction is autophosphorylation of tyrosine residues on the receptor endodomains. This allows each receptor in the dimer to phosphorylate the other, leading to conformational changes in the insulin receptor, as shown in Figure (1-2) (Yunn et al., 2023).

After insulin binds and triggers the initial phosphorylation of the insulin receptor, the phosphorylated activation loop is detached from the catalytic site. This detachment results in an increase in insulin receptor kinase activity, as it allows for interactions between the kinase, substrate, and ATP (Adenosine Triphosate). The downstream molecules, a family of proteins known as Insulin Receptor Substrates (IRS), use active Tyrosine (Tyr) residues as docking sites (Rahman et al., 2021).

Insulin receptor activation regulates two main signaling pathways: the Phosphoinositide 3-Kinase/Protein Kinase B (PI3K/AKT) and Mitogen-Activated Protein Kinase (MAPK) pathways. The PI3K/AKT pathway, is primarily responsible for the majority of the metabolic effects of insulin in peripheral tissues. Furthermore, IRS proteins engage with the regulatory subunit of PI3K, triggering PI3K to generate the second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3). PIP3 then brings PDK1 to the cell membrane, activating AKT serine/threonine kinase. This, in turn, leads to the phosphorylation of different proteins, ultimately resulting in the uptake of glucose by glucose transporter type 4 (GLUT-4) glycogen synthesis, and regulation of fat and protein metabolism (De Meyts, 2016).

The MAPK pathway plays a vital role in controlling cell proliferation, and cell growth. While both Src Homology 2 Domain (SHC) and IRS initiate the MAPK pathway, SHC is the primary initiator. When stimulated by insulin, Extracellular Signal Regulated Kinase (ERK) activates multiple nuclear transcription factors, leading to the expression of genes associated with intermediate metabolism and cell growth (White MF et al., 2021).

To prevent overactivity, the insulin signal is tightly controlled. The receptor itself can be internalized and degraded, and enzymes called phosphatases remove the phosphate groups, effectively turning off the signal **(**Santoro et al., 2021).

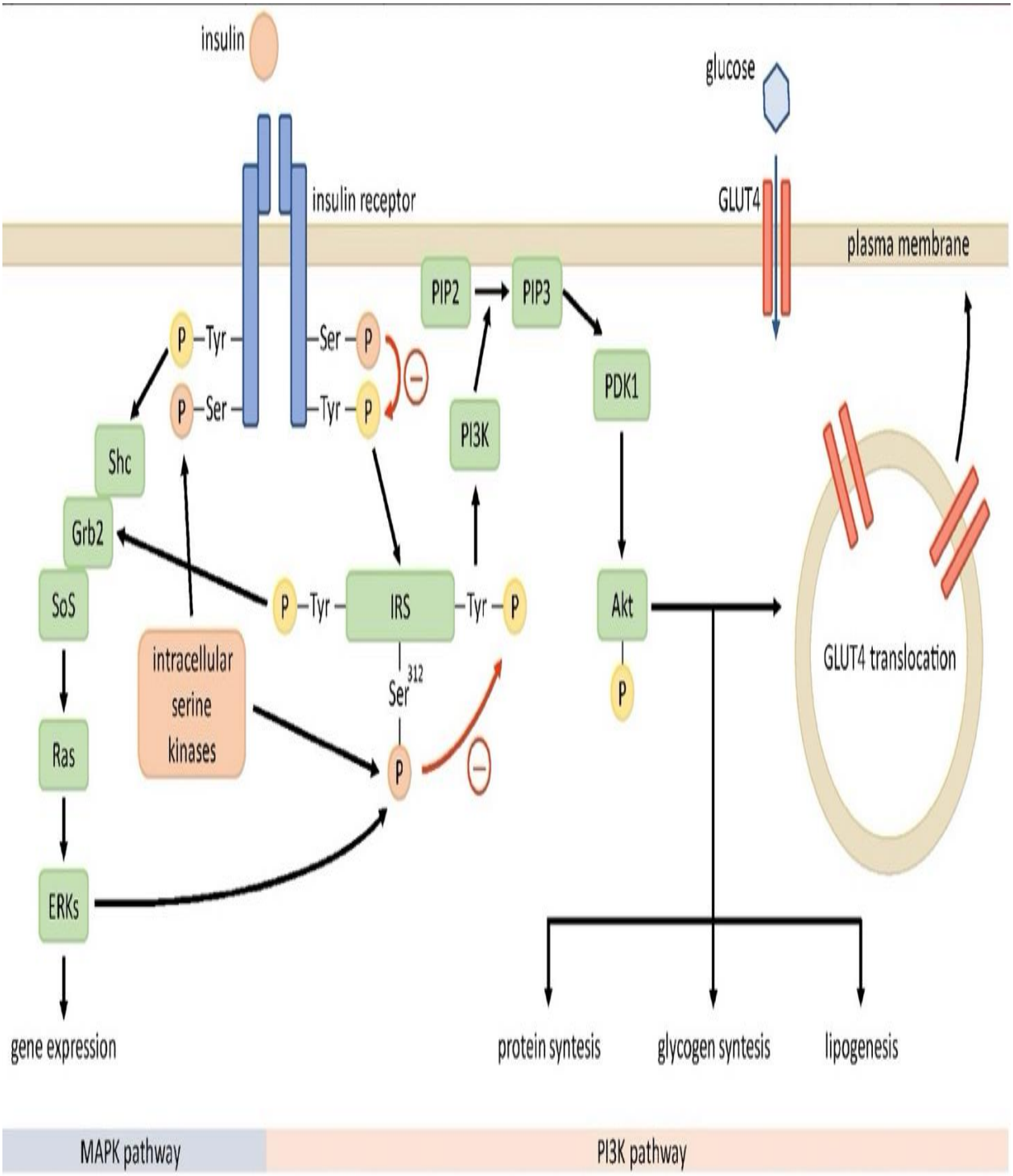


Figure (1-2): Mechanism of Action of Exogenous Insulin. Insulin triggers two main signaling pathways (MAPK and PI3K/AKT) upon activating the INSR (Saltiel, 2021).

1.3. The Insulin Receptor *(INSR)* Gene

The membrane of the majority of human cells contains the insulin receptor, a crucial controller of glucose metabolism. This receptor belongs to the class of receptor Tyrosine Kinases and is encoded by the *INSR* gene. The INSR gene's transcription takes place on chromosome 19 (19p13.2) as illustrated in Figure (1-3A). It is located on the short arm of chromosome 19 and the gene spans over 120 kb consisting of 22 exons and 21 introns (Huang, et al., 2019).

The short exon 11 is alternatively spliced, resulting in two receptor isoforms that differ slightly in affinity for insulin, The first structures of the insulin receptor domains represent a breakthrough in insulin research. The receptor comprises several critical subdomains and two receptor isoforms, the α-subunit and, the β-subunit. Both subunits serve as docking sites for downstream signaling molecules, they collectively facilitate insulin recognition and modulate receptor activity (Rojek et al., 2014; De Meyts, 2016).

The α-subunit ectodomain of the insulin receptor consists of several structural domains, including fibronectin type III domain (FnIII-1), as illustrated in Figure (1-3B), which is essential for receptor dimerization and high-affinity insulin binding. The β-subunit extends from the extracellular domain across the transmembrane region and into the intracellular portion of the insulin receptor, which is involved in receptor internalization, signal regulation (Uchikawa et al., 2019).

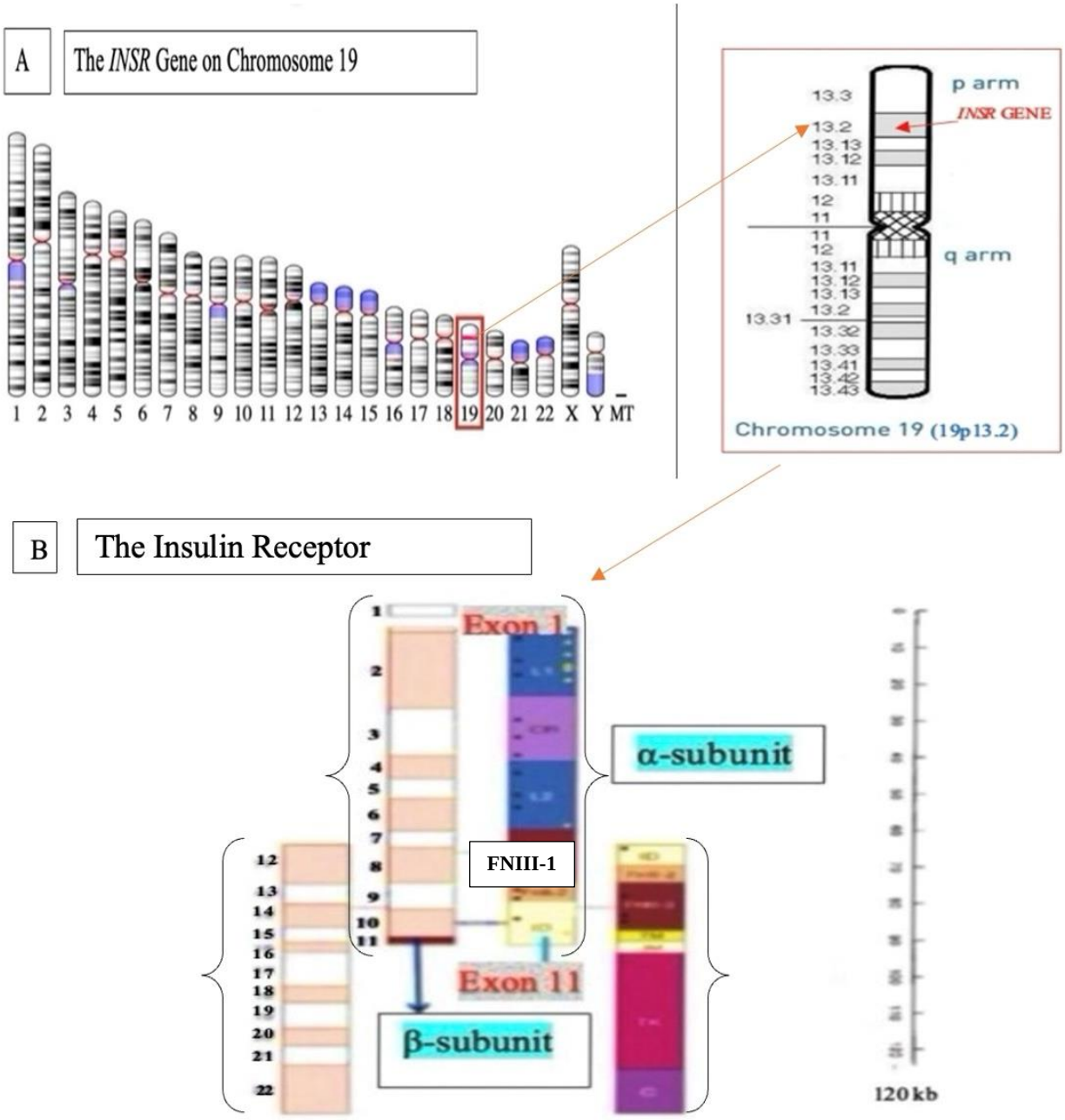


Figure (1-3): The Insulin Receptor Gene Locus and the Insulin Receptor Structure. Figure A shows the *INSR* gene on the short arm of chromosome 19 (19p13.2). Figure B illustrates two receptor isoforms of the insulin receptor which is encoded by the *INSR* gene, the extracellular α-subunit domain (from exon 1 to 11), which mediates ligand binding. The transmembrane along with the intracellular domain belong to the β-subunit (from exon 12 to 22), which mediates downstream signaling pathway (Grimwood et al., 2004; Shimojima et al., 2015; De Meyts, 2016).

Many previous excellent studies reported that the crystal structure of the extracellular domain of the INSR, including the FnIII-1 domains, was found to have an inverted ‘V’ shaped architecture in the absence of insulin, as illustrated in Figure (1-4B). The ectodomain contains key residues (FnIII-1) that directly interact with insulin molecules (Rojek et Al., 2014; Gutmann et al., 2019).

These interactions are essential for stabilizing the INSR complex and initiating downstream signaling events. Upon binding the Insulin to the ectodomain, insulin generates a conformational change in the INSR that brings the two α-subunits closer together (Uchikawa et al., 2019).

The vertical piece of the ‘T’ includes the FnIII-1 domain of the INSR dimer too as shown in Figure (1-4 C). These studies demonstrated that when insulin binds, a significant conformational change occurs in the INSR, transforming the overall architecture of the INSR dimer from an autoinhibited inverted ‘V’ shape to a ‘T’ shape (Weis et al., 2018, Gutmann et al 2018).

This transformation is believed to bring the two kinase domains of the INSR dimer closer together, facilitating efficient trans-autophosphorylation and activation (Scapin et al., 2018).

Understanding the precise binding mechanism of insulin molecules on the α-subunit of the insulin receptor is crucial for elucidating the intracellular signal transduction pathways (Payankaulam et al., 2019).

They coordinate the regulation of glucose transport, glycogen storage, and protein synthesis, while also influencing insulin responsiveness in accordance with variations in circulating glucose concentrations (White MF et al., 2021)

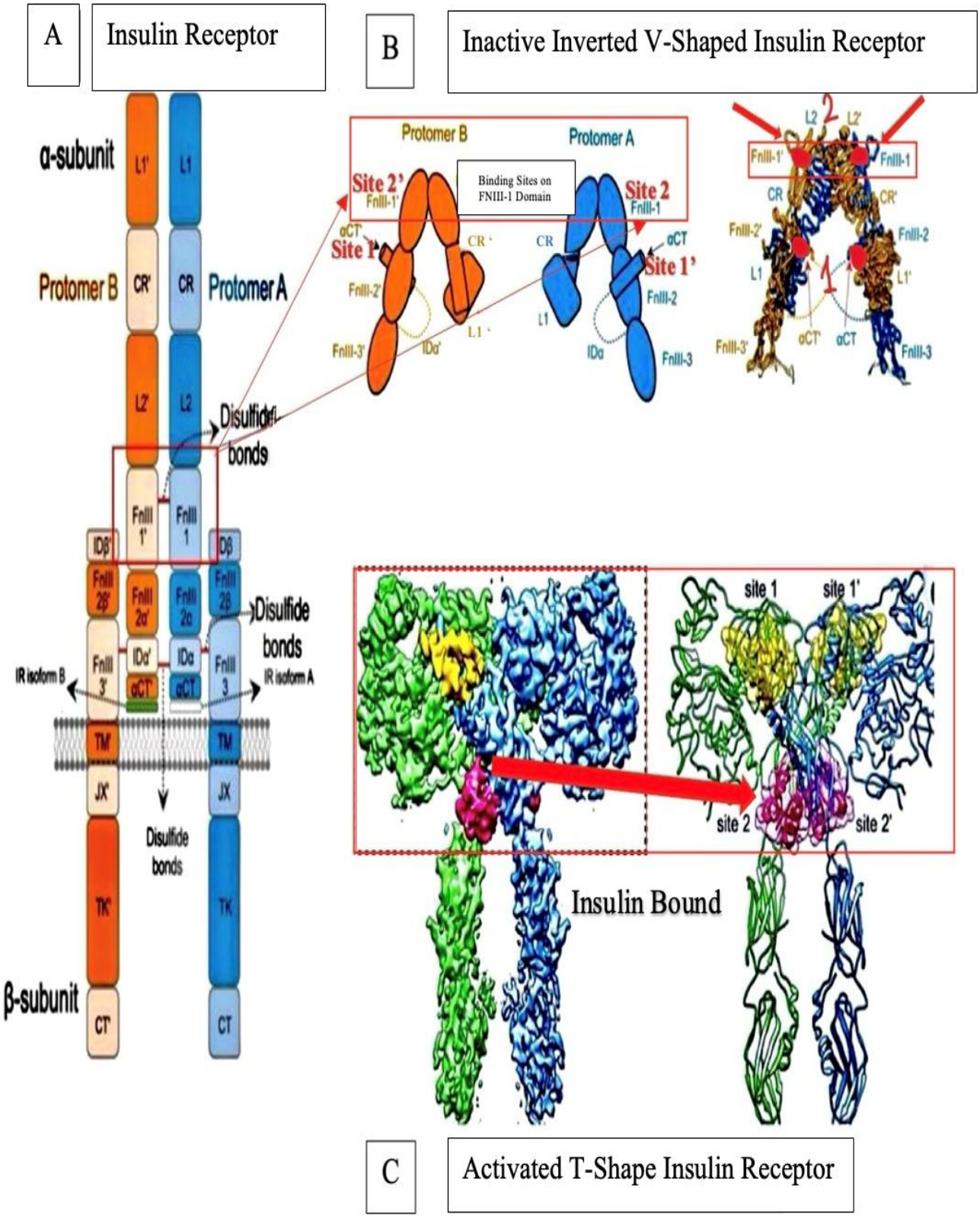

A
Insulin Receptor
B
Inactive Inverted V-Shaped Insulin Receptor
α-subunit
Protomer B
Protomer A
Disulfide bonds
Binding Sites on FNIII-1 Domain
Site 2'
Site 1
Site 2
Site 1'
Disulfide bonds
IR isoform B
IR isoform A
Disulfide bonds
β-subunit
site 1
site 1'
site 2
site 2'
Insulin Bound
C
Activated T-Shape Insulin Receptor

Figure (1-4): Structural Transition of the Insulin Receptor Ectodomain upon Insulin Binding. The FNIII-1 domain of the insulin receptor is presented in Figure (A). The extracellular domain adopts an autoinhibited inverted 'V' shape, illustrated in Figure (B). Insulin binding induces a conformational change to a 'T'-shaped dimer, facilitating activation by bringing the intracellular kinase domains closer together. The horizontal arm of the 'T' consists of four structural binding sites and two functional binding sites, as shown in Figure (C) (Uchikawa et al., 2019; Yunn et al., 2023).

1.3.1. Pathophysiological Significance of Genetic Polymorphisms in the Insulin Receptor Gene

Every individual carries two copies of each gene which may not be the same throughout the population. Approximately 99.9% of nucleotide bases are exactly the same in all people however, the remaining 0.1% account for about 1.4 million individual-specific differences occuring in humans (Teng et al., 2008; Dvornikova et al., 2020).

One of the reasons for human diversity is these scattered single nucleotide polymorphisms (SNPs) that tend to occur in humans every 300–2000 base pairs along the genome, and is found in at least 1 % of the population. These genetic differences can affect how people develop certain diseases, respond to drugs, or display specific physical characteristics (Iffath et al., 2022). They are stable in the sense that they do not change during the life course of an individual and have the potential to be passed on to offspring (Al-Kashwan et al., 2021).

SNPs are categorized into different classes based on their impact on the genetic code and resulting proteins. These classes include synonymous SNPs, nonsynonymous SNPs, and SNPs in non-coding regions (Simeon I et al., 2002; Melkersson et al., 2023).

Many of these polymorphisms are specifically related to mutations in the gene coding for the INSR suggesting a connection with various diseases (Nobakht et al.,

2020; Atoum et al., 2022). These SNPs impact insulin sensitivity on cell surfaces in several ways; by reducing the amount of insulin receptor production, affecting the transportation of existing receptors within cells' surfaces, or speeding the breakdown of current receptors. Specific mutations affect how well the receptor binds to insulin, decreasing its affinity or hindering tyrosine kinase activity, which impairs the receptor's ability to send signals across the membranes. As a result, those who carry the mutation in both copies might have an average level of insulin sensitivity. This suggests that some T1D patients may acquire low insulin sensitivity as a result of homozygosity for mutations in the *INSR* gene (Simeon I et al., 1992; Simeon I et al., 2002; Omar et al., 2021).

Specific SNPs in the α-subunit of the *INSR* gene can lead to significant consequences on the amino acid sequences and subsequently impact the structure and the function of the insulin receptor (IR), which will lead to altered functionality or impaired signaling. Decreased levels of *INSR* gene expression have been documented in people with chronic hyperglycemia. Additionally, decrease in insulin affinity may be shown if the alteration occurs in the α-chain leading to hyperglycemia in T1D (Melkersson et al., 2023).

Mutations that affect the α-subunit of the *INSR* gene can result in health issues related to compromised insulin binding and signaling. These genetic changes impact how the insulin receptor is structured and functions causing problems with glucose absorption and utilization, as shown in Table (1-1) (Massarenti et al., 2022; Melkersson et al., 2023).

Table (1-1): Pathophysiological Significance of Genetic Polymorphisms in the α-Subunit of the *INSR* Gene. This table illustrates various variant locations in the INSR α-subunit compromising over different SNPs and their clinical significance (Al-Gazali et al., 1993; Godarzi et al., 2011; Ardon et al., 2014; Hu et al., 2017; Daghestani et al., 2020; Masserenti et al., 2022).

Variant location	SNP *INSR* Gene	Allele	Gene Consequence	Clinical significance	References
Exon 2	(rs3745546)	G>C	Intron variant	less platinum-based chemotherapy sensitivity in Epithelial ovarian cancer patients	(Hu et al., 2017) (Al-Gazali et al., 1993)
	(rs52836744) (rs121913155)	G>A T>A	Missense Stop gained	Donohue syndrome	(Ardon et al., 2014)
Exon 3	(rs12191314)	T>C	Missense variant	Donohue syndrome	(Ardon et al., 2014)
Exon 5	(rs121913142)	T>G	Missense variant	Diabetes Mellitus,Insu-lin Resistance	(Ardon et al., 2014)
Exon 6	(rs121913160)	T>C	Missense variant	Donohue syndrome	(Ardon et al., 2014)
Exon 8	(rs2229429)	G>A	Synonymous Variant	Insulin resistance	(Massarenti et al., 2022) (Daghestani et al., 2020)
Intron 8	(rs2245649)	T>C	Intron variant	Decreased insulin sensitivity Diabetic patients PCOS	(Massarenti et al., 2022) (Daghestani et al., 2020)
Exon 11	(rs225267)	C>G	Intron variant	PCOS	(Goodarzi et al., 2011)

1.3.2. Impact of Insulin Receptor Gene Polymorphisms on Exogenous Insulin

The impact of the *INSR* gene in insulin signaling and glucose metabolism is crucial with genetic variations affecting insulin sensitivity, structure or receptor function (Malodobra et al., 2011). These alterations might influence how insulin modulate receptor expression levels, binds to its receptor, or disrupt downstream signaling pathways. This in turn influences the efficacy of exogenous insulin in achieving glycemic control leading to chronic hyperglycemia (Gu et al., 2018).

Furthermore, another study identified multiple SNPs, (rs2245649) T>C and (rs2229429) G>A within the *INSR* gene that were linked to insulin resistance and in individuals with T1D (Masserenti et al., 2022). These findings suggest that genetic variations in the *INSR* gene can impact INSR function and play a role in the development and management of hyperglycemia in individuals with T1D (Pociot et al., 2017).

Changes in the nucleotide sequence of (rs2229429) G>A, a synonymous benign variant in the coding region might alter the mRNA's secondary structure due to nucleotide variations. This can reduce the stability of the transcript, potentially affecting the level of protein expression. As a result, this may lead to the production of misfolded or malfunctioning proteins. Such defective proteins are often degraded rapidly and, potentially, influence the duration at which the IR remains intact before degradation. Moreover, misfolded proteins fail to function correctly and ultimately, impair insulin binding (Masserenti et al., 2022; Vihinen, 2022).

Conversely, the intronic SNP (rs2245649) T>C, located in intron 8 of the non-coding region at binding site 2 of the *INSR* gene, does not directly code for protein. However, introns can play important regulatory roles, and this particular variant may significantly reduce gene expression and affect mRNA processing. Such disruptions can impair insulin binding. Studies have shown that the presence of this SNP lowers the receptor's affinity for insulin (Tayler et al., 1992; Vihinen, 2022).

It is found that these specific SNPs in the *INSR* gene were significantly associated with higher fasting blood glucose levels in patients with T1D. To investigate this, patients with T1D and healthy controls were genotyped for two prevalent SNPs in the α-subunit encoding part of *INSR* gene, namely (rs2229429) G>A and (rs2245649) T>C. Subsequently, an evaluation of these SNPs has been performed to explore whether they are linked to the circulating levels of HbA1c or sIR levels in patients who are taking exogenous insulin (Masserenti et al., 2022).

1.4. Rationale of Pharmacogenetic Studies

Around 25-60% of patients exhibit distinct pharmacological reactions to a specific medication. This is referred to as interindividual variability, which influences the efficacy of drugs and the occurrence of side effects in individuals (Sánchez-Pozos et al., 2021).

Pharmacogenetics is an approach in which molecular biomarkers such as SNPs determine expression levels of drug receptors, transporters, metabolic enzymes, ion channels, and others, which might change drug pharmacokinetics and pharmacodynamics leading to low efficacy or adverse drug reactions with negative consequences for patients (Venkatachalapathy et al., 2021).

As additional genes and receptor sequences related to drug response are discovered, the application of advanced, high-throughput DNA testing techniques will facilitate the early identification of numerous genetic variations. This advancement could enable drug dosages to be tailored either prior to or shortly after starting treatment, thereby enhancing the effectiveness of pharmacotherapy and minimizing the risk of adverse effects. Therefore, this study aims to gather existing data on genetic variations in pharmacogenes that influence drug response in T1D, and to integrate this information with their effects on gene expression in order to clarify their contribution to variability in drug efficacy (Iffath et al., 2022).

1.5. Aims of the Study

This study is intended to investigate the following:

1. The distribution of different genotypes of INSR (rs2245649) T>C and INSR (rs2229429) G>A among Iraqi patients with T1D.
2. The impact of genetic polymorphisms of INSR (rs2245649) T>C and (rs2229429) G>A on exogenous insulin-treated patients with T1D.

Chapter Two

Patients, Materials and Methods

2.Patients, Materials, and Methods

2.1. Patients Selection

The cohort includes ninety-nine individuals diagnosed with T1D, fifty-six females and forty-three males, all aged between five and seventeen years. Each participant had been receiving exogenous insulin via a basal-bolus regimen for at least six months prior to the study. Thirty control patients from the same age category have been enrolled in this study. They were recruited during their visits to the Al Hassan Metabolism, Endocrine, and Diabetes Centre in Kerbala; Imam Alhassan AL-Mojtaba Teaching Hospital in Kerbala; Al-Marjan Hospital in Babylon; and Al-Zahraa Hospital in Najaf City.

2.1.1. Patient's Criteria

2.1.1.A. Inclusion Criteria:

Patients included in this study have the following criteria:

- Male and female patients ranging from five to seventeen years old.
- They had previously been diagnosed with T1D for over a year
- All patients with T1D having poor glycemic control (HbA1c $\geq$ 9)
- Used exogenous basal-bolus insulin regimen
- Taking a daily dose of exogenous insulin not exceeding 1 IU/kg for a minimum of six months
- They undergo a gene extraction procedure

2.1.1.B. Exclusion criteria:

- ➢ Diabetic patients on antidiabetic agents in addition to insulin
- ➢ Patients on exogenous Insulin for less than 6 months
- ➢ Diabetic patients with T2D
- ➢ Failure in the gene extraction procedure
- ➢ Pregnancy & Pcos
- ➢ Other endocrine disorders
- ➢ Severe hepatic disease
- ➢ Malignancy
- ➢ lipodystrophy
- ➢ Infections
- ➢ Children on steroid therapy
- ➢ Acromegaly and growth retardation
- ➢ Anemia and Thyroid disease

2.1.2. Collection of Clinical Data:

Direct patient information as well as data from medical records have been collected. Patient's caregivers were provided with an informed consent to gather the following data: age, height, weight, duration of T1D, duration of therapy, use of any additional medications, socioeconomic status, physical exercise, family history and type of regimen, insulin dosages.

2.1.3. Ethical Considerations

The research proposal for this study has undergone ethical and scientific review by the scientific and ethical committee of the faculty of pharmacy, University of Kerbala, and received subsequent approval. Additionally, the study has been approved by Kerbala Health Department, Ministry of Health, Iraq, under approval number 2023167 in 8/09/2023. Imam Alhassan Al-Mujtaba Teaching Hospital in Kerbala, Babylon Health Department and Al-Marjan Hospital, the Health Department and Al-Zahraa Hospital in Najaf City, and the Kerbala Health Department and Al-Hassan Metabolism, Endocrine, and Diabetes Centre all gave their approval for this study to be conducted. Patient's parents or caregivers provided written and verbal consent after receivinf a comprehsenive explanation of the study's objectives and voluntarily completed a questionnaire.

2.2 Materials

2.2.1 Instruments, Equipments, and Their Suppliers

The equipment and instruments employed in this study and their respective suppliers are summarized in Table (2-1).

Table (2-1): Equipments, Instruments and their Manufacturing Companies

Equipments, Instruments and their Suppliers		
Clinical chemistry analyzer Monarch 240	Biorex	UK
Deep Freezer	GFL	Germany
Digital camera	Canon	England
Distillator (Water distiller)	Gel	Germany
Electrophoresis Apparatus	Techin me	England
ELISA Microtiter Plate Reader	HumaReader HS	German
H8 HbA1c analyzer	Lifotronic	China
Hood	LabTech	Korea
Hot plate stirrer	Lab Tech	Korea
Incubator	Memmert	Germany
Micropipettes	Slammed	Japan
Microspin Centrifuge	Hittch	Germany
Microwave oven	Biobase	China
Nano Pac 500 power supplier for electrophoresis	Cleaver	UK
Nanodrop	Thermoscientific	USA
PCR machine (Thermocycler)	Veriti	Singapore
Refrigerator	Hitachi	Japan
Sensitive balance	AND	Taiwan
Tube (1.5ml, 0.5ml, and 0.2ml)	JET BIOFIL	Singapore
UV-Trans illuminator	Syngene	England
Vortex mixer	Human Twist	Germany

2.2.2 Chemicals, Kits, and their Suppliers

The chemicals employed in this study and their respective suppliers are summarized in Table (2-2).

Table (2-2): Chemicals and Kits and their Manufacturing Companies

Chemicals, kits and their Suppliers			
	Chemicals and kits	**Supplier**	**Country**
Chemicals	Absolute ethanol	SDI	Iraq
	Agarose	Intron biotechnology	Korea
	Nuclease Free Water	Bioneer	Korea
	TBE BUFFER	Bioneer	Korea
	Ethidium Bromide Solution (10mg/ml)	Intron	Korea
Biochemical kits	Human Insulin Receptor Elisa Kit	Sunlong	China
	Serum Glucose Kit	Mindray	China
	HbA1c kit	Mindray	China
Kits for genetic study	Favorgen Genomic DNA Extraction Kit	Favorgen	Korea
	PCR premix kit	Bioneer	Korea
	DNA ladder marker	Bioneer	Korea
	Primers	Macrogen	Korea
Medication Used	Insulin (soluble and Lenti)	Novo Nordisk	Denmark

2.3. Methods

2.3.1. Study Design

This study was designed as a cross-sectional study Ninety-Nine type 1 diabetic patients, ranging in age from 5 to 17 years old, and thirty control patients, were retained in this study during their visit to the Al-Hassan Metabolism, Endocrine, and Diabetes Center and ALhassan Al-Mojtaba Teaching Hospital in Kerbala and Al-Marjan Hospital in Babylon as well as Alzahraa hospital in Najaf. Those children were previously diagnosed according to diagnostic criteria; they received a daily dose of the exogenous insulin not exceeding 1 IU/kg/day according to their body weight for at least six months, and they followed both inclusive and exclusive criteria. Caregivers of the patients were informed about the study and asked if they would like to take part. This study was conducted between October 2023 and April 2024.

2.3.2. Samples Collection

Venous blood samples of 5 mL were collected from each individual with T1D. The collected blood was divided into two aliquots for different analyses. Three mL of whole blood were transferred into an ethylene diamine tetraacetic acid (EDTA) tube, used for measuring glycated hemoglobin (HbA1c) and for a complete blood count. The remaining whole blood from the EDTA tube has been frozen for genomic deoxyribonucleic acid (DNA) extraction. For biochemical analysis, two mL was been transferred into a clot activator tube and centrifuged at 5000 rpm for 10 minutes for estimating fasting blood sugar (FBS) and random blood sugar (RBS). The remaining serum was separated appropriately in eppendrofs for analysis of serum Insulin Receptor levels. Urine samples were collected to rule out infections.

2.3.3. Biochemical Assay Methods

2.3.3.1. Determination of Glycemic Indices

2.3.3.1.A. Estimation of Fasting Glucose Level

Clinical chemistry analyzer Monarch 240 had been used for. Analysis of FBS and RBS.

Test principle:

The estimation of fasting serum glucose levels was performed using an in vitro quantitative test on photometric systems using a method known as the glucose oxidase-peroxidase method. The principle behind this method lies in the fact that the enzyme glucose oxidase converts glucose to gluconic acid by oxidation while at the same time reducing oxygen to hydrogen peroxide. The enzymatic agent peroxidase is subsequently accountable for the splitting of hydrogen peroxide into water and oxygen in its atomic form. When this newly formed nascent oxygen combines with 4-aminoantipyrine in the presence of phenol, quinoneimine is produced. Quinonimine forms a red–violet dye as indicator that can be quantified spectophotometrically at 505 to 510 nm. The intensity of the resulting color is directly proportional to the glucose concentration in the sample and is compared against a standard processed in the same manner (Gashoki, 2020; Pasaribu et al., 2021).

$$\text{Glucose} + O_2 + H_2O \xrightarrow[\text{(Glucose Oxidase)}]{} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow[\text{(Peroxidase)}]{} \text{quinoneimine} + 4H_2O$$

2.3.3.1.B. Estimation of Random Glucose Level

Serum glucose testing can be done on a patient at any time during the day, regardless of the timing of the last meal. Random glucose levels have been determined using the same method (glucose oxidase-peroxidase method), as for measuring fasting blood glucose levels (Gashoki, 2020; Pasaribu et al., 2021).

2.3.3.1.C. Estimation of Glycosylated Hemoglobin Level

Glycated Hemoglobin (HbA1c) is a form of hemoglobin that has glucose attached to it. HbA1c is widely recognized as a Gold Standard for monitoring diabetes, indicating the average blood glucose concentration over 8-12 weeks. For patients under the age of 18, the International Society for Paediatric and Adolescent Diabetes and the ADA agreed that a HbA1c level of <7.5% is recommended (Atkinson PhD et al., 2013; Katsarou et al., 2017).

High-Performance Liquid Chromatography (HPLC) Methodology for HbA1c measurement has been used by Chromatographic Separation using H8 HbA1c analyzer. HPLC is considered the gold standard for HbA1c measurement due to its excellent accuracy and precision. It can effectively distinguish HbA1c from other hemoglobin variants, reducing interference. HPLC separates HbA1c directly by measuring the absorbance points continually to form a chromatogram (Chauhan, 2017).

Principle:

HPLC separates molecules based on their different chemical properties and how they interact with a stationary phase (packed inside a column) and a mobile phase (a solvent that flows through the column). In HbA1c testing, the HPLC column is designed to separate HbA1c from other types of hemoglobin. As the separated hemoglobin components pass through a detector (usually UV-Vis), they produce

signals proportional to their concentration, and exhibit strong absorption at 415 nm, which displays a deep red to purple color. The HPLC system is calibrated using standard solutions with known HbA1c concentrations. By comparing the peak area or height of the HbA1c in the patient's sample to the calibration standards, the percentage of HbA1c can be accurately determined (Chauhan, 2017).

2.3.3.2. Determination of Human Soluble Insulin receptor (Sunlong Biotech ™, 2023)

The soluble insulin receptor (sIR) is a truncated version of the insulin receptor found on cell membranes. It's released into the bloodstream through enzymatic cleavage, and is believed to modulate glucose metabolism. Altered sIR levels have been associated with conditions like chronic hyperglycemia. sIR might serve as a biomarker indicating insulin resistance or other metabolic conditions (Soluble Insulin Receptor Study Group, 2007; Hiriart et al., 2019).

The determination of human sIR by ELISA kit involves a series of steps to accurately measure the levels serum samples. The enzyme-linked immunosorbent assay (ELISA) Sunlong Human Insulin Receptor kit SL0936Hu is a highly sensitive technique, specifically designed to detect the sIR concentrations in human samples. According to the manufacturer's kit, sIR lab values normal individuals range from 1.6 to 2 ng/mL, however for diabetic patients levels rise to 2-3 ng/mL (Sunlong Biotech, 2023).

Principle:

The ELISA kit uses Sandwish-ELISA as the method. The Microelisa strip plate provided in this kit has been pre-coated with an antibody specific to INSR. Standards or samples are added to the appropriate Microelisa strip plate wells and combined with the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for INSR is added to each Microelisa well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain INSR and HRP-conjugated INSR antibodies will appear blue and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of sIR. A standard curve is constructed by plotting absorbance values against concentrations of insulin receptor standards, and concentrations of unknown samples are determined using this standard curve. Concentrations of sIR can be calculated in the samples by comparing the OD of the samples to the standard curve.

Procedure:

1. After having collected the whole blood, it has been left for 15 minutes.
2. Dilution of the standards by small tubes, then pipetting the volume of 50 µL from each tube to the well, has been performed, each tube uses two wells, a total of ten wells.
3. In the Microelisa strip plate, a well empty as a blank control has been left.
4. In sample wells, 40 µL sample dilution buffer and 10 µL sample were added and mixed well by gentle shaking.
5. After sealing or coating the wells with a closure plate membrane with a capture antibody specific to the sIR. Incubation of the plate has been performed to allow the antibody to adhere to the surface. Incubating was performed for 30 minutes at 37°C.
6. Dilution of the concentrated washing buffer with distilled water was performed.
7. Carefully peeling off the closure plate membrane and wash solution has been added. The wash solution have been discarded after resting for 30 seconds and the washing process has been repeated for 5 minutes.
8. Adding 50 µL of HRP-Conjugate reagent to each well except the blank control well and incubation has been performed for 30 minutes at 37 °C.
9. Refill with the wash solution and let it rest for 30 seconds.The washing step has been repeated five times.
10. Adding 50 µL of stop solution to each well to terminate the reaction. A clear change from blue color to yellow had occurred.
11. Reading the OD after 15 minutes, at 450 nm using a microtiter plate reader (Sunlong Biotech, 2023).

2.3.4. Measurement of Body Mass Index

Body Mass Index (BMI) is a widely used measure to assess an individual's weight in relation to their height and is calculated using the formula: BMI = weight (kg) / height² (m^2). This simple calculation helps categorize individuals as underweight, normal weight, overweight, or obese. According to Rahman et al. (2010), a BMI below 18.5 is considered underweight, 18.5–24.9 is normal weight, 25–29.9 is overweight, and 30 or above is classified as obese. For children and adolescents, BMI interpretation requires age- and gender-specific percentiles based on the Centers for Disease Control and Prevention (CDC) growth charts. In this context, a BMI between the 5th and < 85th percentile is considered normal, between the 85th and <95th percentile indicates overweight, and above or equal to the 95th percentile is categorized as obese (Al-Hamwandi et al., 2021).

2.3.5. Genetic Analysis

2.3.5.1. DNA Extraction (Favorprep™, 2023)

The DNA (deoxyribonucleic acid) extraction was conducted at Mohamed Saleh laboratory of molecular biology in Kerbala City. Several steps were carried out to extract genomic DNA (gDNA) from a specimen of frozen blood employing the Favorprep™ FABG103-100 Blood gDNA extraction mini kit favorgen.

Procedure:

Step 1- Sample preparation

1. The blood specimen that was frozen has been allowed to melt on the bench at room temperature for fifteen minutes. Two mL of blood was spun at 2000 rpm for one minute to separate the buffy coat. The addition of 200 µL of whole blood into a microcentrifuge tube (1.5 mL) was performed.

Step 2- Cell lysis

2. After dispensing 20 µL of proteinase K (PK) solution into each microcentrifuge tube, the contents were quickly mixed by pulse-vortexing for 15 seconds, to ensure thorough blending, and then incubated for 5 minutes at 60 ºC.

3.When adding 200 µL of FABG buffer to the tube, the contents were allowed to vigorously agitate for 15 seconds using a vortex.

4. Then, incubation was performed for 10 minutes in a 60 ºC water bath. (While incubating, inversion of the sample has been performed every 3 minutes.)

5. The elution buffer had been heated in a water bath at 60 ºC for DNA elution.

Step 3- Binding

6. Two-hundred µL of ethanol (96 %) has been added to the sample and vortexed for 10 seconds. Using a pipette to mix the sample well and ensure no precipitate forms. This step is necessary to guarantee DNA binding.

7. A FABG column was added to a collection tube. The sample mixture was transferred carefully to the FABG column. The mixture was then centrifuged at a speed of 14,000 rpm for 1 minute. Dispose of the collection tube and place the FABG column in a new collection tube.

Step 4- Washing

8. The addition of 400 µL of W1 buffer to the FABG column has been performed and centrifuged for 30 seconds at a speed of 14,000 rpm. The flow-through has been discarded and the FABG column was placed in a new collection tube.

9. The addition of 600 µL of wash buffer to the FABG column has been performed and centrifuged for 30 seconds at a speed of 14,000 rpm. The flow-through has been discarded and the FABG column was placed in a new collection tube. (ethanol has been added previously to the wash buffer when first opened).

10. Spinning the centrifuge for an extra 3 minutes was done at a speed of 14,000 rpm to remove any remaining liquid and prevent enzymatic reactions from being hindered.

Step 5- Elution

11.The dry FABG column was then transferred to a new 1.5 mL microcentrifuge tube.

12. Addition of preheated elution buffer (TE) to the membrane center of the FABG column,for effective elution to make sure that the elution solution is dispensed onto the membrane center absorbed completely, and incubated for 10 minutes at 37 ºC in an incubator.

13. The DNA was eluted by centrifuging at maximum speed (14,000 rpm) for 1 minute.

14. Collection of 100 µL of the DNA elute has been performed and checked for purity by the spectrophotometric method and was then stored at -20 degrees Celsius (Meredith et al., 2011; Yamagata et al., 2021).

2.3.5.2. Quantitation of DNA by spectrophotometric method

The DNA purity and concentration were assessed using the Nano method (Nanodrop). After cleaning the micro detector a 1 µL sample was placed on the Nanodrop micro detector; the concentration and A260/A280 ratio of DNA were documented from the instrument (Hussein., 2023).

2.3.6. Polymerase Chain Reaction

The polymerase chain reaction (PCR) is a process that involves replicating DNA regions using an enzyme called DNA polymerase. This study employs Allele Specific Polymerase Chain Reaction (AS-PCR), which allows the amplification of desired DNA fragments, from one molecule to millions of copies. To carry out a AS-PCR reaction several requirements must be met, including designing primers that bind complementary to the DNA template with the SNP located at the 3' end of the primer. Apart from the DNA template and primers this technique requires components such, as DNA polymerase (Taq polymerase) deoxy nucleotide triphosphates and a buffer solution known as mastermix. The PCR process typically consists of three steps that are repeated around 25-45 times (Wang et al., 1998; Rahman et al., 2013; Hussein., 2023).

This process involved five steps:

1. **Denaturation**:

The first step in AS-PCR was denaturation, where the double-stranded DNA template is heated to around 94-98°C. This high temperature causes the DNA strands to separate, breaking the hydrogen bonds between the complementary bases and creating two single-stranded DNA molecules.

2. **Annealing**:

After denaturation, the reaction mixture was cooled to around 50-65°C. During this step, short DNA primers that are complementary to the sequences flanking the target region anneal (bind) to their respective positions on the single-stranded DNA template.

3. **Extension**:

Once the primers were bound, the temperature is raised to around 72°C, which is the optimal temperature for DNA polymerase activity. The DNA polymerase enzyme extends the primers by adding nucleotides to synthesize new DNA strands complementary to the template.

4. **Cycle Amplification**:

The denaturation, annealing, and extension steps are repeated multiple times in a thermal cycler machine. Each cycle doubled the amount of target DNA present in the reaction mixture, resulting in exponential amplification of the desired DNA fragment.

5. **Final Extension**:

After a set number of cycles (typically 20-40), a final extension step at 72°C was performed to ensure that any remaining single-stranded DNA molecules were fully extended.

2.3.6.1. Primers design

Specific primer pairs were designed for INSR (rs2245649) T>C and (rs2229429) G>A by Asst. Prof. Dr. Hassan Mahmood Mousa Abo Almaali /College of Pharmacy/Kerbala, using primer blast software and depending on https://www.ncbi.nlm.nih.gov/ websites , and were purchased from Macrogen, Korea as lyophilized product of different picomoles concentrations. The primer sequences that were utilized for amplification analysis of INSR genes for SNP identification are shown in Tables (2-3) and (2-4) respectively.

Table (2-3): Primers Sequences of INSR (rs2245649) T>C Genetic Polymorphism

Primers	Primer sequence (5 ’->3 ’)	Primer size (bp)	Product size (bp)	Reference
Forward Primer	CTCGAACAGCTCACTGTCT	19	172	Current study
Reverse Primer Allele T	CCCATTTCCTTTCTCCCTGA	20		
Reverse primer Allele C	CCCATTTCCTTTCTCCCTGG	20		

Table (2-4): Primers Sequences of INSR (rs2229429) G>A Genetic Polymorphism

Primers	Primer sequence (5 ’->3 ’)	Primer size (bp)	Product size (bp)	Reference
Forward Primer	AACCTCACTGCATCAGCCT	19	319	Current study
Reverse Primer Allele G	CAGAATGTGACGGAGTTCGAC	21		
Reverse Primer Allele A	CAGAATGTGACGGAGTTCGAT	21		

2.3.6.2. Polymerase Chain Reaction Optimization Conditions

Optimization of polymerase chain reaction was acquired after several trials.

Optimization of INSR (rs2245649) T>C genetic polymorphism conditions.

INSR (rs2245649) T>C optimization of PCR conditions was prepared by using:

- Different volumes of primers (1μL, 2μL)
- annealing temperatures (59,61 °C)
- Different volumes of DNA sample (1 μL, 3 μL)

The best results of this SNP was obtained in the following conditions:

A. 2 μl outer forward primer

B. 2 μl outer reverse primer

C.3 μl DNA sample

D.8 μl nuclease-free water

E. annealing temperature of 61°C

Optimization of INSR (rs2229429) G>A genetic polymorphism conditions.

INSR (rs2229429) G>A optimization of PCR conditions was formulated by using:

- Different volumes of primers (1μL, 2μL)
- annealing temperatures (59,61 °C)
- Different volumes of DNA sample (1 μL, 3 μL)

The best results of this SNP were achieved in the following conditions:

A. 2 μl OUTER FORWARD PRIMER

B. 2 μl OUTER REVERSE PRIMER

C.3 μl DNA SAMPLE

D.8 μl Nuclease Free Water

E. annealing temperature of 61°C

Lyophilized forward and reverse primers were dissolved in specific volumes of nuclease free water to get a concentration of 100 pmol/ µl (stock solution). To prepare 10 pmol/ µl of working solution of each primer, 10 µl of each primer (stock solution) was diluted with 90 µl of nuclease free water. The primers were kept at 20 °C until further use. The reaction was carried out in a total volume of 25 µL, which was added to a PCR 250 µL premix tube. The premix itself was 10 µL. The sample tubes were then centrifuged at 2000 rpm for 10 seconds in a microcentrifuge to mix the contents before being placed in the thermocycler.

2.3.6.3. Running and Working Solution of Polymerase Chain Reaction

2.3.6.3.A. Allele-specific –Polymerase Chain Reaction Running INSR (rs2245649) T>C polymorphism

The AS-PCR mixture for INSR (rs2245649) T>C was prepared in PCR premix formula by adding: 2 µL of forward primer in each microcentrifuge tube of PCR premix 10 µL of the master mix, 2 µL of R-T in tube 1 of PCR premix, 2 µL of R-C in tube 2 of PCR premix, 3µL of extracted DNA and, the volume was completed to 25 µL with 8 µL of nuclease-free water for each tube,illustrated in Table (2-5). The thermal program for INSR (rs2245649) T>C polymorphism is demonstrated in Table (2-6). DNA bands were determined by using a DNA ladder of 100-1500 bp.

Table (2-5): PCR Tube Components and its Volume for INSR (rs2245649) T>C Polymorphism

Components	Volume (µL)
Forward primer	2 µL
Reverse primer	2 µL
DNA	3 µL
PCR master mix	10 µL
Nucelase free water	8 µL
Total volume	25 µL

Table (2.6): Polymerase Chain Reaction Thermocycler Program for INSR (rs2245649) T>C Polymorphism

Steps	Temperature (°C)	Minutes:seconds	Cycles
Initial denaturation	95	03:00	1
Denaturation	95	00:30	35
Annealing	61	00:30	
Extension	72	00:55	
Final Extension	72	5:00	1

2.3.6.3.B. Allele-specific–Polymerase chain reaction running INSR (rs2229429) G>A polymorphism

The AS-PCR mixture for the INSR (rs2229429) G>A was prepared in a PCR premix formula by adding specific components to each tube. This included 2 µL of forward primer in each tube of PCR premix, 2 µL of R-G in the first tube, 2 µL of R-A in the second tube, 3 µL of extracted DNA, and 10 µL of mastermix. The volume for each tube was adjusted to 25 µL by adding 8 µL of nuclease-free water,found in Table (2-7).The thermal program details for analyzing the INSR (rs2229429) G>A polymorphism can be found in Table (2.8). DNA bands were determined by using a DNA ladder of 100- 1500 bps.

Table (2-7): PCR tube Components and its Volume for INSR (rs2229429) G>A Genetic Polymorphism

Components	Volume (µL)
Forward primer	2 µL
Reverse primer	2 µL
DNA	3 µL
PCR master mix	10 µL
Nucelase free water	8 µL
Total volume	25 µL

Table (2-8): PCR Thermal Program for Genotyping INSR (rs2229429) G>A Genetic Polymorphism

Steps	Temperature (°C)	Minutes:seconds	Cycles
Initial denaturation	95	03:00	1
Denaturation	95	00:30	35
Annealing	61	00:30	
Extension	72	00:55	
Final Extension	72	5:00	1

2.3.6.4. Agarose Gel Electrophoresis

Electrophoresis was performed to assess the integrity of the extracted DNA and to visualize the size of PCR products following the completion of the PCR protocol. The concentration of the agarose gel concentrations was from 2 % (Sambrook, 1989).

1. To prepare Agarose gel, 1,5 g of agarose powder has been dissolved in 75 ml of pre-distilled 1x TBE (Tris-Borate-EDTA) buffer.
2. The mixture was heated in the microwave for a few minutes to dissolve completely, avoiding bubbles until a clear and pure gel solution appeared.
3. The solution was allowed to cool to 45°C, and 2 µL of ethidium bromide was added to the gel.
4. After pouring the agarose solution into the tray, a comb was attached to one end of the tray to create wells for loading the PCR samples, it was left to solidify at 25°C.
5. The comb was gently removed from the tray after it had hardened, which took approximately 20 minutes.
6. The tray was placed into the electrophoresis chamber, and the chamber was filled with 1X TBE-electrolysis buffer with a loading dye that contained a tracking dye Ethidinium Bromide solution for visualization.
7. One of the wells of the agarose gel was loaded with 5 µl of 1000 bp of DNA ladder to serve as a size reference for the DNA fragments, while the others were loaded with 5 µl of each PCR product, loading was done with a micropipette.
8. Connect the electrodes from a power supply to the chamber ensuring correct polarity.

9. To run the electrophoresis process, the voltage of the electrophoresis apparatus was set at 95 volts for 50 minutes, to establish an electrical field of 5 volts per centimeter for a 20 cm distance between the cathode and anode.
10. Analyze band sizes by comparing them to the DNA ladder standards by using a UV trans-illuminator 336 nm, and gel photographs were taken using a Canon digital camera.
11. Interpret results based on band migration distances and relative sizes of DNA fragments (Lee et al., 2012; Rhida et al., 2022).

2.4. Statistical Analysis

The collected data of the present study were analyzed through the Statistical Package for the Social Sciences (IBM/ SPSS version 27 software USA). The data were presented as frequencies and percentages or mean and standard deviation in appropriate tables and graphs. Independent t-test, as well as Anova test with post hoc analysis were used to find out the possible association between the related variables of the current study for the normal distributed data (confirmed by Shapiro-wilk test). Besides correlation test had been done to detect the association among some variables. The distribution of alleles and genotypes in accordance with Hardy-Weinberg equilibrium was examined using the goodness of fit test. Non-numerical data, such as obesity status and sex, were analyzed using the Chi-square (χ^2) test. Multinomial logistic regression was employed to assess the association between genetic variations and the efficacy and safety of exogenous insulin. P-values less than or equal to 0.05 will be considered statistically significant.

Chapter Three

Results

3.Results

3.1. Socio-Demographic and Biochemical Profiles of Controls vs. T1D Patients

3.1.1. Socio-Demographic Data and Related Parameters of Controls vs. T1D Patients

Socio-demographic data had been illustrated in Table (3-1). The study included 99 patients and 30 healthy participants as a control group, matched for age (5–17 years), to compare various diabetes indicators between the two groups and confirm the diagnosis of diabetes. The age range was further subdivided into school-aged children (5–12 years) and adolescents (13–17 years). BMI values ranging from 11 to 30 were converted to BMI percentiles and categorized into underweight, normal weight, overweight, and obese, according to the ADA BMI classification criteria. The duration of DM and basal-bolus monotherapy duration and doses (≤ 1 IU/kg/day) have been recorded for patients along with the family history. As illustrated in the below table, 59.5 % of patients had no family history of diabetes, while 40.5 % reported a positive family history. The majority of patients (86.8%) come from middle-income households, with 12.12% from low-income and the remainder from high-income backgrounds (1.1%). Patients have been categorized into three groups based on levels of physical activity and lifestyle, labeled (sedentary activity and lifestyle) were 9.1% , (moderate activity and lifestyle) were 85.8% and (vigorous activity and healthy) were 5.1 %.

Table (3-1): Descriptive Statistics of the Socio-Demographic Data and Related Parameters of the 129 Participants.

Variables		Control (30) n (%)	Patients (99) n (%)	Range (min-max)
Age Groups	5-12 13-17	18 (60) 12 (40)	45 (45.5) 54 (54.5)	12 (5-17)
Obesity Status	Underweight Healthy weight Overweight Obese	4 (13) 26 (87) 0 (0) 0 (0)	55 (55.5) 31 (31.3) 7 (7.1) 6 (6.1)	19 (11-30)
Duration of DM (year)	1-12 years	-	99	11 (1-12)
Treatment Duration (year)	1-9 years	-	99	8 (1-9)
Insulin Bolus (Soluble) IU	≤ 1 IU/kg/day	-	99	45 (5-50)
Insulin Basal (Lenti) IU	≤ 1 IU/kg/day	-	99	34 (4-38)
Family History of DM	Absent present	26 (86.7) 4 (13.3)	59 (59.5) 40 (40.5)	55 (4-59)
Socio-Economic status	Poor Middle high	2 (6.7) 25 (83.3) 3 (10)	86 (86.8) 12 (12.1) 1(1.1)	84 (2-86)
Physical activity	Sedentary moderate active	9 (30) 18 (60) 3 (10	9 (9.1) 85 (85.8) 5 (5.1)	82 (3-85)

The distribution of 129 participants according to sex, with 47.3% total males participants and 52.7 % total females. T1D male patients of 43.4% have been enrolled while 56.6% were T1D females. Sex distribution showed no significance between the both groups (p-value=0.11), as illustrated in Table (3-2).

Table (3-2): The Distribution of Participants According to Sex

Sex	Healthy control n=30 (%)	T1D n=99 (%)	Total n=129(%)	Chi-square value	p-value
Male	18 (60)	43 (43.4)	61 (47.3)	2.53	0.11
Female	12 (40)	56 (56.6)	68 (52.7)		

Chi-square value used, p-value <0.05* is indicated as significant, numbers of patients presented as numbers and percentages

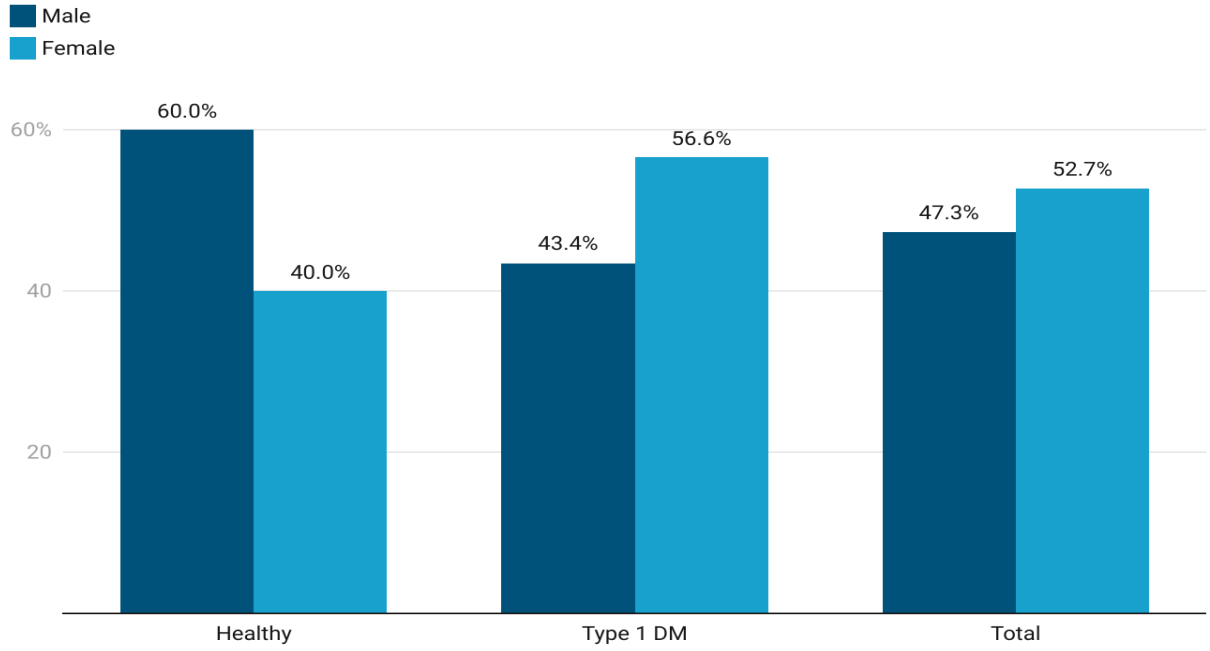


Figure (3-1): The Distribution of Participants According to Gender. The distribution of 129 participants according to gender, with 47.3% total males participants and 52.7% total females.

Table (3-3) summarized the descriptive statistics for continuous variables among study participants. No statistically significant difference in age was observed between the patient group (n = 99) and the control group (n = 30) (p = 0.16). However, both BMI and BMI percentile showed statistically significant differences between the two groups (p < 0.001).

Table (3-3): Socio-Demographic Data of Patients and Control

Variables	Control (n=30) mean ± SD	T1D (n=99) mean ±SD	Total (n=129) mean ± SD	p-value
Age (years)	11.43±3.41	12.60±3.23	12.33±3.29	0.16
BMI ($kg/m^{2)}$	20.15 ± 1.78	19.67 ± 4.27	19.79 ± 3.84	**< 0.001***
BMI Percentile (%)	72 ± 13.8	63 ± 23.9	65 ± 23.2	**< 0.001***

BMI: body mass index, BMI Percentile: body mass index percentile, p-values are based on the Independent Samples T test, p-value <0.05* is indicated as significant, n=number of participants

3.1.2. Comparative Analysis of Biochemical Profiles of Controls vs. T1D Patients

Table (3-4) presented a comparison between patients and controls, and revealed statistically significant differences in FBS, RBS, and HbA1c levels, all of which were significantly higher in the patient group (p < 0.001).

Table (3-4): Comparison of the Biochemical Parameters between Patients and Control

Variables	Control (n=30) mean ± SD	T1D (n=99) mean ±SD	Total (n=129) mean ± SD	p-value
FBS (mg/dL)	104.97±7.89	204.26±87.32	181.17±87.32	**<0.001***
RBS (mg/dL)	117.73±9.81	305.95±130.51	262.18±139.40	**<0.001***
HbA1c %	4.98 ± 0.41	11.01 ± 1.46	9.61±2.94	**<0.001***

FBS: Fasting Blood Sugar, RBS: Random Blood Sugar, HbA1c: Glycated Hemoglobin, p-values are based on the Independent Samples T test, p-value <0.05* is indicated as significant, n=number of participants

Patients exhibited a significantly higher mean sIR level (3.08 ± 0.96) compared to controls (1.56 ± 0.26), with a p-value of <0.001, as shown in Table (3-5).

Table (3-5): Soluble Insulin Receptor Levels in Control and T1D Patients

Variables	control (n=30) mean ± SD	T1D (n=99) mean ±SD	Total (n=129) mean ± SD	p-value
sIR (ng/mL)	1.56 ±0.26	3.0 ±0.96	2.72±1.06	**<0.001***

sIR: soluble insulin receptor, Normal lab values of sIR are ≤ 1.5 ng/mL according to manufactrer's kit, p-values are based on the Independent Samples T test, n=number of participants, p-value <0.05* is indicated as significant

3.2. Socio-Demographic and Biochemical Parameters of T1D Patients

3.2.1. Socio-Demographic Parameters of T1D Patients

In the current study, patients were divided into two age groups: 5–12 years (school-age group) and 13–17 years (adolescent group), as illustrated in Table (3-6). These age groups showed statistically significant differences in age ($p < 0.001$), as well as in BMI and BMI percentile (both $p < 0.001$). Additionally, insulin therapy requirements differed substantially between the two groups, with significant differences observed in both bolus insulin ($p = 0.007$) and basal insulin ($p < 0.001$).

Table (3-6): Socio-Demographic Parameters in Two Age Groups of T1D Patients

Variable	(School-age Group) n=45 mean ± SD	(Adolescent Group) n=54 mean ± SD	p-value
Age (years)	9.69±2.20	15.02±1.43	**< 0.001***
BMI (kg/m^2)	17.12±3.21	21.82±3.87	**< 0.001***
BMI Percentile (%)	34.8 ± 21.6	53.98±17.0	**< 0.001***
DM duration (years)	3.83±2.25	4.73±3.24	0.13
Duration of Rx (years)	2.56±1.02	2.57±1.54	0.95
Insulin bolus (IU)	19.02±8.79	24.44±10.53	**0.007***
Insulin basal (IU)	14.73±6.54	22.20±9.31	**< 0.001***

Independent sample T test, n=number of patients, p-value <0.05* is indicated as significant, Duration of Rx: duration of nsulin basal bolus monotherapy,Insulin dose in International Unit

3.2.2. Comparative Analysis of Biomarker Profiles of T1D Patients

The age groups (5–12 years and 13–17 years) showed no statistically significant differences in any glycemic parameters nor in soluble insulin receptor (sIR) levels, as illustrated in Table (3-7).

Table (3-7): Comparison of The Biochemical Parameters between Patient's Age Groups

Variable	(School-age Group) n=45 mean ± SD	(Adolescent Group) n=54 mean ± SD	p-value
FBS (mg/dL)	212.76±85.25	197.19±89.19	0.38
RBS (mg/dL)	325.18±125.54	289.93±133.54	0.18
HbA1c %	11.05±1.73	10.98±1.58	0.84
sIR (ng/mL)	2.99±0.70	3.15±1.13	0.43

p-values are based on the Independent Samples T test, p-value <0.05* is indicated as significant
n=number of patients

The correlational matrix revealed significant relationships between several key variables in diabetes management, as demonstrated in Table (3-8). Including Age, BMI percentile, DM Duration, Insulin monotherapy (bolus and basal), FBS, RBS, sIR, and HbA1c, emerged as tightly linked factors.

Table (3-8): Correlation of the Study Variables Among Each Other

Correlation		Age (year)	BMI Percentile (%)	DM Duration (year)	Duration of Rx (year)	Insulin Bolus (IU)	Insulin Basal IU)	FBS (mg/dL)	RBS (mg/dL)	HbA1c (%)	sIR ng/mL)
Age (year)	r	1									
	p										
BMI Percentile (%)	r	0.514**	1								
	p	**0.001****									
DM Duration (year)	r	0.223*	0.171	1							
	p	**0.026***	0.090								
Duration of Rx (year)	r	0.006	0.108	0.516**	1						
	p	0.951	0.288	**0.001****							
Insulin Bolus (Soluble) IU	r	0.225*	0.370**	0.048	0.131	1					
	p	**0.025***	**0.001****	0.637	0.195						
Insulin Basal (Lenti) IU	r	0.413**	0.562**	0.203*	0.310**	0.554**	1				
	p	**0.001****	**0.001****	**0.044***	**0.002****	**0.001****					
FBS (mg/dL)	r	-0.018	-0.017	-0.010	-0.009	0.215*	0.147	1			
	p	0.843	0.847	0.923	0.932	**0.032***	0.147				
RBS (mg/dL)	r	0.033	-0.033	0.002	-0.056	0.188	0.119	0.498**	1		
	p	0.712	0.712	0.984	0.585	0.062	0.242	**0.001****			
HbA1c (%)	r	0.166	-0.045	0.223*	0.101	0.252*	0.320**	0.492**	0.674**	1	
	p	0.061	0.610	**0.026***	0.318	**0.012***	**0.001****	**0.001****	**0.001****		
sIR (ng/mL)	r	0.057	-0.029	0.070	0.072	-0.095	-0.013	0.289**	0.374**	0.624**	1
	p	0.523	0.745	0.489	0.481	0.349	0.902	**0.001****	**0.001****	**0.001****	

Correlation is significant at the 0.05* level, Correlation is strongly significant at 0.01** level

3.3. Genetic Analysis of INSR Genetic Polymorphisms

3.3.1. Genotype Status of INSR (rs2245649) T>C Genetic Polymorphism

3.3.1.1. Results of Amplification Reaction

The results of AS-PCR analysis were presented for the genetic polymorphism of INSR (rs2245649) T>C within this study showcasing all genotypes.

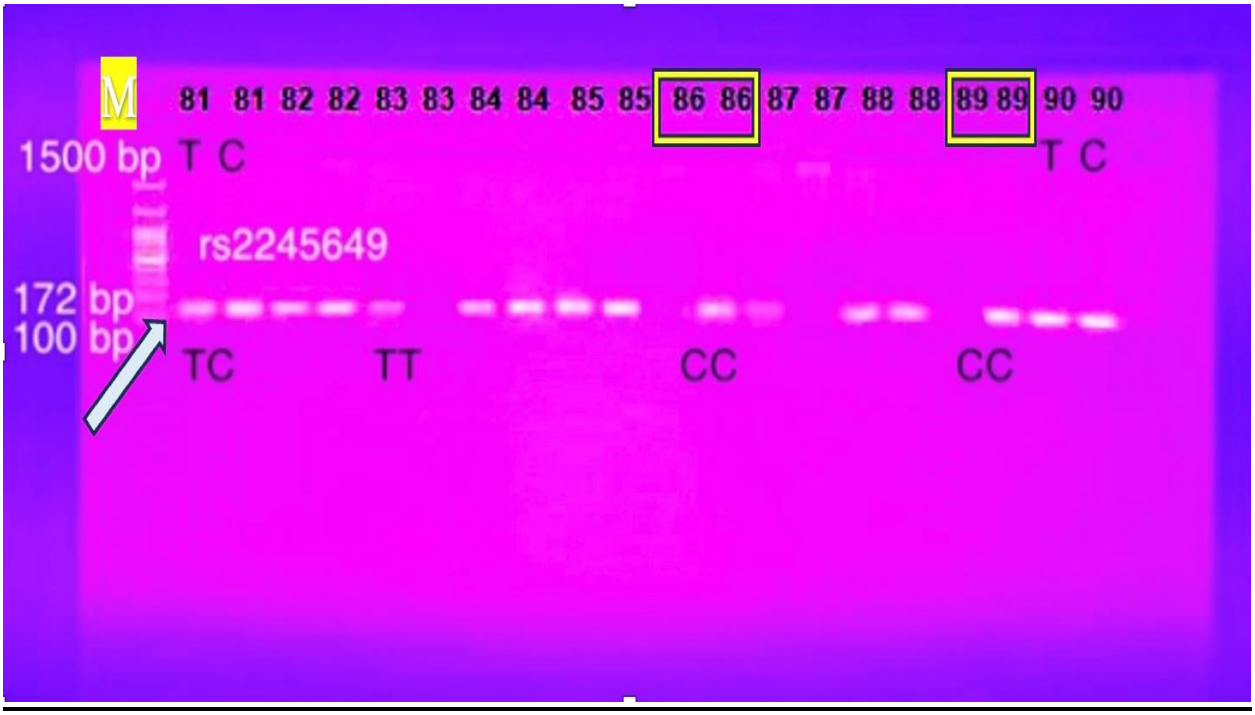


Figure (3-2): Genotyping of (rs2245649) T > C Applying Allele-Specific PCR Technique. Agarose gel electrophoresis was performed by using 2 % agarose per TBE buffer lane M represents the DNA ladder 100-1500 bp. Gene polymorphism of INSR (rs2245649) T > C produced a clear band with a molecular size of 172 bp (white arrow), as illustrated in Figure (3-2). lane 81 represent TC genotype (heterozygous), lane 82 represents TT genotype (wild), lane 86 and 89 represents CC genotype (homozygous mutant).

3.3.1.2. Genotype and Allele Frequencies Analysis for INSR (rs2245649) T>C in T1D patients

The provided data in the Table (3-9) presented findings of the genotype and allele frequencies of the (rs2245649) T>C from 99 individuals. Analysis of genotypic distribution revealed 74 % TT of participants carried the wild-type TT genotype, with smaller percentages for heterozygous TC (12 %) and homozygous CC (14 %) genotypes, illustrated in Table (3-9) and Figure (3-9). The major allele frequencies indicated 74 % for allele T and 26 % for allele C. The (rs2245649) genotype distribution may be significant in evaluating genetic contributions that T1D patients carrying C allele (p <0.0001) are at a high risk for establishing poor glycemic control and insulin resistance.

Table (3-9): Distribution of Genetic Variants in INSR (rs2245649) T>C Among T1D Patients

Gene	Genotype	Patients n	Frequency %	Allele		p-value
				T	C	
(rs2245649) T>C	TT wild	66	66.6	(147) 0.74	(51) 0.26	**<0.0001***
	TC heterozygous	15	15.2			
	CC homozygous	18	18.2			
	Total	99	100			

p-value <0.05***** is indicated as significant, patients are presented in numbers and percentages

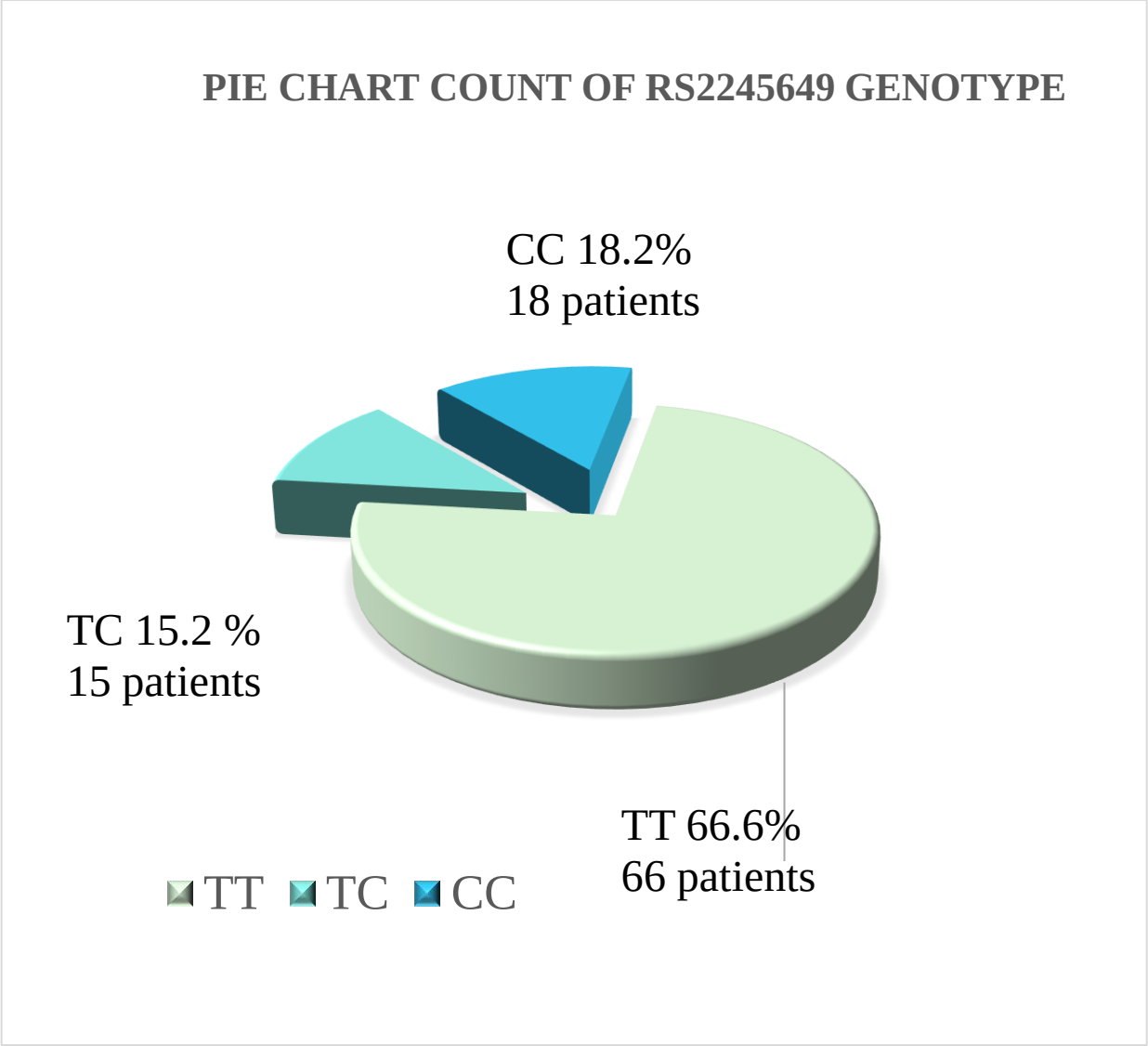


Figure (3-3): Distribution of Genetic Variants INSR (rs2245649) T>C Across Three Genotypes Among T1D Patients. Analysis of genotypic distribution revealed that 66.6 % (66 patients) carried the wild-type TT genotype, with smaller percentages for heterozygous TC 15.2 % (15 patients) and homozygous CC 18.2 % (18 patients).

3.3.1.3.Hardy-Weinberg equilibrium Analyses for INSR (rs2245649) T>C Genotypes

The Hardy-Weinberg equilibrium (HWE) test was used to show the expected frequenct and percent of genotype groups which is statistically significant (p-value < 0.05). These findings underscored the presence of genetic deviations from the Hardy-Weinberg equilibrium within this sample across all three genotypes, indicating potentially significant genetic factors play a role such as random mating, genetic drift, life style and environmental factors. According to the aim of this study, the patients were classified according to one of three genotypes for the rs2245649 T>C Genotypes genetic polymorphism, the wild type homozygous for T allele (TT), heterozygous (TC) and homozygous for the C allele (CC) mutant type. Table (3-9) and Figure (3-3) show the different genotypes among the enrolled 99 patients.

Table (3-10): Hardy-Weinberg Equilibrium Analyses for INSR (rs2245649) T>C Genotype

Genotypes	Observed	Expected	HWE-Frequency	P-value
TT	66	54.57	55.12%	**<0.0001***
TC	15	37.86	38.25%	
CC	18	6.57	6.63%	

p-value <0.05***** is indicated as significant, patients are presented in numbers and percentages, HWE:Hardy-Weinberg Equilibrium:We reject or the null hypothesis that the population is at H-W equilibrium

3.3.2. Genotype Status of INSR (rs2229429) G>A Genetic Polymorphism

3.3.2.1. Results of Amplification Reaction

This analysis successfully distinguished between different genotypes of the (rs2229429) G>A polymorphism, providing valuable genetic information that may have clinical implications, such as drug response.

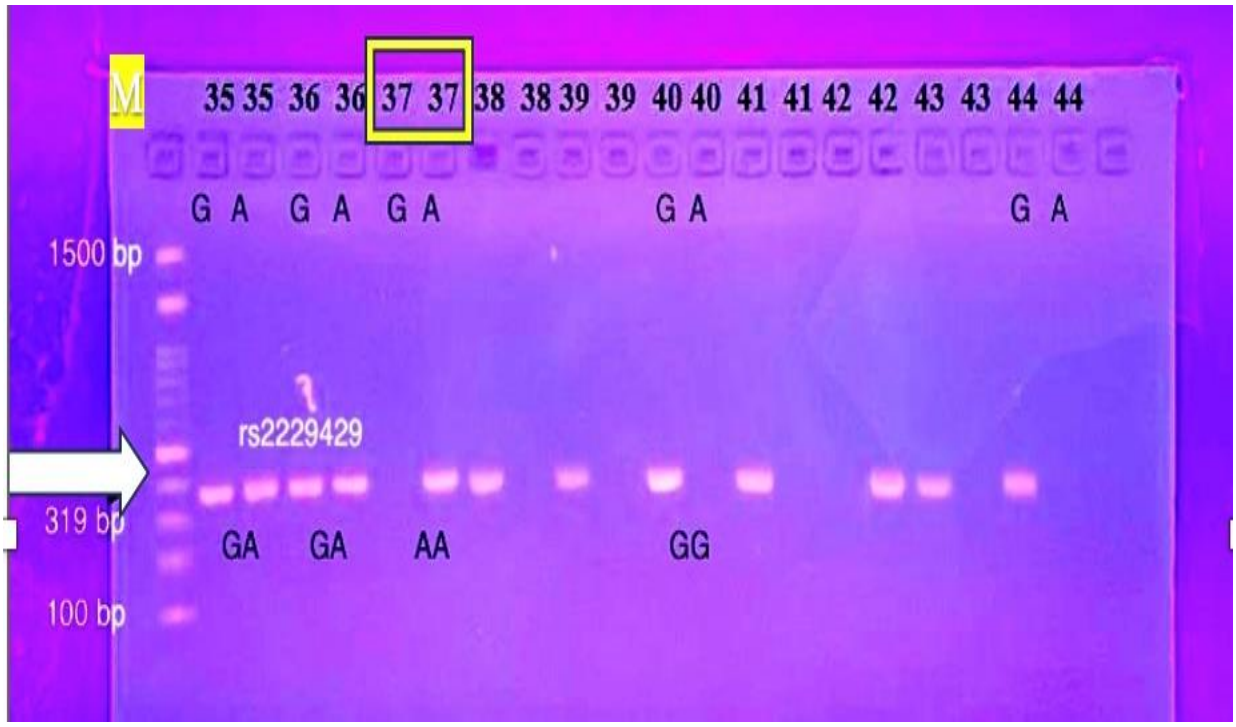


Figure (3-4): Genotyping of INSR (rs2229429) G>A Genetic Polymorphism Allele-specific PCR Technique. Agarose gel electrophoresis was performed by using 2 % agarose per TBE buffer. Lane M represents the DNA ladder of 100-1500 bp as size reference for the amplicon. The gene polymorphism (rs2229429) G>A produced a clear band with a molecular size of 319 bps amplicon, as shown in Figure (3-4). Lane 35 and 36 represent GA genotype (heterozygous). Lane 37 represents AA genotype (homozygous mutant) and lane 40 represents GG genotype (wild).

3.3.2.2 Genotype and Allele Frequencies Analysis for INSR (rs2229429) G > A in T1D patients

Consistent with the aim of this study, the patients were classified according to one of three genotypes for the (rs2229429) G > A genetic polymorphism, the wild type homozygous for G allele GG (83.8%), heterozygous GA (8.1%) and homozygous for the A allele AA (8.1%) mutant type, alongside allele frequencies of 88 % for allele G and 12 % for allele A. Table (3-11) and Figure (3-5) show the different genotypes among the 99 enrolled patients. Clear significant association is presented by a P-value <0.05, indicating that INSR SNP is strongly linked to hyperglycemia and insulin resistance in T1D patients on exogenous insulin as illustrated in Table (3-11).

Table (3-11): Distribution of Genetic Variants in INSR (rs2229429) G>A among T1D patients

Gene	Genotype	Patients n	Frequency %	Allele		p-value
(rs2229429) G>A	GG wild	83	83.8	G	A	**<0.0001***
	GA heterozygous mutant	8	8.1	(174) 0.88	(24) 0.12	
	AA homozygous mutant	8	8.1			
	Total	99	100			

p-value <0.05***** is indicated as significant, patients are presented in numbers and percentages

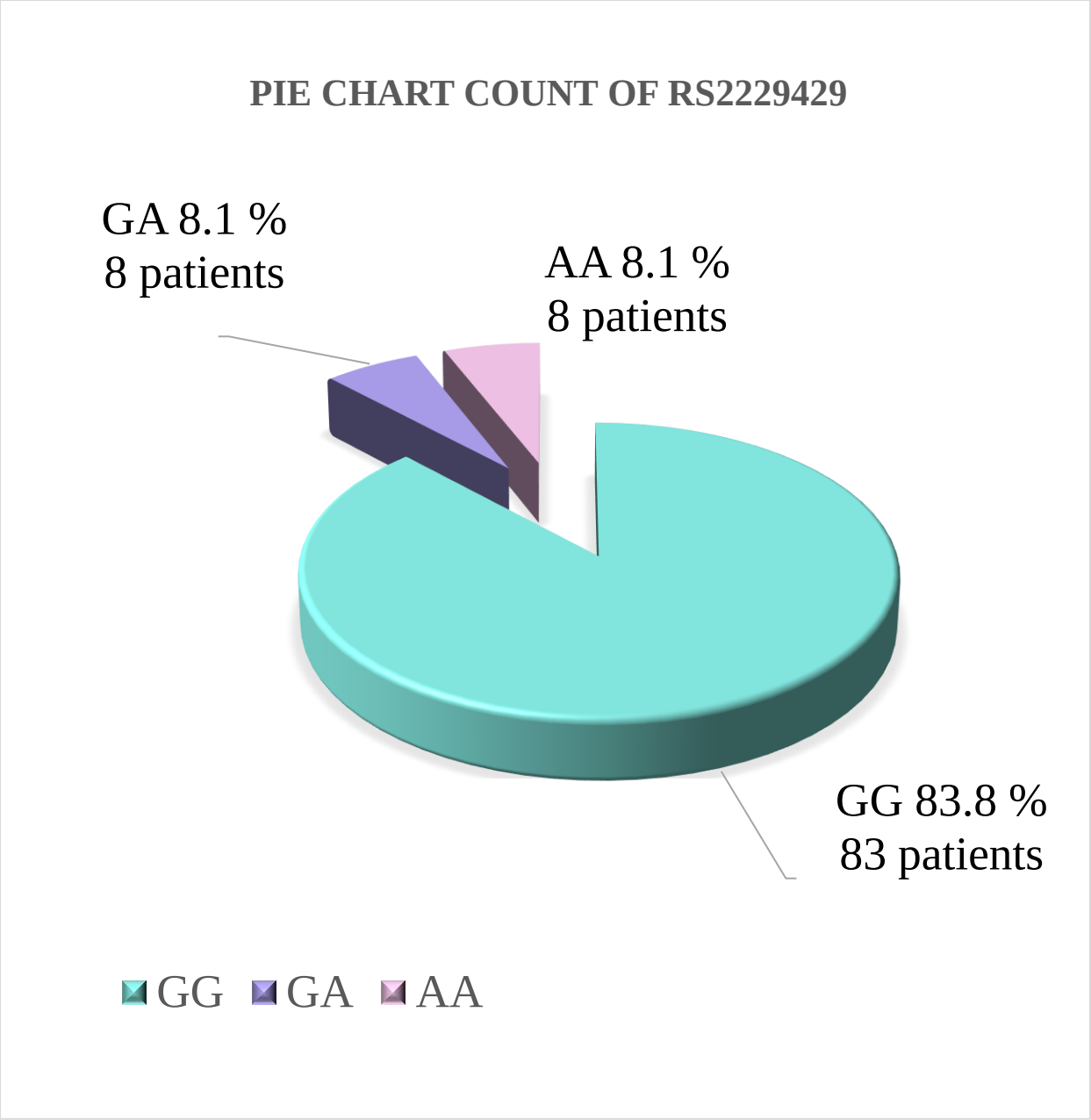


Figure (3-5): Distribution of Genetic Variants INSR (rs2229429) G>A Across Three Genotypes Among T1D Patients. Distribution of genetic variants of (rs2229429) G>A among patients showed that 84 % (83 patients) had the wild-type GG genotype, while the remaining patients were split evenly between heterozygous GA 8 % and homozygous AA 8 % genotypes.

3.3.2.3 Hardy-Weinberg Equilibrium Analyses for INSR (rs2229429) G>A Genotypes

The HWE test was used to show the expected frequency and percent of genotype groups which is statistically significant (p-value < 0.05). The following findings underscore the presence of genetic deviations from the Hardy-Weinberg equilibrium within this sample across all three genotypes, indicating potentially significant genetic factors play a role such as random mating, genetic drift, life style and environmental one of three genotypes for the rs2229429 G>A Genotypes, the wild type homozygous for G allele GG, heterozygous GA and homozygous for the A allele AA mutant type. Table (3-12) shows the HWE of expected populations and the observed genotypes among the enrolled 99 patients.

Table (3-12): Distribution of Genotypes of the INSR (rs2229429) G>A Genotypes

Genotype Observed % n=99		HWE Expected %			Fisher exact test	P-value
G	A	G	A	GA/AG	GG/observed vs GG/expected	**<0.0001***
88	12	77.23	1.47	21.3	GA/observed vs GA/expected	**<0.0001***
					AA/observed vs AA/expected	**<0.0001***

p-value <0.05***** is indicated as significant, patients are presented in numbers and percentages, HWE:Hardy-Weinberg Equilibrium:We reject or the null hypothesis that the population is at HWE

3.4. Impact of INSR (rs2245649) T>C and (rs2229429) G>A Gene Variations on Exogenous Insulin in T1D

3.4.1. Impact of the INSR (rs2245649) T>C Gene Variations on Insulin Therapy in T1D

3.4.1.A. Association of INSR (rs2245649) T>C Genotypic Variations with Socio-Demographic Variables in T1D

Table (3-13) compared basal characteristics, including age, BMI percentile, duration of diabetes and therapy, across three genotypes TT (66), TC (15), CC (18) of the rs2245649 gene in 99 T1D patients. Characteristics such as age, diabetes duration and duration of therapy and dose did not show statistically significant differences across genotypes. However the BMI percentile of the normal weighted patients carrying TC genotype showed significant difference compared to the CC genotype (p=0.01). Additionally, patients with healthy weight carrying TT genotype showed significant diiference compared to the CC genotype (p=0.05). Overweight patients with TT allele showed significant differences compared to CC genotype (p=0.04).

Table (3-13) : Distribution of INSR (rs2245649) T>C Genotypes Across Socio-Demographic Variables

Variable		INSR (rs2245649) T>C Genotypes mean (±SD)						p-value		
		n	TT (n=66)	n	TC (n=15)	n	CC (n=18)	TT vs TC	TT vs CC	TC vs CC
Age (years)	5-12	26	9.96±2.05	8	10.38±2.01	11	9.96±2.05	0.62	0.08	0.10
	13-17	40	15.00±1.36	7	15.14±1.77	7	15.00±1.73	0.81	0.99	0.88
BMI Percentile (%)	Underweight (<5th %ile)	39	2.58 ± 2.53	8	3.96 ± 1.99	8	2.18 ± 2.32	0.28	0.72	0.35
	Healthy weight (5th to <85th %ile)	21	31.16 ± 19.95	4	36.09 ± 12.71	6	43.05±2.22	0.63	**0.05***	**0.01***
	Overweight (85th to <95th %ile)	3	85.00 ± 0.02	2	89.91 ± 5.09	2	92.26 ± 2.74	0.56	**0.04***	0.17
	Obese (≥95th ile)	3	97.69 ± 4.39	1	100.00 ± 0.00	2	98.79 ± 1.30	0.61	0.75	0.76
DM duration (years)	1 -12	66	4.12±2.74	15	5.27±3.43	18	4.33±2.77	0.17	0.77	0.39
Duration of Rx (years)	1-9	66	2.58±1.50	15	2.43±0.78	18	2.61±0.98	0.71	0.94	0.57
Daily Dose of Rx (IU/kg)	≤ 1	66	1.06±0.25	15	1.27±0.17	18	1.16±0.14	0.61	0.69	0.72

BMI percentile %ile: body mass index percentile in percentage, DM Duration: diabetes duration, Duration of Rx: duration of insulin therapy, Daily Dose of Rx= Dose of Basal-Bolus Insulin monotherapy in International Units per kilograms (IU/kg), n=number of patients, Anova test used, p-value <0.05* is indicated as significant

3.4.1.B. Association of INSR (rs2245649) T>C Genotypic Variations with Laboratory Parameters in T1D

HbA1c levels varied significantly among genotypes (p = 0.01), with patients carrying the TC genotype exhibiting a notably higher mean HbA1c (11.69%), followed closely by the CC genotype (11.67%), compared to the TT genotype (10.84%). Similarly, sIR levels showed significant differences across genotypes (p = 0.02), with the TC group displaying the highest mean sIR concentration (3.48 ng/mL) compared to the TT group (2.88 ng/mL), as presented in Table (3-14). FBS levels also differed significantly among the three genotypes (p = 0.03).

Table (3-14): Impact of Exogenous Insulin on Laboratory Profiles in Relation to INSR (rs2245629) T>C Gene Variations

Variable	INSR (rs2245649) Genotypes mean (±SD)			p -value
	TT (n=66) mean ± SD	TC(n=15) mean ± SD	CC(n=18) mean ± SD	
FBS (mg/dL)	239.15±65.12	227.20±91.11	245.89±57.94	**0.03***
RBS (mg/dL)	313.52±134.45	282.47±114.79	297.78±132.02	0.68
HbA1c (%)	10.84±1.65	11.69±1.28	11.67±1.58	**0.01***
sIR (ng/ml)	2.88±0.80	3.48±1.17	3.43±1.14	**0.02***

n=numbers of patients, p-value <0.05* is indicated as significant, Anova test used

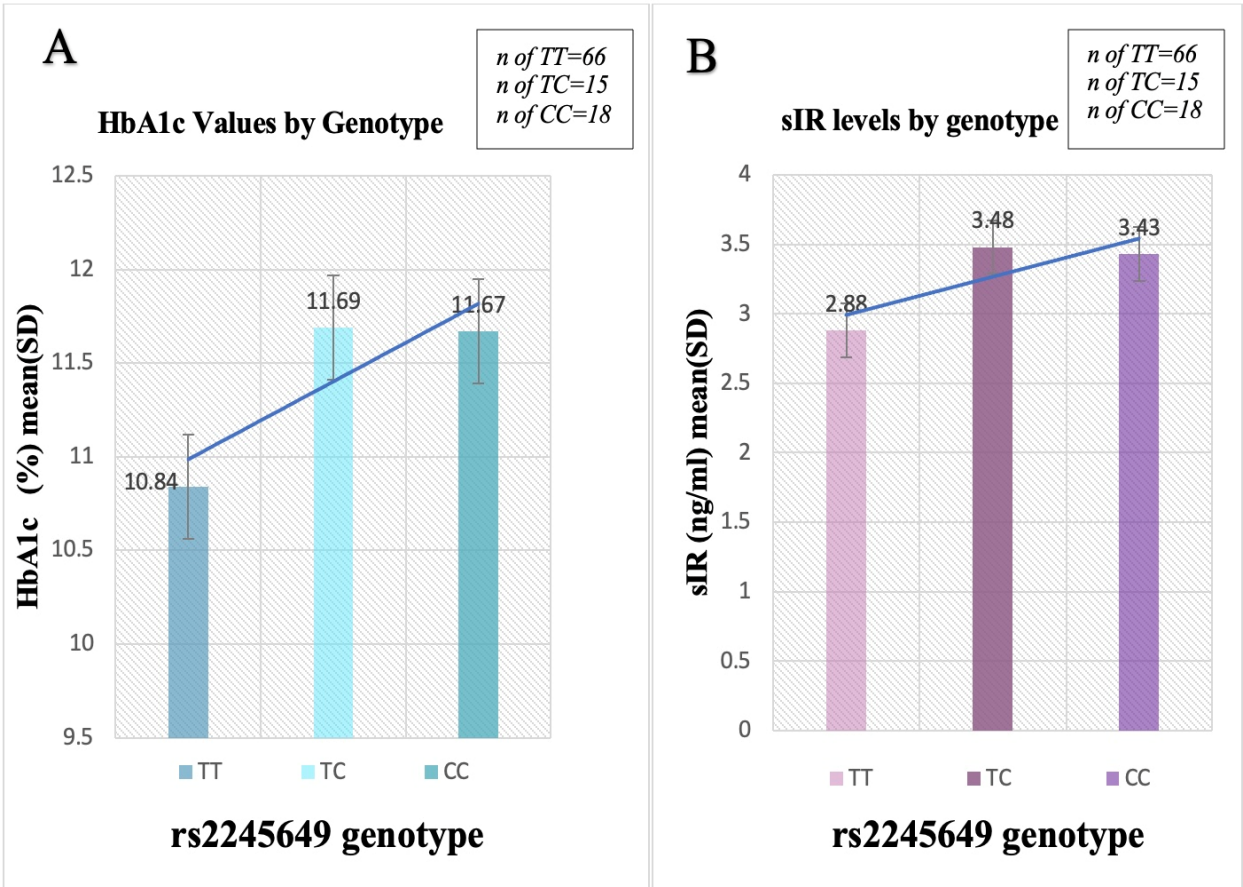


Figure (3-6 A and 3-6 B) HbA1c levels (A) and sIR Levels (B) by INSR (rs2245649) T>C: Figure (A) illustrated the relationship between HbA1c levels and INSR (rs2245649) genotypes in patients with T1D. A significant increase in HbA1c was observed in individuals with the heterozygous TC genotype (11.69%, turquoise bar) compared to those with the homozygous TT genotype (10.85%, teal bar). Similarly, patients with the homozygous mutant CC genotype exhibited elevated HbA1c levels (11.67%, emerald green bar). Figure (B) displays the variation in sIR concentrations across the same genotypes. Individuals with the TC (bordeaux bar) and CC (amethyst bar) genotypes showed significantly higher sIR levels compared to those with the TT genotype (lavender bar), suggesting a genotype-dependent impact on insulin receptor.

3.4.1.C. Distribution of INSR (rs2245649) T>C Genotypes by HbA1c Levels in T1D

Table (3-15) presented the association between (rs2245649) T>C genotypes and HbA1c levels (< 10 % and ≥ 10 %), along with the Odds Ratios (OR), and p-value. Highly unstable glycemic control was defined as HbA1c ≥ 10.0%, according to the ADA 2020 guideline.

- Moderate control: HbA1c ≤ 9.0%
- Poor control: HbA1c > 9.0% to < 10.0%
- Severe poor control: HbA1c ≥ 10.0%

Another citation classifies moderate hyperglycemia as A1c <10% (FBS and RBS ≤ 250 mg/dL), and severe hyperglycemia as A1c ≥10% (FBS and RBS >250 mg/dL) (Agiostratidou et al., 2017, Delgado et al., 2021).

In the current study we classified moderate hyperglycemia (A1c <10%) as responders, and severe hyperglycemia (A1c ≥10%) as non-responders. The distribution of these genotypes was notably high in the non-responders group, with 13 patients carrying the TC genotype and 14 carrying the CC genotype. TC and CC carriers exhibited severe hyperglycemia. The data suggested that the (rs2245649) C genotype had a significant association with decompensated diabetes**,** as evidenced by an OR of 2.523 and a p-value of 0.04. Carrier of the minor allele C were about 2.5 times more likely to develop insulin resistance than the homozygous wild type.

Table (3-15): Association Between INSR (rs2245629) T>C Genotypes and Glycemic Control

Gene		n	Responders (HbA1c < 10 %)			n	Non-Responders (HbA1c ≥ 10 %)			OR 95%CI	p-value
			(rs2245649) T>C Genotypes				(rs2245649) T>C Genotypes				
			TT	TC	CC		TT	TC	CC		
(rs2245649) T>C	T	27 (81.8%)	27 (81.8%)	0	0	39 (59.1)	39 (59.1)	0	0	**2.523** *(1.171-5.434)*	**0.04***
	C	6 (18.2%)	0	2 (6.1%)	4 (12.1%)	27 (40.9%)	0	13 (19.7%)	14 (21.2%)		
Total patients (99)		33 (100%)				66 (100%)					

OR: Odds ratio, 95 % CI: 95 % Confidence Interval, Haldane-Anscombe correction used for OR, HbA1c < lower than 10 % and ≥ equal or higher than 10 %, n=numbers of patients are shown as numbers and frequencies, p-value <0.05* is indicated as significant

A significant association between INSR (rs2245649) genotypes and mean HbA1c levels was demonstrated in Table (3-16). Mean HbA1c levels were higher in individuals with the TC ($11.95 \pm 1.17\%$) and CC ($12.25 \pm 1.23\%$) genotypes compared to those with the TT genotype ($11.67 \pm 1.41\%$) within the non-responders group. A similar trend was observed for the non-responders TC genotype, where individuals had higher HbA1c levels than the responders group. The CC genotype in the non-responders group was also associated with severely poor glycemic control, compared to the responders group.

Table (3-16): HbA1c Levels by INSR (rs2245649) T>C Genotypes

Variable Genotype		Responders (HbA1c < 10 %) Mean (±SD) (n=33 patients)	Non-Responders (HbA1c ≥ 10 %) Mean (±SD) (n=66 patients)	p-value
(rs2245649) T>C	TT	9.25±0.55	11.67±1.41	**<0.001***
	TC	9.90±0.01	11.95±1.17	**0.04***
	CC	9.63±0.62	12.25±1.23	**0.001***

HbA1c: glycated hemoglobin presented in percentage, independent sample t-test, p-value <0.05* is indicated as significant

Table (3-17) presented the mean HbA1c (%) values for school-aged children (5–12 years) and adolescents (13–17 years), categorized by INSR (rs2245649) genotypes (TT, TC, CC). No statistically significant differences in HbA1c were observed between the two age groups across any genotype, as all p-values exceeded 0.05. This suggests that age alone does not significantly impact glycemic control within each genotype group. However, both TC and CC genotypes were consistently associated with higher mean HbA1c levels compared to TT in both age groups, although these differences were not statistically significant, indicating generally poor glycemic control irrespective of age.

Table (3-17): Age Group Comparison According to INSR (rs2245649) T>C Genotype Distribution

Variable		School-Age Group	Adolescent Group	p-value
		HbA1c %, mean ± SD	HbA1c %, mean ± SD	
(rs2245649) T>C	TT	10.59±1.75	10.74±1.59	0.72
	TC	11.71±0.99	11.65±1.64	0.93
	CC	11.66±1.86	11.68±1.16	0.98
	Total	11.05±1.73	10.98±1.58	

HbA1c: glycated hemoglobin presented in percentage, independent sample t-test, p-value <0.05* is indicated as significant

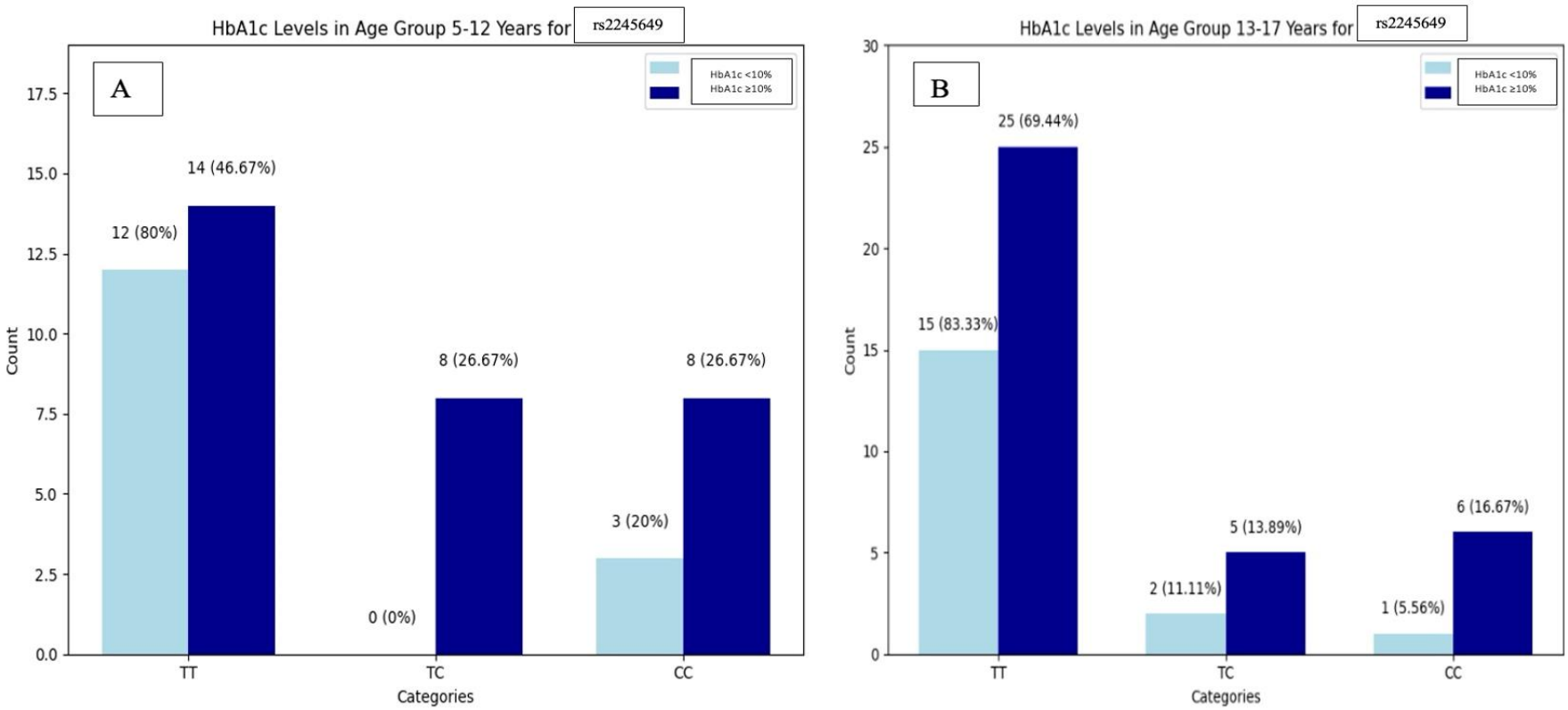


Figure (3-7A) and (3-7B): Distribution of T1D Individuals by Age Group and HbA1c Levels According to INSR (rs2245649) T>C Genotypes. Patients carrying the heterozygous (TC) and homozygous (CC) genotypes with HbA1c levels ≥ 10% were distributed across two age groups: school-aged children and pubertal individuals. Notably, the TC genotype was more frequently observed among school-aged patients with elevated HbA1c levels, while the CC genotype showed a slightly higher prevalence in school-aged children compared to adolescents.

3.4.1.D. Impact of INSR (rs2245649) T>C Gene Variations on Soluble Insulin Receptor in T1D

TC heterozygotes showed a significant increase in sIR levels and exhibited markedly severe poor glycemic control (HbA1c ≥ 10%), compared to those with HbA1c <10 % (4.69 ± 0.42 ng/ml vs. 2.60 ± 0.72 ng/ml; p = 0.002).In contrast, among CC homozygotes, sIR levels remained elevated regardless of glycemic status (3.39 ± 0.72 vs. 3.44 ± 1.26; p = 0.94).

Table (3-18): Relationship Between sIR Levels and HbA1c Categories According to INSR (rs2245649) T>C Genotypes in T1D

Variable		Responders (HbA1c < 10 %)	Non-Responders (HbA1c ≥ 10 %)	p-value
		sIR (ng/ml) **mean (±SD)**	**sIR** (ng/ml) **mean (±SD)**	
(rs2245649)	TT	2.54±0.26	3.13±0.96	**0.003***
	TC	2.60±0.72	4.69±0.42	**0.002***
	CC	3.39±0.72	3.44±1.26	0.94

sIR: soluble insulin receptor in ng/ml, independent sample t-test used, p-value <0.05* is indicated as significant

3.4.2. Impact of INSR (rs2229429) G>A Gene Variation on Insulin Therapy in T1D

3.4.2.A. Association of INSR (rs2229429) G>A Genotypic Variations with Socio-Demographic Variables in T1D

Table (3-19**)** explored the potential relationship between INSR rs2229429 genotypes (GG, GA, AA) and various characteristics of 99 T1D patients, including age, BMI percentile categories, duration of diabetes, duration of insulin therapy, and insulin dose. The aim is to assess whether this genetic variation is associated with demographic or treatment-related parameters. While age and most BMI percentile comparisons across genotypes did not reach statistical significance, notable exceptions were observed: the GG genotype was significantly associated with AA genotypes in underweight status ($p = 0.02$). The GG genotype showed a significant compared to GA genotype in overweight status ($p = 0.01$) .

Table (3-19): Distribution of INSR (rs2229429) G>A Genotypes Across Socio-Demographic Variables

Variable			INSR (rs2229429) genotypes mean (±SD)					p-value		
		n	GG (n=83)	n	GA (n=8)	n	AA (n=8)	GG vs GA	GG vs AA	GA vs AA
Age (years)	5-12	38	9.58±2.29	2	10.50±2.12	5	10.20±1.79	0.48	0.56	0.86
	13-17	45	15.02±1.44	6	15.00±1.41	3	15.00±2.00	0.97	0.98	>0.99
BMI Percentile (%)	Underweight (< 5th %ile)	52	3.25 ± 0.78	1	3.30 ± 0.00	2	4.65 ± 1.45	0.98	**0.02***	0.06
	Healthy Weight (5th to <85th %ile	27	51.80±6.90	2	55.20 ± 5.80	2	52.90±2.70	0.55	0.71	0.73
	Overweight (85th to<95th %ile)	2	88.60±2.20	2	91.20 ± 2.80	3	90.30±2.30	**0.01***	0.55	0.69
	Obese (≥95th %ile)	2	97.80±0.90	3	97.80 ± 0.70	1	97.50±0.00	0.99	0.72	0.73
DM duration (years)	1 -12	83	4.40±2.96	8	4.00±2.62	8	3.94±1.94	0.71	0.67	0.96
Duration of Rx (years)	1-9	83	2.60±1.39	8	2.25±1.04	8	2.56±1.04	0.49	0.94	0.51
Daily Dose of Rx (IU/kg)	≤ 1	83	1.27±0.10	8	1.05±0.12	8	1.10±0.15	0.11	0.26	0.48

BMI percentile: body mass index, DM Duration: diabetes duration, Duration of Rx: duration of insulin therapy, Daily Dose of Rx= Dose of Basal-Bolus Insulin monotherapy in International Units per kilograms (IU/kg), n=number of patients, Anova test used, p-value <0.05* is indicated as significant

3.4.2.B. Association of INSR (rs2229429) G>A Genotypic Variations with Laboratory Parameters in T1D

Patients received a mean daily insulin dose of 1.16 ± 0.08 IU/kg, with none exceeding 1 IU/kg/day**.** As shown in Table (3-20)**,** mean FBS and HbA1c levels differed significantly among genotypes, with p-values of 0.03 and <0.001**,** respectively. The AA genotype group exhibited the highest mean HbA1c level. Although the GA genotype group had the highest mean RBS, no statistically significant association was observed between RBS and genotype. In contrast, plasma sIR levels showed a significant difference ($p < 0.001$)**,** with GA and AA genotypes presenting higher mean values compared to the GG genotype**.**

Table (3-20): Impact of Exogenous Insulin on Laboratory Profiles in Relation to INSR (rs2229429) G>A Gene Variations

Variable	INSR (rs2229429) genotypes			p- value
	GG (n=83) Mean (± SD)	GA (n=8) mean (± SD)	AA (n=8) mean (± SD)	
FBS (mg/dL)	188.76±52.99	222.38±90.32	233.1±25.78	**0.03***
RBS (mg/dL)	317.27±73.79	375.63±72.19	344.00±112.20	0.09
HbA1c %	10.64±1.44	12.43±1.03	13.43±1.39	**< 0.001***
sIR (ng/ml)	2.91±0.87	3.59±0.92	4.32±0.89	**< 0.001***

n=numbers of patients, Anova test used, p-value <0.05* is indicated as significant

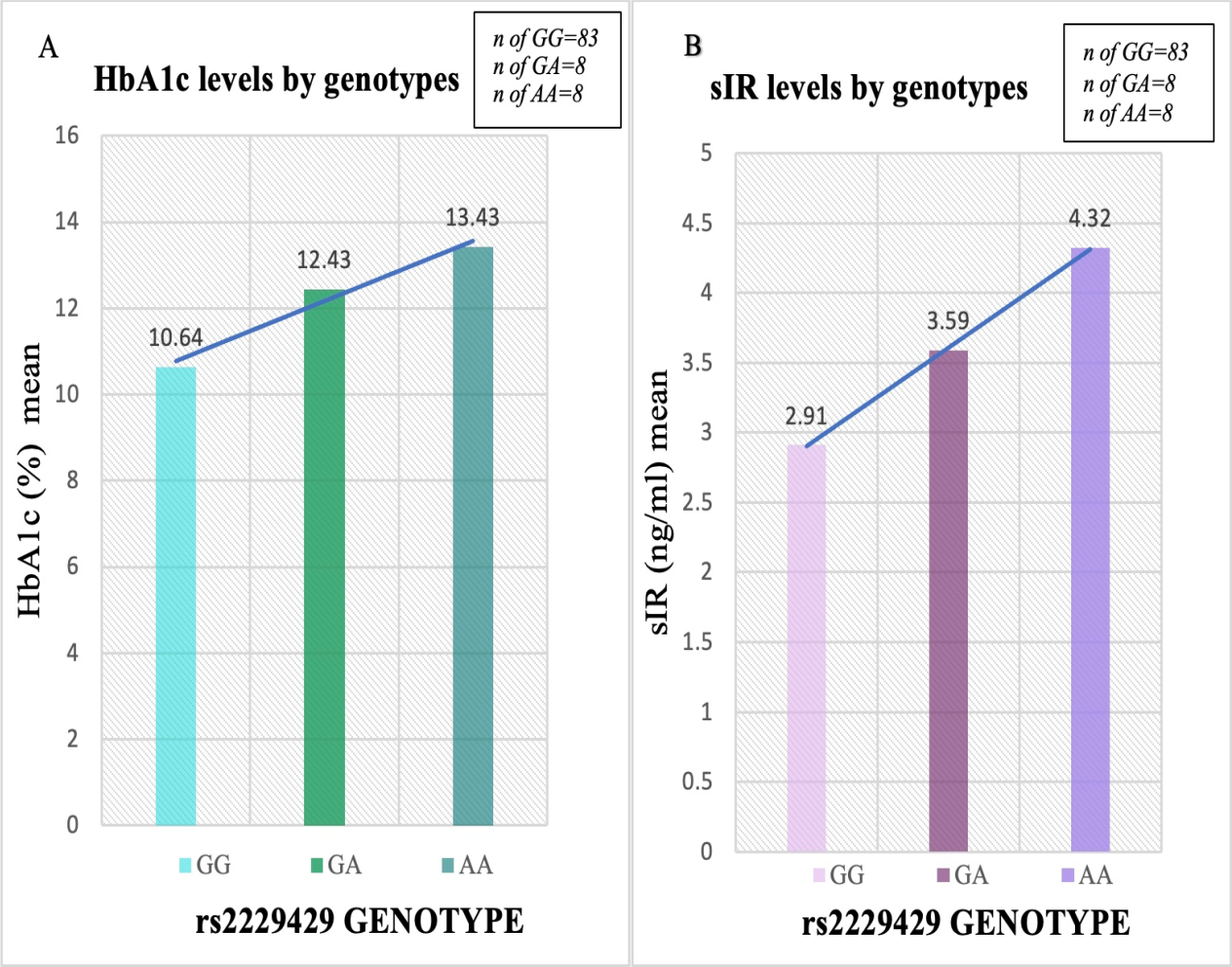


Figure (3-8A and 3-8B): HbA1c levels (A) and sIR Levels (B) by INSR (rs2229429) G>A. Figure (A) Serum HbA1c levels in T1D patients according to INSR (rs2229429) genotypes. A significant increase in HbA1c is observed in heterozygous GA carriers (jade green bar) compared to homozygous GG individuals (turquoise bar). Patients with the mutant AA genotype (teal bar) also show elevated HbA1c levels. Figure (B) Soluble insulin receptor (sIR) levels among rs2229429 genotypes. Elevated sIR levels are seen in patients with the mutant GA (lilac bar) and AA (amethyst purple bar) genotypes compared to GG genotype carriers (lavender bar).

3.4.2.C. Distribution of INSR (rs2229429) G>A Genotypes by HbA1c Levels in T1D

Table (3-21) compared the distribution of the rs2229429 (G>A) genotypes among T1D patients grouped by HbA1c levels: HbA1c < 10% (moderate hyperglycemia = responders) and HbA1c ≥ 10% (severe hyperglycemia = non-responders). A total of 99 patients were included (33 responders and 66 non-responders). The odds ratio (OR) and 95% confidence interval (CI) were calculated using the Haldane-Anscombe correction to account for zero values. A p-value < 0.05 was considered statistically significant. The GA and AA genotypes were highly prevalent in the non-responders group, with equal distributions of 8 patients each. These findings reveal a strong and highly significant association between the A allele of rs2229429 and poor glycemic control in Iraqi T1D patients. Individuals carrying the GA or AA genotypes are over 10 times more likely to develop severe hyperglycemia compared to GG homozygotes, supported by a p-value of 0.008 and a confidence exceeding one, confirming statistical significance.

Table (3-21): Association Between INSR (rs2229429) G>A Genotypes and Glycemic Control

SNP allele	n		Responders (HbA1c < 10 %)			n	Non-Responders (HbA1c ≥ 10 %)			OR 95% CI	p-value
			(rs2229429) G>A Genotypes				rs2229429 G>A Genotypes				
			GG	GA	AA		GG	GA	AA		
rs2229429 G>A	G	33	33 (100 %)	0	0	50	50 (75.8%)	0	0	**10.479** (1.964-55.916)	**0.008***
	A	0	0	0	0	16	0	8 (12.1%)	8 (12.1%)		
Total Patients (99)	33 (100 %)					66 (100%)					

OR: Odds ratio, 95 % CI: 95 % confidence interval, Haldane-Anscombe correction used for OR, HbA1c: glycated hemoglobin (HbA1c < less than 10 % and HbA1c ≥ equal or higher than 10 %), n=numbers of patients are shown as numbers and frequencies, p-value <0.05* is indicated as significant

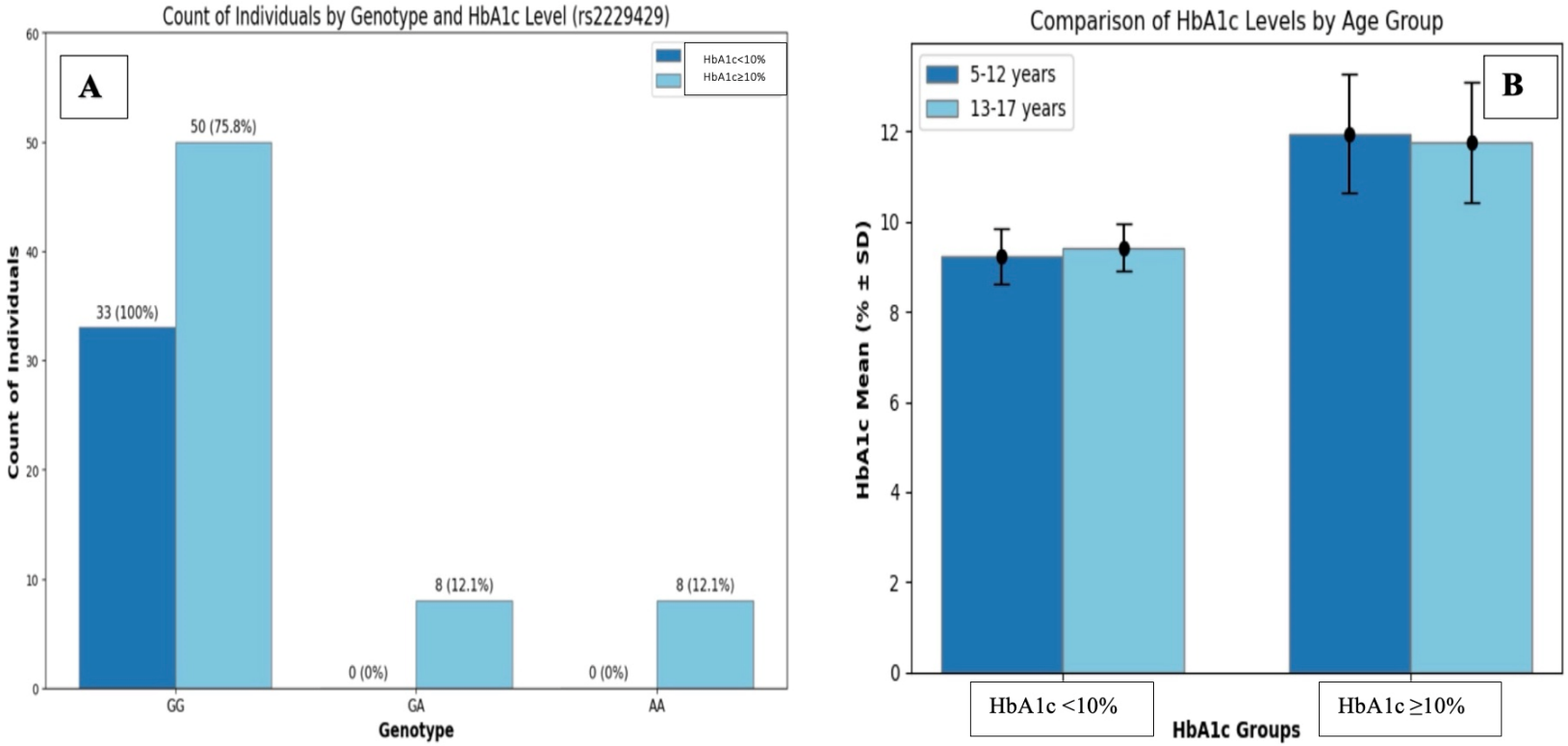


Figure (3-9A) and (3-9B): Distribution of INSR (rs2229429) G>A Genotypes Across HbA1c and Age Groups

Figure (A) illustrates the distribution of INSR (rs2229429) genotypes across HbA1c level groups. The GA and AA genotypes are highly prevalent in the HbA1c ≥ 10% group, indicating a strong association with poor glycemic control. Figure (B) shows comparable HbA1c levels between the two age groups.

The data clearly demonstrated that the A allele of INSR (rs2229429) was strongly associated with elevated HbA1c levels and severe poor glycemic control, as illustrated in Table (3-22). Mean HbA1c levels for the GA (12.43 ± 1.03) and AA (13.43 ± 1.39) genotypes were higher than those for the GG genotype (11.51 ± 1.16) in the HbA1c ≥10% group. The complete absence of GA and AA genotypes in the HbA1c <10% group, combined with significantly elevated HbA1c values in A allele carriers. These findings suggest that genotyping for rs2229429 may offer clinical value for risk stratification and the development of personalized diabetes management strategies in patients with T1D.

Table (3-22): HbA1c Levels by INSR (rs2229429) G>A Genotypes

Variable Genotype		Responders (HbA1c < 10 %) Mean (±SD) (n=33 patients)	Non-Responders (HbA1c ≥ 10 %) Mean (±SD) (n=66 patients)	p-value
(rs2229429) G>A	GG	9.34±0.57	11.51±1.16	**<0.001***
	GA	0±0	12.43±1.03	**<0.001***
	AA	0±0	13.43±1.39	<**0.001***

HbA1c < less than 10 % and HbA1c ≥ equal or higher than 10 %,independent sample t-test, p-value (zero-inflated model), p<0.05* is indicated as significant

Table (3-23) compares mean HbA1c levels (±SD) across two age groups: school-aged children (5–12 years) and pubertal adolescents (13–17 years), within the different genotypes of the INSR (rs2229429) G>A polymorphism. The aim is to assess whether age and genotype interact to influence glycemic control in patients with T1D. An independent samples t-test was performed to compare HbA1c levels between the two age groups for each genotype. Although the differences in HbA1c across age groups within each genotype were not statistically significant, both the GA and AA genotypes were associated with poorer glycemic control, particularly during puberty. These findings age alone does not appear to be a determining factor for glycemic variation across the rs2229429 genotypes.

Table (3-23): Age Group Comparison According to INSR (rs2229429) G>A Genotype Distribution

Variable Genotype		School-Age Group HbA1c %, mean (±SD)	Adolescent Group HbA1c %, mean (±SD)	p-value
(rs2229429)	GG	10.74±1.59	10.57±1.3	0.60
	GA	11.95±0.07	12.59±1.17	0.49
	AA	13.1±1.63	13.99±0.87	0.43
	Total	11.05±1.73	10.98±1.58	
independent sample t-test used, p-value <0.05* is indicated as significant				

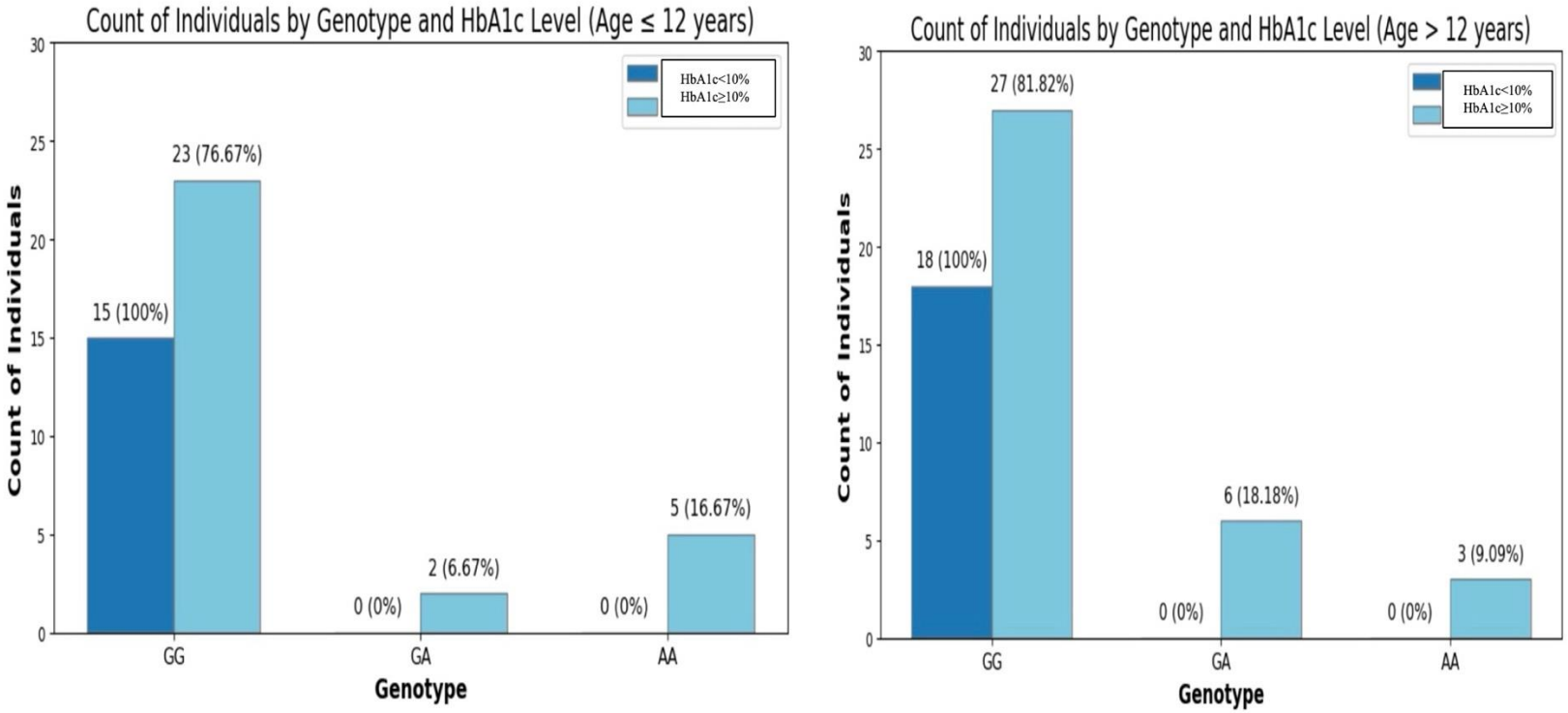


Figure (3-10): Distribution of T1D Individuals by Age Group and HbA1c Levels According to INSR (rs2229429) G>A Genotypes: Patients carrying the heterozygous (GA) and homozygous (AA) genotypes with HbA1c levels ≥10% were distributed across two age groups: school-aged children and pubertal individuals. The findings showed an almost equal distribution of poor glycemic control among patients with the GG genotype in both age groups. However, pubertal individuals exhibited overall poorer glycemic control compared to school-aged children. Notably, the GA genotype was more frequently observed among pubertal patients with elevated HbA1c levels, while the AA genotype showed a slightly higher prevalence among school-aged children.

3.4.2.D. The Impact of the INSR (rs2229429) G>A Gene Variations on Soluble Insulin Receptor in T1D

Table (3-24) presents the mean sIR levels across different genotypes of the INSR gene SNP rs2229429 (G>A), stratified by two HbA1c groups: <10% and ≥10%. Each genotype is examined within both HbA1c categories to assess the influence of genetic variation on sIR levels in relation to glycemic control. Statistical analysis was conducted using an independent samples t-test with a zero-inflated model to account for the high number of zero sIR values observed in the moderate control group. A p-value < 0.05 indicates statistical significance.

Table (3-24): Relationship Between sIR Levels and HbA1c Categories According to INSR (rs2229429) G>A Genotypes in T1D

Variable		Responders (HbA1c < 10 %)	Non-Responders (HbA1c ≥ 10 %)	p-value
		sIR (ng/ml) **Mean (±SD)**	**sIR** (ng/ml) **Mean (±SD)**	
(rs2229429)	GG	2.74±0.56	3.02±1.01	0.16
	GA	0±0	3.59±0.92	**<0.001***
	AA	0±0	4.32±0.89	**<0.001***

sIR: soluble insulin receptor in ng/ml , independent sample t-test used (zero-inflated model), p-value <0.05* is indicated as significant

Chapter Four

Discussion

4. Discussion

T1D represents a global health problem, because of the high number of cases reported globally and the low adherence to therapeutic management (Sánchez-Pozos et al., 2021). Based on data from the WHO, lifelong use of insulin is crucial for T1D population. Previous researches have shown that exogenous insulin is highly effective to control blood sugar levels in T1D. However, even with strict adherence to exogenous insulin guidelines, many patients can still not achieve the desired blood sugar control (Jiráček et al., 2017).

Consequently, the impact of SNPs on the *INSR* gene can significantly affect the interaction between insulin and its receptor, potentially influencing the efficacy of exogenous insulin in achieving glycemic control leading to insulin resistance (Gu et al., 2018; Sánchez-Pozos et al., 2021; Massarenti et al., 2022).

Therefore, we explored the predictive role of *INSR* gene polymorphisms in influencing insulin binding, downstream cellular responses, and *INSR* expression levels in Iraqi patients with T1D undergoing exogenous insulin therapy. Understanding how these genetic variations contribute to insulin resistance may aid in identifying patients who exhibit poor response to insulin treatment. Such insights could support the use of additional or alternative therapeutic strategies to improve clinical outcomes.

4.1. Socio-Demographic and Biochemical Profiles of Controls vs. T1D Patients

4.1.A. Socio-Demographic Parameters of Controls vs. T1D Patients

In Tables 3-1, 3-2, and 3-3, the results from 99 patients with T1D and 30 age-matched healthy controls are presented. Age and sex distributions were comparable between the groups ($p = 0.16$ and $p = 0.11$, respectively), ensuring a balanced representation. However, the BMI range among patients (11–30 kg/m²) highlights the coexistence of both undernutrition and excess weight within this population. A significant difference in nutritional status was observed: 55.5% of T1D patients were underweight compared to 13% of the controls. This finding aligns with the hypotheses of previous studies, which suggest that impaired glucose metabolism leads to increased catabolism and weight loss as a result of lipolysis, proteolysis, and glycosuria (Ardestani, 2018; Villegas-Valverde et al., 2018; James et al., 2023).

All cases of overweight and obesity were observed within the diabetic group, reflecting a broad spectrum of nutritional outcomes likely influenced by multiple factors. Notably, reduced insulin sensitivity or the presence of genetic polymorphisms in the *INSR* gene may significantly contribute to obesity in individuals with T1D (Wilkin et al., 2001; Pozzilli et al., 2014).

All diabetic patients were treated with insulin, receiving average daily doses of 45 IU (bolus) and 34 IU (basal), with total doses not exceeding 1 IU/kg/day, consistent with recommended dosing guidelines (Blonde et al., 2022). A family history of diabetes was significantly more common among patients than controls, supporting the role of genetic predisposition and highlighting the importance of early screening (Zaccardi et al., 2015).

4.1.B. Laboratory Parameters of the Control Group and T1D Patients

Tables 3-4 and 3-5 compare glycemic and biochemical markers between T1D patients and healthy controls, revealing significant differences across all measured parameters. The mean FBS level in T1D patients was significantly elevated compared to the control group ($p < 0.001$), consistent with the autoimmune destruction of pancreatic beta cells and the resulting disruption of glucose homeostasis due to insulin deficiency. A similarly significant difference was observed in RBS levels ($p < 0.001$). Long-term glucose regulation, as measured by HbA1c levels, also differed significantly between patients and controls ($p < 0.001$), with values far exceeding pediatric targets. These findings are consistent with a previous study, highlighting ongoing challenges in glycemic control. The elevated HbA1c confirms persistent hyperglycemia over recent months and underscores patients' difficulty in managing both pre- and postprandial glucose levels, even under intensive basal-bolus insulin therapy (Chiang et al., 2018).

Soluble IR levels also showed notable differences between groups. Patients exhibited a significantly higher mean sIR level compared to controls ($p < 0.001$). The elevated sIR in patients may result from increased receptor degradation. Insulin receptors continuously cycle between the cell surface and intracellular compartments. This mechanism regulates receptor availability and insulin signaling. During this process, protease enzymes cleaves the extracellular portion of the receptor on the membrane, releasing it into the bloodstream as a soluble form, a process known as ectodomain shedding. Under certain metabolic conditions, particularly hyperglycemia, the activity of proteases is increased, leading to accelerated receptor shedding (Umehara et al., 2009).

4.2. Biochemical Profiles of T1D Patients

4.2.A. Laboratory Parameters of T1D Patients

Table 3-7 showed no statistically significant differences across all parameters between children and adolescents. Despite the known physiological changes during puberty, school-aged children had slightly higher mean FBS and RBS levels than adolescents. HbA1c and sIR levels were similarly elevated in both groups, indicating persistent hyperglycemia and suboptimal glycemic control. This suggests that multiple factors, such as chronic stress, psychiatric issues, seasonality, poor treatment adherence, dietary miscalculations may contribute to disparities in glycemic control. Most importantly, however, insulin resistance appears to be a key contributor, as supported by several studies (Paschou et al., 2018; Chaudary et al., 2025).

4.2.B. Correlation of the Study Variables Among Each Other in T1D

According to Donner et al. (2023), as age increases, BMI, FBS, and HbA1c levels tend to rise, necessitating higher insulin doses in adolescents with T1D. This is supported by the strong positive correlation between age and BMI shown in Table 3-8, likely reflecting the physiological increase in BMI during puberty. The trend is further confirmed by the significant difference observed in Table 3-6 between school-aged children and adolescents ($p < 0.001$), which can be attributed to developmental, hormonal, and metabolic changes occurring during adolescence (Donner et al., 2023).

Correspondingly, insulin requirements, both bolus and basal were significantly higher in adolescents ($p = 0.007$ and $p < 0.001$, respectively), reinforcing the association between age, BMI, and insulin demand. These correlations are further supported by previous studies, which highlight the

cumulative impact of diabetes duration on metabolic outcomes. They underscore the increased insulin requirements during puberty, a period when rising levels of growth hormone and sex steroids can worsen glycemic control. Additionally, excess adiposity during this stage may contribute to insulin resistance (Katsarou et al., 2017).

Basal insulin showed a stronger correlation with HbA1c and FBS, suggesting that basal insulin therapy plays a critical role in achieving long-term glycemic control. In contrast, the weak correlation between bolus insulin and RBS implies that postprandial glucose spikes may not be as directly influenced by bolus insulin doses in this cohort. A moderate correlation between FBS and RBS was observed, which is expected, as both are important markers of glycemic status. While HbA1c remains a reliable long-term indicator of average blood glucose, it may not fully capture daily fluctuations. This highlights the importance of integrating both short-term (e.g., FBS, RBS) and long-term (HbA1c) measures when evaluating and optimizing diabetes management (Blonde et al., 2022).

The positive correlation between sIR levels and both RBS and HbA1c suggests that elevated sIR levels may be associated with poorer glycemic control, potentially reflecting underlying insulin resistance. This association reinforces the role of insulin resistance in the pathophysiology and management of diabetes. These findings are consistent with previous evidence highlighting the contribution of impaired insulin signaling to suboptimal glycemic outcomes (Chaudary et al., 2025).

4.3. Genetic Analysis of INSR Polymorphisms (rs2245649 T>C and rs2229429 G>A) in T1D

In the present investigation, three key elements influencing blood glucose regulation were examined in Iraqi patients with T1D: genetic variations in the α-subunit of the *INSR* gene (rs2245649 T>C and rs2229429 G>A), HbA1c levels, and sIR levels. These SNPs were selected based on prior literature indicating their association with persistent hyperglycemia in individuals with T1D undergoing insulin therapy. It was hypothesized that these SNPs in the α-subunit of the *INSR* gene may interfere with insulin binding to its receptor, thereby contributing to insulin resistance. We investigated whether the putative intronic splice variant SNP rs2245649 (T>C), located in intron 8 of the non-coding region of the *INSR* gene, within the FnIII-1 domain of the α-subunit on chromosome 19 (locus chr19:7166377), is associated with elevated HbA1c levels in individuals with T1D. In contrast, the SNP rs2229429 (G>A), also located in the FnIII-1 domain of the α-subunit but situated in exon 8, is considered a benign synonymous mutation in the coding region (Massarenti et al., 2022).

The results of DNA amplification for the investigated polymorphisms revealed three genotypes for rs2245649: TT, TC, and CC. In the sampled Iraqi population (from Kerbala, Najaf, and Babylon), the minor allele frequency (MAF) of the C allele was 26%. The most prevalent genotype was the homozygous TT variant (66.6%), followed by nearly equal frequencies of the heterozygous TC (15.2%) and homozygous CC (18.2%) genotypes. Similarly, genotyping analysis for rs2229429 identified three genotypes: GG, GA, and AA. The GG homozygous genotype was the most common, observed in 83.8% of individuals. Both the GA heterozygous and AA homozygous genotypes were detected at equal frequencies (8.1% each), with a minor allele (A) frequency of 12%.

Consistent with findings from previous studies, the current investigation indicates a significant deviation from Hardy-Weinberg equilibrium (HWE) for both SNPs. The results provide strong evidence against the null hypothesis of HWE, suggesting that the observed genotype distributions are not due to random mating alone. These discrepancies may reflect various factors influencing the genetic structure of the *INSR* gene in this cohort of individuals with T1D. Potential contributing factors include the ethnic and environmental background of the study population, limitations in genotyping methodology, and the relatively small sample size (Massarenti et al., *2022;* Hussein et al., 2023).

These findings align with previous population-based data from the 1000 Genomes Project Consortium, which support the presence of population-specific and disease-associated influences on allele distribution. According to Massarenti et al.(2022), both SNPs showed a heterozygous prevalence of approximately 8% in individuals of European ancestry. In comparison, the C allele of rs2245649 exhibited a minor allele frequency (MAF) of 29% in the Qatari population (Qatar Genome Project, 2024), 0.05% in both Korean and Japanese populations (gnomAD, 2023), and 19% among Caucasians, highlighting notable ethnic variability in its distribution (1000 Genomes Project Consortium, 2015).

Similarly, the A allele of rs2229429 demonstrated a MAF of 13% in the Qatari population (Qatar Genome Project, 2024), 2% in both Korean and Japanese populations (gnomAD, 2023), and 16% in the broader Asian population. These data emphasize the importance of considering ethnic diversity in genetic association studies (1000 Genomes Project Consortium, 2015; Massarenti et al., 2022).

4.3.1. The Impact of *INSR* Gene Variations (rs2245649 T>C and rs2229429 G>A) on Socio-Demographic Factors and Laboratory Parameters in T1D

4.3.1.A. Genotypic Variations of INSR (rs2245649 T>C and rs2229429 G>A) Across Socio-Demographic Variables in T1D

The findings presented in Tables 3-13 and 3-19 did not reach statistical significance for variables such as age, diabetes duration, treatment duration, and mean basal-bolus insulin dosage across both genotypes. This lack of significance may be due to limitations in sample size, as well as variability in factors like age, pubertal status, and lifestyle, which could obscure stronger associations. Although BMI percentile did not differ significantly among rs2245649 genotypes, there was a notable trend toward higher BMI in CC carriers within the healthy and overweight categories compared to the wild type, with p-values of 0.05 and 0.04, respectively. Similarily, homozygous mutant carriers in the healthy weight category showed a significant increase compared to the heterozygous carriers from the same BMI category (p=0.01). These findings might indicate insulin resistance potentially linked to double diabetes (Kietsiriroje et al., 2019).

A notable exception was observed in the underweight category in, where individuals with the AA genotype exhibited slightly higher BMI percentile values compared to GG carriers of the *INSR* (rs2229429) G>A polymorphism (p = 0.02). This finding may reflect impaired insulin receptor signaling in AA carriers, potentially leading to reduced glucose uptake and altered fat metabolism. Similarily, the heterozygous type showed a significant increase with p=0.01 compared to the wild type (Shulman, 2000).

The genotype-related BMI percentile variations observed in this study may represent early phenotypic signs of double diabetes, particularly among obese carriers of the minor alleles. These individuals could have a genetic predisposition to insulin resistance, as highlighted in previous studies, placing them at greater risk for progressive metabolic deterioration (Sesti et al., 2001).

In the current cohort, 39% of participants reported a positive family history of diabetes. Among these, one obese individual with the TC genotype and two with the CC genotype of the rs2245649 variant (Table 3-13), as well as three obese individuals with the GA genotype and one with the AA genotype of the rs2229429 variant (Table 3-19), all reported parental diabetes history. Although mean BMI percentile values in the obese category was around 30 kg/m^2 across all genotypes, no statistically significant differences were detected (Kietsiriroje et al., 2019; Chaudhary et al. 2025).

Based on these observations, it is strongly recommended to conduct further investigations, including lipid profiles, metabolic markers, blood pressure measurements, and waist circumference, in the three obese carriers of the studied INSR SNPs. These additional assessments are essential for exploring the potential presence of double diabetes. Obese patients carrying the minor alleles may have an increased likelihood of insulin resistance due to defective insulin receptor signaling, where disrupted binding of exogenous insulin impairs glucose uptake into cells (Chaudhary et al., 2025).

This leads to excess circulating glucose being redirected to visceral adipose tissue and stored as fat. Excessive fat accumulation in visceral tissue leads to chronic inflammation and increased release of free fatty acids and adipokines, which contribute to insulin receptor desensitization and impair downstream signaling

pathways, ultimately promoting systemic insulin resistance. Chronically elevated glucose levels in response to resistance further promote elevated lipogenesis and inhibit lipolysis, contributing to obesity (Zhang et al., 2015).

This aligns with previous studies reporting associations between INSR genetic variants, BMI regulation, and insulin resistance, highlighting the importance of early detection and the potential for personalized treatment strategies in T1D patients predisposed to double diabetes. Further research involving larger and more diverse cohorts is warranted to validate and expand upon these preliminary findings (Wilkin, 2001; Pozzilli et al., 2014).

4.3.1.B. Genotypic Variations of INSR (rs2245649 T>C and rs2229429 G>A) Across Laboratory Parameters in T1D

4.3.1.B.1. Association of HbA1c Levels with INSR (rs2245649 T>C and rs2229429 G>A) Genotypes in T1D

The presence of the (rs2245649) C allele was significantly associated with elevated HbA1c and sIR levels, along with a slight increase in FBS ($p = 0.03$). HbA1c levels varied significantly among genotypes ($p = 0.01$), with patients carrying the TC genotype showing notably higher mean HbA1c values, followed by the CC genotype, compared to the TT genotype, as shown in Table 3-14 and Figure 3-6A. Similarly, as illustrated in Figure 3-6B, sIR concentrations were significantly elevated in individuals with TC and CC genotypes ($p = 0.02$), which aligns with a previous study (Masserenti et al., 2022) .

Analysis in Table 3-20 revealed that individuals heterozygous for the minor A allele of (rs2229429) exhibited higher HbA1c levels compared to those homozygous for the G allele. This finding suggests, in line with previous studies, a potential association between this SNP and long-term glycemic control. Statistically

significant differences in both HbA1c and sIR levels were observed among the genotypes ($p < 0.001$), with the GA and AA genotype groups showing higher values than the GG group, as presented in Figures 3-8A and 3-8B (Simeon et al., 1992; Simeon et al., 2002).

Our findings are strongly supported by previous studies, which suggest that individuals carrying the minor alleles of both SNPs within the α-subunit of the *INSR* gene are more likely to experience chronic hyperglycemia. This highlights a potential functional role of these *INSR* variants in the pathophysiology of insulin resistance, likely due to disruptions in insulin receptor structure and function. These observations are consistent with our results, which show significantly elevated mean HbA1c levels in minor allele carriers of both SNPs compared to wild-type carriers (Malodobra et al., 2011; Omar et al., 2021).

Previous studies reported similar findings, demonstrating that the *INSR* variants (rs2245649) T>C and (rs2229429) G>A contribute to impaired receptor stability and reduced folding efficiency. These structural alterations disrupt downstream insulin signaling and glucose uptake, ultimately leading to elevated HbA1c levels and persistent hyperglycemia. Our results are consistent with these findings and further underscore the role of both SNPs in the development of insulin resistance in T1D patients, particularly among minor allele carriers, as reflected by their significantly increased HbA1c and sIR levels (Malodobra et al., 2011; Massarenti et al., 2022).

4.3.1.B.2. Association of INSR (rs2245649 T>C and rs2229429 G>A) Genotypes with Poor Glycemic Control in T1D

As shown in Table 3-15, two responders carried the TC genotype, and four CC homozygotes were observed in the responders group. In contrast, the non-responder group included 13 individuals with the TC genotype and 14 with the CC genotype, suggesting a significantly higher prevalence of the C allele among non-responders. These findings support a potential genetic role of the C allele in contributing to severe hyperglycemia, consistent with previous research. Statistical analysis revealed that carriers of the C allele (TC or CC) had a 2.5-fold increased odds of having insulin resistance compared to individuals with the TT genotype (Vorm et al., 1992).

The analysis in Table 3-16 demonstrated a statistically significant increase in HbA1c across all genotype groups as glycemic control worsened from poor to severely poor. Individuals with the TT genotype showed a significant rise in HbA1c ($p < 0.001$), indicating that even wild-type carriers experience deteriorating glycemia under progressively uncontrolled conditions. This trend may be partly attributed to the high proportion of pubertal individuals in the cohort, as illustrated in Figure 3-7B, who are known to undergo significant metabolic and hormonal fluctuations. Factors such as elevated growth hormone levels, increased sex steroids, psychological stress, and reduced physical activity during puberty can contribute to insulin resistance and poor glycemic control, thereby affecting HbA1c levels independently of genotype (Melkersson et al., 2023).

Individuals with the TC genotype demonstrated significantly higher HbA1c levels than TT carriers, even under poor glycemic control, indicating a potential early effect of the C allele on glucose regulation ($p = 0.04$). Moreover, CC

homozygotes in the non-responder group showed the highest HbA1c levels, with a statistically significant elevation compared to responders ($p = 0.001$). This pattern aligns with findings from a previous study, where homozygosity for the C allele was linked to greater glycemic deterioration, likely due to more pronounced insulin receptor dysfunction or resistance. These results demonstrate a clear genotype-dependent effect of the (rs2245649) C allele on HbA1c levels and is reinforced by a previous study. The stepwise increase in HbA1c from TT to TC to CC genotypes among non-reponders suggests that this SNP may contribute to insulin resistance in T1D. Consequently, it could serve as a potential genetic marker for identifying patients at higher risk of persistent hyperglycemia, underscoring the need for further research in larger cohorts (Massarenti et al., 2022).

The distribution of GA and AA genotypes in Table 3-21 revealed a marked predominance among non-responders. These findings suggest that carriers of the A allele have a 10.479-fold increased risk of severe hyperglycemia associated with insulin resistance, compared to individuals carrying the G allele. As shown in Table 3-22, a clear genotype-related increase in HbA1c levels was observed, with the presence of the A allele (in both GA and AA genotypes) significantly associated with elevated HbA1c among non-responders ($p < 0.001$). The complete absence of GA and AA genotypes in the responder group further supports a potential genetic influence on the development of hyperglycemia and difficulties in achieving effective diabetes management in A allele carriers (Uchikawa et al., 2019).

Collectively, our findings underscore the potential role of the (rs2229429) variant in influencing glycemic control, likely through mechanisms involving impaired insulin receptor function or heightened insulin resistance. These results align with previous studies that have shown polymorphisms in the INSR gene, particularly within the FnIII-1 binding domain, which is essential for ligand

specificity and high-affinity insulin binding, can render this region structurally vulnerable to disruption (Rojek et al., 2014; Uchikawa et al., 2019).

Such disruptions may impair mRNA splicing or post-transcriptional processing, resulting in defective receptor maturation or mislocalization. This can reduce the number of functional insulin receptors on the plasma membrane by up to fivefold. As a consequence, structurally compromised receptors exhibit decreased insulin-binding capacity and increased ectodomain cleavage. These alterations diminish insulin-receptor interactions, lower insulin sensitivity, and substantially impair insulin signaling in individuals carrying the minor alleles (Bergman et al., 2002; Zhang et al., 2018).

These impairments hinder AKT pathway activation, restrict glucose uptake, and promote hepatic gluconeogenesis, which is a key mechanisms driving insulin resistance. Consistent with previous studies, our findings support the hypothesis that the minor alleles of both SNPs are linked to altered insulin receptor function, which may reduce the effectiveness of exogenous insulin therapy. This contributes to insulin resistance and underscores their potential role as genetic risk factors in its pathophysiology (Uchikawa et al., 2019; Melkersson et al., 2023).

4.3.1.B.3. Comparison of INSR (rs2245649 T>C and rs2229429 G>A) Genotypes Between Two Age Groups in T1D

No significant differences in HbA1c levels were observed between school-age and adolescent groups across (rs2245649) genotypes, as showed in Table 3-17, suggesting that age did not significantly modify the SNP's effect on glycemic control. Although HbA1c typically increases during puberty, slightly higher HbA1c levels were seen in school-aged children across genotypes in Figure 3-7A, indicating the possibility that the (rs2245649) SNP's influence may not be entirely independent of age. However, the absence of statistically significant differences and the relatively small sample size might have limited the detection of subtle age-related effects on glycemic control (Paschou et al., 2018).

Additionally, Table 3-23 compared mean HbA1c levels between two age groups, school-aged children and adolescents. Results showeds no statistically significant differences within any genotype category, as all p-values exceeded 0.05. Figure 3-10 indicated that most individuals with the heterozygous GA genotype were adolescents undergoing puberty, while the homozygous mutant AA group mainly consisted of school-aged children. This distribution explained the near-equal mean HbA1c values observed across both age groups in Figure 3-9B. Despite the hormonal and pubertal changes typically seen in adolescence, wild-type (GG) genotype carriers exhibited hyperglycemia likely influenced by non-genetic factors such as poor treatment adherence, dehydration, psychological or physical stress, inactivity, hormonal fluctuations, or a sedentary lifestyle. Although a slight upward trend in HbA1c with increasing age was noted among individuals with GA and especially AA genotypes, this variation was not substantial enough to reach statistical significance in this dataset. These findings suggest that the effect of the (rs2229429) A allele on glycemic control remains relatively stable across age

groups, with age having minimal influence on HbA1c levels within this genetic context (Chaudary et al., 2025).

4.3.1.B.4. Association of sIR Levels with HbA1c Groups According to INSR (rs2245649 T>C and rs2229429 G>A) Genotypes in T1D

Our data demonstrated a clear genotype-dependent association between both SNPs and sIR concentrations. In Table 3-18, sIR levels were significantly higher ($p = 0.002$) in non-responders carrying the TC genotype of the rs2245649 SNP, indicating insulin resistance. Similarly, Table 3-24 showed a pronounced and statistically significant increase in sIR levels among non-responders with the GA and AA genotypes of (rs2229429), highlighting a strong association between these genotypes and severe hyperglycemia ($p < 0.001$). Soluble insulin receptor, comprising the ectodomain of the INSR, was found at significantly elevated plasma levels in carriers of the minor alleles, particularly those with the AA and CC genotypes of the INSR gene, consistent with findings by a previous study (Masserenti et al., 2022).

Our results strongly support the role of glycemic stress in elevating sIR levels among genetically susceptible individuals. This finding aligns with previous hypotheses proposed by who suggested that polymorphisms in the INSR gene may disrupt downstream signaling cascade, ultimately leading to severely poor glycemic control (Vorm et al. 1992 and Umehara et al. 2009).

Such disruption potentially results in increased sIR levels in diabetic patients, which may serve as a more immediate indicator of glycemic fluctuations than traditional markers like HbA1c, and is closely associated with insulin resistance. This aligns with previous studies that identified the synonymous SNP (rs2229429) G>A and the intronic splice variant (rs2245649) T>C, located in exon and intron 8

of the INSR gene's α-subunit, as contributors to insulin resistance through their effects on receptor structure and function (Hiriart et al., 2014; Masserenti et al., 2022).

Furthermore, our own findings demonstrated higher sIR in hyperglycemic minor allele carriers which aligns with a previous study (Masserenti et al., 2022). This pattern is further explained by previous studies, that the rapid shedding of sIR is closely linked with insulin resistance in diabetic individuals (Umehara et al., 2009; Soluble Insulin Receptor Study Group, 2007). The observed increase in INSR shedding indicates that certain SNPs within the INSR gene may disrupt normal gene expression or protein folding, thereby reducing the functionality of insulin receptor proteins (Malodobra et al., 2011).

Furthermore, they might influence trafficking to the plasme membrane, by reducing receptor availability on the cell surface, functional, integrity binding efficiency and increasing receptor shedding. Resulting in elevated sIR levels, and disrupted insulin receptor signaling, which aligns with our results showcasing significantly elevated sIR for the minor allele carriers of both SNPs. Contributing to the pathogenesis of insulin resistance and persistent hyperglycemia observed in the non-responders to exogenous insulin therapy (Umehara et al., 2009).

The High sIR levels in circulation have been proposed as a marker of insulin receptor dysfunction, thereby acting weakly without triggering downstream signaling. This contributes to reduced bioavailability of insulin at functional membrane-bound receptors, a hallmark feature of peripheral insulin resistance, which aligns with our findings (Vorm et al., 1992; Malodobra et al. 2011).

4.4. Conclusion

- The study identified the presence of homozygous minor alleles (C/C for rs2245649 and A/A for rs2229429) among a sample in Iraqi patients with T1D, indicating a clear distribution pattern of these genotypes in the target population.
- It was found that T1D patients carrying the minor alleles of both SNPs were strongly associated with severe hyperglycemia.
- Carriers of the minor alleles of both SNPs were at a higher risk of developing insulin resistance. These polymorphisms are therefore strongly associated with chronic hyperglycemia despite insulin treatment.

4.5. Recommendations for Future Perspectives

- Future research should adopt a multidimensional approach that integrates clinical, metabolic, and immunological profiling to better elucidate genotype-phenotype relationships in T1D in a larger sample size and in diverse regions in Iraq.
- Long-term, large-scale clinical trials are also warranted to assess the safety and effectiveness of non-insulin antidiabetic therapies, particularly those targeting insulin resistance in T1D.
- In addition, gene-editing technologies like CRISPR-Cas9 offer promising therapeutic potential for correcting pathogenic mutations in stem cells; however, their translation to clinical practice necessitates thorough investigation into ethical, regulatory, and biosafety concerns, including genomic stability and immune responses.
- Complementary to these efforts, the development of computational models to simulate the dynamics of the soluble insulin receptor could enhance our understanding of its role in insulin sensitivity and glucose regulation, thereby informing future therapeutic strategies.

References

References

1000 Genomes Project Consortium (2015). *A global reference for human genetic variation*. Nature,526(7571),pp. 68–74.

Agiostratidou, G., Anhalt, H., Ball, D. et al., (2017). Standardizing clinically meaningful outcome measures beyond HbA1c for type 1 diabetes: a consensus report of the American Association of Clinical Endocrinologists, the American Association of Diabetes Educators, the American Diabetes Association, the Endocrine Society. Diabetes Care, 40(12), pp.1622–1630.

Ahmed, I., Siddiqui, H.I., Qureshi, G.S. et al., (2022). A review of literature on the pharmacogenomics of single-nucleotide polymorphisms. *Biomedical and Biotechnology Research Journal*, 6(1), pp.14–20.

Al-Gazali, L.I., Khalil, M. and Devadas, K. (1993). A syndrome of insulin resistance resembling leprechaunism in five sibs of consanguineous parents. *Journal of Medical Genetics*, 30(6), pp.470–475.

Al-Hamwandi, A. M. H., Mohammad, H. F. & Ahmed, S. A. (2021). Pattern of Nutritional Obesity Among 4-6-Year-Old Children in Sulaymaniyah City, Iraq. *Crescent Journal of Medical and Biological Sciences*, 8.

Al-Kashwan, T.A., Algenabi, A.H.A., Omara, A.M. et al., (2021). Association of vitamin D receptor gene polymorphisms BsmI (rs 1544410) and TaqI (rs 731236) with the type 2 diabetes mellitus in Iraqi patients from the middle. *Meta Gene*, *28*, p.100854.

Alam, S., Hasan, M.K., Neaz, S. et al., (2021). Diabetes mellitus: insights from epidemiology, biochemistry, risk factors, diagnosis, complications and comprehensive management. *Diabetology*, 2(2), pp.36–50.

Almahfoodh, D., Alabbood, M., Alali, A. et al., (2017). Epidemiology of type 1 diabetes mellitus in Basrah, Southern Iraq: A retrospective study. *Diabetes Research and Clinical Practice*, 133, pp.104–108.

Alsabbagh, F.F., Mosa, A.U., HM, A.A. et al., (2023). ORAI1 Genetic Polymorphism and its Correlation with Erythropoietin Resistance, Hemoglobin, and Erythropoietin Serum Level in Iraqi Patients with Chronic Renal Failure on Hemodialysis. *Karbala Journal of Pharmaceutical Sciences*, *14*(23), pp.74-83.

Alyas, J., Rafiq, A., Amir, H. et al., (2021). Human insulin: history, recent advances, and expression systems for mass production. *Biomedical Research and Therapy*, *8*(9), pp.4540-4561.

American Diabetes Association, (2019). Standards of medical care in diabetes—2019 abridged for primary care providers. *Clinical Diabetes*: A Publication of the American Diabetes Association, 37(1), pp.11–34.

American Diabetes Association, (2020). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, 43(Supplement 1), pp.S14–S31.

American Diabetes Association, (2022). *Diabetes care*: A manual for healthcare professionals, pp.S1–S130.

Antar, S.A., Ashour, N.A., Sharaky, M. et al., (2023). Diabetes mellitus: Classification, mediators, and complications; a gate to identify potential targets for the development of new effective treatments. *Biomedicine & Pharmacotherapy*, 168, p.115734.

Ardon, O., Procter, M., Tvrdik, T. et al., (2014). Sequencing analysis of insulin receptor defects and detection of two novel mutations in INSR gene. *Molecular Genetics and Metabolism Reports*, 1, pp.71–84.

Atkinson, M.A., Eisenbarth, G.S. and Michels, A.W. (2013). Type 1 diabetes. *The Lancet*, 381(9861), pp.2449–2462.

Atoum, M.F. (2022). Association of leptin receptor Q223R gene polymorphism and breast cancer patients: a case control study. *Asian Pacific Journal of Cancer Prevention*, *23*(1), p.177.

Baltimore, D., Berg, P., Botchan, M. et al., (2015). A prudent path forward for genomic engineering and germline gene modification. *Science*, *348*(6230), pp.36-38.

Barrett, J.C., Clayton, D.G., Concannon, P. et al., (2009). Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nature Genetics*, 41(6), pp.703–707.

Bedair, R.N., Magour, G.M., Ooda, S.A. et al., (2020). Could insulin receptor H1085H C > T single nucleotide polymorphism predict insulin resistance in type 2 diabetic and chronic hepatitis C virus patients in Egypt? *Egyptian Liver Journal*, 11, pp.1-11.

Bergman, R.N., Ader, M., Huecking, K. et al., (2002). Accurate assessment of β-cell function: the hyperbolic correction. *Diabetes*, *51*(suppl_1), pp.S212-S220.

Bielka, W., Przezak, A., Molęda, P. et al., (2024). Double diabetes—when type 1 diabetes meets type 2 diabetes: definition, pathogenesis and recognition. *Cardiovascular Diabetology*, 23, p.62.

Blonde, L. (2023). American Association of Clinical Endocrinology Clinical Practice Guideline: Developing a Diabetes Mellitus Comprehensive Care Plan. *Endocrine Practice*, *29*(1), pp.80-81.

Blonde, L., Umpierrez, G.E., Reddy, S.S. et al., (2022). American Association of Clinical Endocrinology Clinical Practice Guideline: Developing a Diabetes Mellitus Comprehensive Care Plan. *Endocrine Practice*, 28(10), pp.923–1049.

Chaudhary, H., Patel, J., Jain, N.K. et al., (2021). The role of polymorphism in various potential genes on polycystic ovary syndrome susceptibility and pathogenesis. *Journal of Ovarian Research*, 14(1), p.125.

Chaudhary, R.K., Ali, O., Kumar, A. et al., (2025). Double diabetes: a converging metabolic and autoimmune disorder redefining the classification and management of diabetes. *Cureus*, 17(3), p.e80495

Chauhan, N. and Jain, U. (2017) 'Laboratory diagnosis of HbA1c', *Journal of Nanomedicine Research*, 5(4), pp. 1–10

Chiang, J.L., Maahs, D.M., Garvey, K.C. et al., (2018). Type 1 diabetes in children and adolescents: a position statement by the American Diabetes Association. *Diabetes care*, 41(9), p.2026.

Choi, E., Kikuchi, S., Gao, H. et al., (2019). Mitotic regulators and the SHP2-MAPK pathway promote IR endocytosis and feedback regulation of insulin signaling. *Nature Communications*, 10(1), p.1473.

Chrudinová, M., Žáková, L., Marek, A. et al., (2018). A versatile insulin analog with high potency for both insulin and insulin-like growth factor 1 receptors: structural implications for receptor binding. *The Journal of Biological Chemistry*, 293(43), pp.16818–16829.

Cleland, S.J. (2012). Cardiovascular risk in double diabetes mellitus–when two worlds collide. *Nature Reviews Endocrinology*, 8(8), pp.476–485.

Cleland, S.J., Fisher, B.M., Colhoun, H.M. et al., (2013). Insulin resistance in type 1 diabetes: what is 'double diabetes' and what are the risks? *Diabetologia*, 56(7), pp.1462–1470.

Cobelli, C. Giovanni, S., Magni, L., De Nicoloa, G. et al., (2009). Diabetes: models, signals, and control. *IEEE Reviews in Biomedical Engineering*, *2*, pp.54-96.

Daghestani, M.H. (2020). RS1799817 in INSR associates with susceptibility to polycystic ovary syndrome. *Journal of Medical Biochemistry*, 39(2), pp.149–159.

De Meyts, P. (2015). Insulin/receptor binding: the last piece of the puzzle? What recent progress on the structure of the insulin/receptor complex tells us (or not) about negative cooperativity and activation. *BioEssays*, 37(4), pp.389–397.

De Meyts, P. (2016). The insulin receptor and its signal transduction network. *Endotext* [online]. South Dartmouth (MA): MDText.com, Inc. Available at: https://www.ncbi.nlm.nih.gov/books/NBK378978

Del Chierico, F., Rapini, N., Deodati, A. et al., (2022). Pathophysiology of type 1 diabetes and gut microbiota role. *International Journal of Molecular Sciences*, 23(23), p.14650.

Delgado, J.A. and Bauça, J.M., (2021). Monitoring of diabetic patients with poor glycemic control: Are international recommendations met? *EJIFCC*, 32(1), pp.78–84.

Diabetes.co.uk. (2025). *Type 5 Diabetes Formally Recognised by IDF*. [online] Available at: https://www.diabetes.co.uk/news/2025/apr/type-5-diabetes-formally-recognised.html.

DiMeglio, L.A., Evans-Molina, C. and Oram, R.A. (2018). Type 1 diabetes. *The* Lancet, 391(10138), pp.2449–2462.

Donner, T. and Sarkar, S., (2015). Insulin–pharmacology, therapeutic regimens, and principles of intensive insulin therapy. *Endotext* [online]. South Dartmouth (MA): MDText.com, Inc. Available at: https://www.ncbi.nlm.nih.gov/books/NBK279408

Duckworth, W.C. and Kitabchi, A.E. (1981). Insulin metabolism and degradation. *Endocrine Reviews*, 2(2), pp.210–233.

Dvornikova, K.A., Bystrova, E.Y., Platonova, O.N. et al., (2020). Polymorphism of toll-like receptor genes and autoimmune endocrine diseases. *Autoimmunity Reviews*, 19(4), p.102496.

ElSayed, N.A., Aleppo, G., Aroda, V.R. et al., (2022). Introduction and Methodology: Standards of Care in Diabetes—2023. *Diabetes Care*, 46, pp.S1–S4.

Eng, S., Michonova-Alexova, E. and Alexov, E. (2008). Approaches and resources for prediction of the effects of non-synonymous single nucleotide polymorphism on protein function and interactions. *Current Pharmaceutical Biotechnology*, 9(2), pp.123–133.

Ewald, N. and Hardt, P.D. (2013). Diagnosis and treatment of diabetes mellitus in chronic pancreatitis. *World Journal of Gastroenterology*, 19(42), pp.7276–7281.

Farrukh, A., Khattak, S.H., Kaleem, I. et al., (2022). Plant based nanotechnology–a new trend in therapeutic approaches of diabetes. *Endocrinology & Diabetes Open Access Journal*, 1(1), p.2-501.

Favorgen Biotech Corp. (2023) *FavorPrep™ Tissue Genomic DNA Extraction Micro Kit* [online]. Available

at: https://www.favorgen.com/en/product.php?act=view&cid=12&id=26 (Accessed: 8 August 2024).

Ferreira-Hermosillo, A., Ibarra-Salce, R., Rodríguez-Malacara, J. et al., (2020). Comparison of indirect markers of insulin resistance in adult patients with double diabetes. *BMC Endocrine Disorders*, 20(1), p.87.

Gachoki, J.M., (2020). Comparative analysis of random blood glucose levels in serum, plasma and whole blood using glucose oxidase and hexokinase methods under spectrophotometric and electrochemical techniques. *Medical Science and Clinical Research*, 7(2), pp. 44-52.

Gagnon, C.A., Schmitt, J.A. and Ashraf, A.P (2023). Children and adolescents with hybrid diabetes: A management conundrum. *Endocrine Practice*, 30(1), pp.49–56.

Gelal, B., Poudel, B. & Campus, M.M., (2014)**.** Association of estimated average glucose (eAG) with fasting, post-prandial and average of fasting and PP serum glucose levels. *Clinics*, 11, pp.1–18.

Gerstein, H.C. and Rutty, C.J., (2021). Insulin therapy: the discovery that shaped a century. *Canadian Journal of Diabetes*, *45*(8), pp.798-803.

Giri, B., Dey, S., Das, T. et al., (2018). Chronic hyperglycemia mediated physiological alteration and metabolic distortion leads to organ dysfunction, infection, cancer progression and other pathophysiological consequences: an update on glucose toxicity. *Biomedicine & Pharmacotherapy*, 107, pp.306–328.

gnomAD Consortium (2023) Genome Aggregation Database (gnomAD). *Available at: https://gnomad.broadinstitute.org (Accessed: 29 January 2025).*

Goodarzi, M.O., Louwers, Y.V., Taylor, K.D. et al., (2011). Replication of association of a novel insulin receptor gene polymorphism with polycystic ovary syndrome. *Fertility and Sterility*, 95(5), pp.1736–1741.e1–11.

Goyal, S., Rani, J., Bhat, M.A. et al., (2023). Genetics of diabetes. *World Journal of Diabetes*, 14(6), pp.656–679.

Gregory, G.A., Robinson, T.I., Linklater, S.E. et al., (2022). Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040: a modelling study. *The Lancet Diabetes & Endocrinology*, 10(10), pp.741–760.

Grimwood, J., Gordon, L.A., Olsen, A.S. et al., (2004). The DNA sequence and biology of human chromosome 19. *Nature*, 428(6982), pp.529–535.

Gu, X., Shen, Y., Fu, L. et al., (2018). Association between rs1799817 polymorphism of INS-R gene and type 1 diabetes mellitus complicated with diabetic nephropathy. *Journal of Hainan Medical University*, 24(20), pp.43–46.

Gutmann, T., Schäfer, I.B., Poojari, C. et al., (2019). Cryo-EM structure of the complete and ligand-saturated insulin receptor ectodomain. *Journal of Cell Biology*, 219(1), p.e201907210.

Hadi, Z.S., Al-Kaseer, E.A. and Al-Zubaidi, M.A. (2018). Growth of diabetic children in post-conflict Baghdad, Iraq. *Journal of the Faculty of Medicine Baghdad*, 60(1), pp.69–73.

Haller, M.J., Atkinson, M.A. and Schatz, D. (2005). Type 1 diabetes mellitus: Etiology, presentation, and management. *Pediatric Clinics of North America*, 52(2), pp.391–410.

Hiriart-Urdanivia, M., Sánchez-Soto, C., Velasco, M. et al., (2019). Soluble insulin receptor and metabolic syndrome. *Gaceta médica de México*, 155(5), pp.500-503.

Hiriart, M., Sanchez-Soto, C., Diaz-Garcia, C.M. et al., (2014). Hyperinsulinemia is associated with increased soluble insulin receptors release from hepatocytes. *Frontiers in Endocrinology*, 5, p.95.

Hu, J.L., Hu, X.L., Han, Q. et al., (2017). INSR gene polymorphisms correlate with sensitivity to platinum-based chemotherapy and prognosis in patients with epithelial ovarian cancer. *Gene Therapy*, 24(7), pp.392–398.

Huang, G., Song, C., Wang, N. et al., (2019). RNA-binding protein CUGBP1 controls the differential INSR splicing in molecular subtypes of breast cancer cells and affects cell aggressiveness. *Carcinogenesis*, 41, pp.1294–1305.

Hussain, A.F., (2023). Tamoxifen Therapy in Postmenopausal Iraqi Women with Breast Cancer: Evaluating Side Effects and Hot Flash Incidence. *Ann Clin Med Case Rep*, *12*(3), pp.1-6.

Hussein, S.A., Ibrahim, B.A. and Abdullah, W.H. (2023). Nutritional status of children and adolescents with Type 1 Diabetes Mellitus in Baghdad: A case-control study. *Journal of Medicine and Life*, 16(2), pp.254–260.

James, E.A., Joglekar, A.V., Linnemann, A.K. et al., (2023). The beta cell-immune cell interface in type 1 diabetes (T1D). *Molecular Metabolism*, p.101809.

Jayakrishnapillai, P.V., Shantikumar, V.N. and Kamalasanan, K. (2017). Current trend in drug delivery considerations for subcutaneous insulin depots to treat diabetes. *Colloids and Surfaces B: Biointerfaces*, 153, pp.123–131.

Jiráček, J. and Žáková, L. (2017). Structural perspectives of insulin receptor isoform-selective insulin analogs. *Frontiers in Endocrinology*, 8, p.167.

Katsarou, A., Gudbjörnsdottir, S., Rawshani, A. et al., (2017). Type 1 diabetes mellitus. *Nature Reviews Disease Primers*, 3, pp.1–17.

Kavran, J.M., McCabe, J.M., Byrne, P.O. et al., (2014). How IGF-1 activates its receptor. *Elife*, 3, p.e03772.

Kietsiriroje, N., Pearson, S., Campbell, M. et al., (2019). Double diabetes: A distinct high-risk group? *Diabetes, Obesity and Metabolism*, 21(12), pp.2609–2618.

Kumar, R., Saha, P., Kumar, Y., Sahana, S. et al., (2020). A review on diabetes mellitus: type 1 & Type 2. *World Journal of Pharmacy and Pharmaceutical Sciences*, 9(10), pp.838–850.

Lek, M., Karczewski, K.J., Minikel, E.V. et al., (2016). Analysis of protein-coding genetic variation in 60,706 humans. *Nature*, 536, pp. 2.

Liu, X., Wang, K., Hou, S. et al., (2022). Insulin induces insulin receptor degradation in the liver through EphB4. *Nature Metabolism*, 4(9), pp.1202–1213.

Malodobra, M., Pilecka, A., Gworys, B. et al., (2011). Single nucleotide polymorphisms within functional regions of genes implicated in insulin action and association with the insulin resistant phenotype. *Molecular and cellular biochemistry*, *349*, pp.187-193

Marcovecchio, M.L. (2017). Complications of acute and chronic hyperglycemia. *US Endocrinol*, 13(1), pp.17–21.

Marcovecchio, M.L. (2020). Importance of identifying novel biomarkers of microvascular damage in type 1 diabetes. *Molecular Diagnosis & Therapy*, 24(5), pp.507–515.

Massarenti, L., Aniol-Nielsen, C., Enevold, C. et al., (2022). Influence of insulin receptor single nucleotide polymorphisms on glycaemic control and formation of anti-insulin antibodies in diabetes mellitus. *International Journal of Molecular Sciences*, 23(12), p.6481.

Mayer, J.P., Zhang, F. and DiMarchi, R.D. (2007). Insulin structure and function. *Biopolymers*, 88(5), pp.687–713.

Mazucanti, C., Kennedy, V.L., Cho, A., Liu, Q.-R. et al., (2022). 1320-P: Insulin receptors and their signal transduction pathways in choroid plexus. *Diabetes*, 71, p.A1320.

Melkersson, K. and Persson, B. (2023). Associations between heredity, height, BMI, diabetes mellitus type 1 or 2 and gene variants in the insulin receptor (INSR) gene in patients with schizophrenia. *Neuroendocrinology Letters*, 44(1).

Meng, X., Chen, B. and Zhang, J. (2017). Intracellular insulin and impaired autophagy in a zebrafish model and a cell model of type 2 diabetes. *Ivyspring International Publisher*, 13(8), pp.985–995.

Misra, A., Joshi, S. & Mithal, A., (2025). Malnutrition-related diabetes mellitus: Rushing toward "type 5" amid unresolved questions and limited evidence. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 19(5), p.103250

Mo, R., Jiang, T., Di, J. et al., (2014). Emerging micro-and nanotechnology based synthetic approaches for insulin delivery. *Chemical society reviews*, *43*(10), pp.3595-3629.

Mobasseri, M., Shirmohammadi, M., Amiri, T. et al., (2020). Prevalence and incidence of type 1 diabetes in the world: A systematic review and meta-analysis. *Health Promotion Perspectives*, 10(2), p.98.

Nobakht, H., Mahmoudi, T., Sabzikarian, M. et al., (2020) .Insulin and insulin receptor gene polymorphisms and susceptibility to nonalcoholic fatty liver disease. *Arquivos de Gastroenterologia*, 57(3), pp.203–208.

Norris, J.M., Johnson, R.K. and Stene, L.C. (2020). Type 1 diabetes—early life origins and changing epidemiology. *The Lancet Diabetes & Endocrinology*, 8(3), pp.226-238.

Nyaga, D.M., Vickers, M.H., Jefferies, C. et al., (2018). The genetic architecture of type 1 diabetes mellitus. *Molecular and Cellular Endocrinology*, 477, pp.70–80.

Ogrotis, I., Koufakis, T. and Kotsa, K. (2023). Changes in the global epidemiology of type 1 diabetes in an evolving landscape of environmental factors: causes, challenges, and opportunities. *Medicina*, 59(4), p.668.

Oleiwi Jasim, A.R., Abdul Razzaq, N., Thoulfikar A. Imeer, A. et al., (2023). Epidemiological profile and diabetes control of Type 1 Diabetes Mellitus patients in Karbala Governorate, Iraq. *F1000Research*, 12, p.409.

Omar, G.A., Hegab, W.S. and Ghanem, A.I. (2021). Association among insulin receptor substrate1 genetic polymorphism, sulfonylurea therapeutic efficacy, and insulin resistance in patients with type 2 diabetes mellitus. *Journal of Medicine in Scientific Research*, 4(3), pp.196.

Pasaribu, S.F., Wiboworini, B. and Kartikasari, L.R. (2021). Effect of germinated black rice Krisna extract on fasting blood glucose and body weight in diabetes mellitus rats. *International Journal of Nutrition Sciences*, 6(4), pp.194–200.

Paschou, S.A., Papadopoulou-Marketou, N., Chrousos, G.P. et al., (2018). On type 1 diabetes mellitus pathogenesis. *Endocrine Connections*, 7(1), pp.R38-R46.

Patterson, C.C., Karuranga, S., Salpea, P. et al., (2019). Worldwide estimates of incidence, prevalence and mortality of type 1 diabetes in children and adolescents: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*, 157, p.107842.

Payankaulam, S., Raicu, A.M. and Arnosti, D.N. (2019). Transcriptional regulation of INSR, the insulin receptor gene. *Genes*, 10(12), p.984.

Pitt, J.P., McCarthy, O.M., Wellman, B.M. et al., (2020). Factors influencing insulin absorption around exercise in type 1 diabetes. *Frontiers in Endocrinology*, 11, p.573275.

Pleus, S., Tytko, A., Landgraf, R. et al., (2024). Definition, classification, diagnosis and differential diagnosis of diabetes mellitus. *Experimental and Clinical Endocrinology & Diabetes*, 132(3), pp.112-124.

Pociot, F. and McDermott, M.F. (2002). Genetics of type 1 diabetes mellitus. *Genes & Immunity*, 3, pp.235-249.

Pociot, F., Akolkar, B., Concannon, P. et al., (2010). Genetics of type 1 diabetes: what's next? *Diabetes*, 59(7), pp.1561–1571.

Pociot, F., Lernmark, Å. and Genetic Factors Research Group. (2017). Genetic risk factors for type 1 diabetes. *The Lancet*, 387(10035), pp.2331-2339.

Popoviciu, M.S., Kaka, N., Sethi, Y. et al., (2023). Type 1 diabetes mellitus and autoimmune diseases: a critical review of the association and the application of personalized medicine. *Journal of Personalized Medicine*, 13(3), p.422.

Pozzilli, P. and Guglielmi, C., (2014). Double diabetes: a mixture of type 1 and type 2 diabetes in youth. *Diabetes Care*, 37(2), pp.593-599.

Qatar Genome Project (2024) *Qatar population allele frequencies, Qatar Precision Health Institute Report*. Available at: https://www.qphi.org.qa/genetic-findings (Accessed: 29 January 2025).

Quattrin, T., Mastrandrea, L.D. and Walker, L.S. (2023). Type 1 diabetes. *The Lancet*, 401(10394), pp.2149-2162.

Quon, M.J., Guerre-Millo, M., Zarnowski, M.J. et al., (1994). Tyrosine kinase-deficient mutant human insulin receptors (met1153-to-ile) overexpressed in transfected rat adipose cells fail to mediate translocation of epitope-tagged GLUT4. *Proceedings of the National Academy of Sciences*, 91, pp.5587-5591.

Rahman, M. and Berenson, A.B. (2010). Accuracy of current body mass index in women aged 10–21. *Obstetrics & Gynecology*, 115(5), pp.982-988.

Rahman, M.S., Hossain, K.S., Das, S. et al., (2021). Role of insulin in health and disease: an update. *International Journal of Molecular Sciences*, 22(12), p.6403.

Redondo, M.J., Krischer, J.P., Type 1 Diabetes TrialNet Study Group. Et al., (2008). Autoantibody "subspecificity" in type 1 diabetes: risk for organ-specific autoimmunity clusters more by autoantibody specificities than by family history. *Diabetes Care*, 31(1), pp.17-22.

Regnell, S.E. and Lernmark, Å. (2017). Early prediction of autoimmune (type 1) diabetes. *Diabetologia*, 60(8), pp.1370-1381.

Renna, L.V., Bosè, F., Iachettini, S. (2017). Receptor and post-receptor abnormalities contribute to insulin resistance in myotonic dystrophy type 1 and type 2 skeletal muscle. *PLoS One*, *12*(9), p.e0184987.

Ridha, I.A., Khalaf, B.H. and Mahdi, J.F., (2022). The Influence of rs2943641 of Insulin Receptor Substrate-1 on Metformin Response in Iraqi Male with Type 2 Diabetes Mellitus. *Biochemical & Cellular Archives*, *22*(1).

Roep, B.O., Thomaidou, S., Van Tienhoven, R. and Zaldumbide, A. (2021). Type 1 diabetes mellitus as a disease of the β-cell (do not blame the immune system?). *Nature Reviews Endocrinology*, *17*(3), pp.150-161.

Rojek, A. and Niedziela, M. (2014). Insulin receptor and its relationship with different forms of insulin resistance. *Medical Journal of Cell Biology*, 2, pp.59–90

Roy, L. and Bhattacharjee, M. (2020). Overview of novel routes of insulin: current status. *Int J Adv Med*, 7, p.1597.

Saberzadeh-Ardestani, B., Karamzadeh, R., Basiri, M. et al., (2018). Type 1 Diabetes Mellitus: *Cellular and Molecular Pathophysiology at A Glance*. *Cell Journal*, 20, pp.294-301.

Saltiel, A.R., (2021). Insulin signaling in health and disease. *The Journal of Clinical Investigation*, 131(1), pp.e143789.

Sambrook, J. (1989). Molecular cloning: a laboratory manual. *Cold Spring Habor Laboratory*.

Sánchez-Pozos, K., de los Ángeles Granados-Silvestre, M. and Guadalupe Ortíz-López, M. (2021). From Pharmacogenetics to Gene Expression: Implications for *Precision Medicine in Diabetes.* Drug Metabolism, 2021. ISBN: 9781839688805.

Santoro, A., McGraw, T.E. and Kahn, B.B (2021). Insulin action in adipocytes, adipose remodeling, and systemic effects. *Cell Metabolism*, 33(4), pp.748-757.

Scapin, G., Dandey, V.P., Zhang, Z. et al., (2018). Structure of the insulin receptor–insulin complex by single-particle cryo-EM analysis. *Nature*, 556(7699), pp.122-125.

Seino, S., Seino, M., Nishi, S. et al., (1989). Structure of the Human Insulin Receptor Gene and Characterization of Its Promoter. *Proceedings of the National Academy of Sciences*, 86, pp.114-118.

Sesti, G., Federici, M., Hribal, M.L., et al., (2001). Defects of the insulin receptor substrate (IRS) system in human metabolic disorders. *FASEB Journal*, 15(12), pp.2099-2111.

Sesti, G., Federici, M., Lauro, D. et al., (2001). Molecular mechanism of insulin resistance in type 2 diabetes mellitus: role of the insulin receptor variant forms. *Diabetes/metabolism research and reviews*, *17*(5), pp.363-373.

Shimojima, K., Okamoto, N., Tamasaki, A. et al. (2015). An association of 19p13.2 microdeletions with Malan syndrome and Chiari malformation. American Journal of Medical Genetics Part A, 167A(4), pp.724–730.

Shulman, G.I., (2000). Cellular mechanisms of insulin resistance. *Journal of Clinical Investigation*, 106(2), pp.171–176.

Soluble Insulin Receptor Study Group et al., (2007) 'Soluble insulin receptor ectodomain is elevated in the plasma of patients with diabetes', *Diabetes*, 56(8), pp. 2028–2035.

Stümvoll, M., Goldstein, J.B. and Haeften, V.W.T., (2005). Type 2 diabetes: principles of pathogenesis and therapy. *The Lancet*, 365(9467), pp.1333-1346.

Sunlong Biotech (2023) Rat CuZn-SOD ELISA Kit [online]. Available at: Sunlong Biotech website (Accessed: 1 August 2024).

Syed, F.Z., (2022). Type 1 diabetes mellitus. *Annals of Internal Medicine*, 175(3), pp.ITC33-ITC48.

Taylor, S.I., Cama, A., Accili, D. et al., (1992). Mutations in the Insulin Receptor Gene. *Endocrine Reviews*, 13(3), pp.566–595.

Taylor, S.I., Kadowaki, T., Kadowaki, H. et al., (2010). Mutations in Insulin-Receptor Gene in Insulin-Resistant Patients. Mutations in insulin-receptor gene in insulin-resistant patients. *Diabetes Care*, 13(3), pp.257–279.

Teh, C.P.W., Nizam, N.A., Nee, C.P. et al., (2016). Association between polymorphisms of insulin and insulin receptor gene with childhood obesity in Malay population. *Journal of Endocrinology and Metabolism*, 6(1), pp.20-25.

Teng, S., Michonova-Alexova, E., Alexov, E. (2008). Approaches and resources for prediction of the effects of non-synonymous single nucleotide polymorphism on

protein function and interactions. *Current Pharmaceutical Biotechnology*, 9(2), pp.106-112.

Tomic, D., Shaw, J.E. and Magliano, D.J. (2022). The burden and risks of emerging complications of diabetes mellitus. *Nature Reviews Endocrinology*, 18(9), pp.525-539.

Uchikawa, E., Choi, E., Shang, G. et al., (2019). Activation mechanism of the insulin receptor revealed by cryo-EM structure of the fully liganded receptor–ligand complex. *eLife*, 8, p.e48630.

Umehara, A., Nishioka, M., Obata, T. et al., (2009). A novel ultra-sensitive enzyme immunoassay for soluble human insulin receptor ectodomain and its measurement in urine from healthy subjects and patients with diabetes mellitus. *Clinical Biochemistry*, 42, pp.1468–1475.

Van der Vorm, E.R., Van der Zon, G.C., Müller, W., Krans, H.M. et al., (1992). An Arg for Gly substitution at position 31 in the insulin receptor, linked to insulin resistance, inhibits receptor processing and transport. *Journal of Biological Chemistry*, 267(1), pp.66-71.

Venkatachalapathy, P., Padhilahouse, S., Sellappan, M. et al., (2021). Pharmacogenomics and personalized medicine in type 2 diabetes mellitus: Potential implications for clinical practice. *Pharmacogenomics and Personalized Medicine*, 14, pp.1441-1455.

Vihinen, M. (2022). When a synonymous variant is nonsynonymous. *Genes*, 13(8), p.1485.

Villegas-Valverde, C.C., Kokuina, E. and Breff-Fonseca, M.C. (2018). Strengthening national health priorities for diabetes prevention and management. *MEDICC Review: International Journal of Cuban Health and Medicine* , 20(4), p.5.

Villegas, R., Delahanty, R., Williams, S. et al., (2015). Genetic variation and insulin resistance in middle-aged Chinese men. *Annals of Human Genetics*, 79(5), pp.357-365.

Wang, T., Wang, J., Hu, X. et al., (2020). Current understanding of glucose transporter 4 expression and functional mechanisms. *World Journal of Biological Chemistry*, 11(3), pp.76-98.

Weis, F., Menting, J.G., Margetts, M.B. et al., (2018). The signalling conformation of the insulin receptor ectodomain. *Nature Communications*, 9(1), p.4420.

White, M.F. and Kahn, C.R. (2021). Insulin action at a molecular level - 100 years of progress. *Molecular Metabolism*, 52, p.101304.

Wilkin, T.J., (2001). The accelerator hypothesis: weight gain as the missing link between type I and type II diabetes. *Diabetologia*, 44(7), pp.914-922.

Wolosowicz, M., Lukaszuk, B. and Chabowski, A. (2020). The causes of insulin resistance in type 1 diabetes mellitus: Is there a place for quaternary prevention? *Diabetologia*, 63(9), pp.1713-1718.

Xu, Y., Kong, G.K.W., Menting, J.G. et al., (2018). How ligand binds to the type 1 insulin-like growth factor receptor. *Nature Communications*, 9(1), p.821.

Yunn, N., Kim, J., Ryu, S.H. et al., (2023). A stepwise activation model for the insulin receptor. *Experimental & Molecular Medicine*, 55(10), pp.2147-2161.

Zaccardi, F., Webb, D.R., Yates, T. et al., (2016). Pathophysiology of type 1 and type 2 diabetes mellitus: a 90-year perspective. *Postgraduate Medical Journal*, 92(1084), pp.63–69.

Zhang, L., J Li, Y., Lu, J. et al., (2018). PI4KIIα regulates insulin secretion and glucose homeostasis via a PKD-dependent pathway. *Springer Science+Business Media*, 4(1), pp.25.

Zhang, M., Hu, T., Zhang, S. and Zhou, L., (2015). Associations of different adipose tissue depots with insulin resistance: A systematic review and meta-analysis of observational studies. *Scientific Reports*, 5, p.18495.

References

Appendices

Ministry of Higher Education
and Scientific Research
University of Karbala
College of Pharmacy
Department of Pharmacology & Tox.

جمهـــورية العـــراق
وزارة التعليم العالي والبحث العلمي
جامـــعة كربلاء
كلـــية الصيـــدلـــة
شعبة الدراسات العليا

Issue No.:
Date:

العدد: د.ع/ 6 / ١٤١٠
التاريخ: 17/ 7/ 2023

6	حسام صالح حسن	Genetic Polymorphisms of UDP-Glucuronate UGT1A1 enzyme in response of thalassemia patient treated with deferasirox therapy	المشرف الأول :- أ.م.د. احمد حقي اسماعيل
7	محمد سهيل عبد	Impact of genetic polymorphism of GLUT4 on response to exogenous insulin therapy in type 1 diabetic Iraqi patients.	المشرف الأول :- م.د. محمد إبراهيم رسول
8	فرح علي مهدي	Investigation of genetic polymorphism in insulin receptor gene (INSR) on response to exogenous insulin therapy in type 1 Diabetic patients in a sample of iraqi patients	المشرف الاول :- :- أ.م.د. محمد إبراهيم رسول
9	رحاب كاظم صادق	the impact of CYP19A1 genetic polymorphism on therapeutic response of letrozole in Iraqi infertile women	المشرف الاول :- أ.م. آمال عمران موسى
10	نوار رياض كريم	impact of genetic polymorphism of Cytochrome P450 2A6 on therapeutic response of letrozole in infertile women	المشرف الاول :- أ.م. آمال عمران موسى
11	خالد قاسم محمد	Impact of PCSK9 genes polymorphism on atrovastatin response in hyperlipidemic patients in kerbala province	أ.م. مازن حامد عودة أ.م.د. سوزان جبير عباس

أ.د. احمد صالح الخزعلي
عميد كلية الصيدلة
2023 / 7 / 17

نسخة منه الى:
- مكتـــب السيـــد العميد , للتفضـــل بالاطـــلاع .
- مكتـــب معـــاون العميـــد للشـــؤون العـــلميـــة .
- فـــرع الادويـــــة والســـــموم.
- شـــعبـــة الدراســـات العليـــا للحفـــظ مع الاوليـــات .
- الصـــــــــــــــــادرة

Ministry of Health & Environment
Karbala Health Directorate
Training and Human Development Center
Research Committee

Form number 53

Decision number:2023169

Date18/9/2023

Research committee decision

The Research Committee of Karbala Health Directorate has examined the research protocol number(**2023167**/Karbala) entitled:

Investigation of genetic Polymorphism in insulin receptor gene (INSR) on response to exogenous insulin therapy in type 1 Diabetic Patients in a sample of Iraqi

Submitted by researchers: **Farah Ali Mahdi Alquraishy** to the research and Knowledge Management Unit at the Training and Human Development Center of Karbala Health Directorate on 18/9/2023

The unit has decided to:

*** Accept the above-mentioned research protocol as it meets the standards adopted by the Ministry of Health for the implementation of research, and there is no objection to implementing it in the Directorate's institutions.**

الدكتور
نعيم عبيد المشهداني
طبيب اختصاص
Rapporteur of the committee

18/09/2023

Notes:

- The committee member (Dr. Taqwa Khudhur Abdulkareem)/ committee rapporteur (Dr. Naeem Obaid. Talal) were authorized to sign this decision on behalf of the remaining members of the committee under the rules of procedures of the research committee.
- The research committee approval means that the research project submitted to the aforementioned committee has fulfilled the ethical and methodological standards adopted by the Ministry of Health for conducting a research. As for the implementation of the research, it depends on the researchers adherence to the instructions of the health institution in which the research will be implemented as well as the laws, instructions and recommendations in force that govern the practice of medical and health action in Iraq.

Ministry of Higher Education
and Scientific Research
University of Karbala
College of Pharmacy
Department of Postgraduate Studies

جمهـــورية العـــراق
وزارة التعليم العالي والبحث العلمي
جامـــعة كربلاء
كلـــية الصيـــدلـــة
شعبة الدراسات العليا

Issue No.: العدد: د.ع/ 6 / ١٦٥٢
Date: التاريخ: ١٢/ 9 / 2023

إلى/ دائرة صحة كربلاء / مركز الغدد الصم وامراض السكري
مستشفى الامام الحسن المجتبى (ع)
مستشفى الامام الحسين(ع) التعليمي
مستشفى الامام الحجة (ع)

م/تسهيل مهمة

تحية طيبة ..

يرجى تفضلكم بالموافقة على تسهيل مهمة طالبة الدراسات العليا / ماجستير/ الادوية والسموم في كليتنا (فرح علي مهدي) لغرض اكمال اجراءات بحث الماجستير الموسوم:

Investigation of genetic polymorphism in insulin receptor gene (INSR) on response to exogenous insulin therapy in type 1 Diabetic patients in a sample of iraqi patients

شاكرين تعاونكم معنا مع التقدير....

أ.م.د. جمال علي عاشور
معاون العميد للشؤون العلمية
2023 / 9 /

نسخة منه الى:
- مكتـــب السيـــد العميد ، للتفضـــل بالاطـــلاع .
- مكتـــب معـــاون العميـــد للشـــؤون العـــلميـــة .
- شـــعبـة الدراســات العليـــا للحفـــظ مع الاوليـــات .
- الصـــــــــــــــادرة .

Email: Pharmacy@uokerbala.edu.com
العراق- محافظة كربلاء- مكتب بريد كربلاء- ص ب 1125

College of Pharmacy
Department of Postgraduate Studies

كلـــية الصيـــدلـــة
شعبة الدراسات العليا

Issue No.:
Date:

العدد: د.ع/ 6 / ٧٨٧
التاريخ: ١ / 10 / 2023

الى/ دائرة صحة بابل
مستشفى مرجان التعليمي / مركز امراض السكري والغدد الصماء
م/تسهيل مهمة

تحية طيبة ..

يرجى تفضلكم بالموافقة على تسهيل مهمة طالبة الدراسات العليا / ماجستير/ الادوية والسموم في كليتنا (فرح علي مهدي) لغرض اكمال اجراءات بحث الماجستير الموسوم:

Investigation of genetic polymorphism in insulin receptor gene (*INSR*) on response to exogenous insulin therapy in type 1 Diabetic patients in a sample of iraqi patients

شاكرين تعاونكم معنا مع التقدير....

أ.م.د. جمال علي عاشور
معاون العميد للشؤون العلمية
/ 10 / 2023

نسخة منه الى:
- مكتب السيد العميد ، للتفضل بالاطلاع .
- مكتب معاون العميد للشؤون العلمية .
- شعبة الدراسات العليا للحفظ مع الاوليات .
- الصادرة .

العراق- محافظة كربلاء- مكتب بريد كربلاء- ص ب 1125
Email: Pharmacy@uokerbala.edu.com

Republic of Iraq
Ministry of health
Najaf Health Directorate
Training and Human Development Center
No.
Date:

جمهورية العراق
وزارة الصحة
مديرية صحة النجف
مركز التدريب و التنمية البشرية
العدد: ١٧ ١٢
التاريخ: ١٤/ ١/ ٢٠٢٤

إلى / جامعة كربلاء_ كلية الصيدلة

م / تسهيل مهمة

تحية طيبة ...

إشارة الى كتابكم ذي العدد **٩٣في ٢٠٢٤/٠١/١٠** بخصوص تسهيل مهمة الباحثة (**فرح علي مهدي**)للحصول على الموافقة الاخلاقية لإجراء البحث العلمي الموسوم :

Investigation of genetic polymorphism in insulin recepter gene (INSR) on response to exogenous insulin therapy in type1 diabetic patients in a sample of iraqi patients

حصلت موافقة اللجنة العلمية للبحوث في مركز دائرتنا على اجراء البحث في(**مستشفى الزهراء التعليمي**)في دائرتنا مع التأكيد على الالتزام الكامل بتعليمات السلامة الحيوية والضوابط الاخلاقية والحصول على موافقة المشاركين قبل الشروع بالبحث والحفاظ على خصوصيتهم وعدم افشاء البيانات او استخدام العينات لغير اغراض البحث العلمي ... على ان لا تتحمل دائرتنا اية تبعات مادية ولا يسمح بإخراج العينات خارج مختبرات المؤسسة .

للتفضل بالاطلاع .....مع الاحترام

الدكتور
حيدر خضير عباس

الدكتور
عبدالله محمد الغزالي
المدير العام
٢٠٢٤/١/١٤

نسخة منه الى/
- مركز التدريب و التنمية البشرية /شعبة ادارة المعرفة والبحوث..... مع الأوليات

University of Kerbala

Consent to be in Research

Study Title: Investigation of the effectof Genetic Polymorphism in Insulin receptor (INSR) Gene on Response to Exogenous Insulin Therapy in a sample of Iraqi Type 1 Diabetic Patients

The Researcher Name: Farah Ali AlQuraishi

This is a medical research study, and you do not have to take part. The researcher and doctor Samaher will explain this study to the parents of the patient. If you have any questions, you may ask me and/or the doctor. You are being asked to let your child participate in this study because they have type 1 diabetes mellitus with chronic hyperglycemia and are treated with exogenous insulin. In this study, the researcher are collecting blood samples from your child to learn more about the association of genetic polymorphism of INSR with therapeutic response of exogenous insulin. If you agree to be in this study, you will go to the laboratory and give a blood sample for one time only. The blood will be drawn by putting a needle into a vein in your arm. One small tube of blood will be taken. This will take about five minutes.

The risks?

The needle stick may hurt. There is a small risk of bruising and fainting, and a rare risk of infection.

Will my medical information be kept confidential?

We will do our best to protect the information we collect from you and your medical record. Information that identifies you will be kept secure and restricted. If information from this research is published or presented at scientific meetings, your name and other identifiers will not be used. Information that identifies you will be

destroyed when this research is complete. You have been given copies of this consent form to keep.

The Consent:

If you wish to be in this study, please sign below.

Name of participant parent :

Date Participant Parent’s Signature for Consent

Date Person Obtaining Consent (Researcher)

Questionnaire for Type 1 Diabetic Patients Demographic Characterization

Parameters	Variables
Name	
Phone Number	
Age	
Sex	Male/Female
Weight	
Height	
Dietary Lifestyle	Sedentary/Healthy
Physical Activity	Active/moderate/Sedentary
Family History of Diabetes Mellitus	Absent/Presents
Duration of T1D	
Duration of Insulin Monotherapy	
Socio-Economic Status of Caregivers	High/Middle/Poor
Occupation of Caregivers	
Educational Status of Caregivers	Academic Degree/High school Degree/ None
Insulin Basal-Bolus Dose	
Other Diseases/Infections	
Other Medications	
Pregnant girls	

Parameters	Unit
FBS	mg/dL
RBS	mg/dL
HbA1c	%
sIR	ng/mL
BMI Percentile	%ile
Genotyping of INSR (rs2245649)T>C and (rs2229429)	

الملخص

الخلفية: يُعد الإنسولين الخارجي مدى الحياة حجر الزاوية في علاج داء السكري من النوع الأول وتعتمد فعالية الإنسولين في تحقيق التحكم في مستوى السكر في الدم على مدى ارتباطه بمستقبلات الإنسولين. قد تؤثر التعددات الشكلية للنيوكليوتيد المفرد (SNPs) في جين مستقبل الإنسولين (INSR) على فعالية الإنسولين الخارجي في تحقيق السيطرة على سكر الدم لدى الأفراد المصابين بداء السكري من النوع الأول الذين يتلقون علاجًا خارجيًا بالإنسولين، مما قد يؤدي إلى مقاومة الإنسولين.
تُعد هذه الدراسة الدوائية-الوراثية المقطعية ذات أهمية كبيرة، حيث تقدم طرقًا مبتكرة في الطب الدقيق، وتُفكك المواقع الوراثية المحددة التي تسهم في انخفاض حساسية الإنسولين الخارجي، مما يوفر فهمًا واضحًا لتطور فرط سكر الدم المزمن، على الرغم من الالتزام الصارم بإرشادات استخدام الإنسولين الخارجي.
ويمكن أن تساهم النتائج المتعلقة بالتعددات الشكلية المدروسة في تطوير تطبيقات جديدة ضمن تقنيات هندسة الجينوم، بهدف التعديل الدقيق للجينات أو تنظيم التعبير الجيني.

الهدف: تهدف هذه الدراسة إلى التحقق من تأثير التعددات الشكلية (rs2245649) و(rs2229429) في جين INSR على الاستجابة العلاجية للإنسولين الخارجي في عينة من الأفراد العراقيين المصابين بداء السكري من النوع الأول.

الطريقة: تم دراسة تأثير التعددات الشكلية T>C (rs2245649) و(rs2229429) G>A في جين INSR على 99 فردًا مصابًا بالسكري من النوع الأول يعانون من ضعف في التحكم بمستوى السكر في الدم، ويُعالجون بنظام أحادي يشمل جرعات أساسية وعالية من الإنسولين الخارجي.
بالإضافة إلى ذلك، تم تضمين 30 فردًا سليمًا كمجموعة ضابطة، وكانت الفئة العمرية المتوسطة لكلا المجموعتين 12.3 سنة.
تم إجراء النمط الجيني باستخدام تقنية تفاعل البوليميراز المتسلسل النوعي للأليلات، وتم تحليل البيانات إحصائيًا.

النتائج: أظهرت النتائج أن الأفراد المصابين بالنوع الأول من السكري والحاملين للنمط الجيني الطافر المتماثل INSR (rs2245649) T>C لديهم تحكم ضعيف في سكر الدم مقارنةً بالحاملين للنمط البري المتماثل ($p = 0.01$).
كما أظهرت النتائج أن تكرار الأليل الطفيف للتعدد الشكلي INSR (rs2245649) T>C كان بنسبة 26%، بينما بل INSR (rs2229429) G>A نسبة 12% في عينة من السكان العراقيين وقد ارتبطت الأليلات الطفيفة لـ INSR (rs2245649) بزيادة خطر مقاومة الإنسولين بمقدار 2.523 مرة لدى مرضى النوع الأول من السكري($p = 0.04$) .
أما الحاملون الطافرون المتماثلون للتعدد الشكلي (rs2229429) G>A، فقد كانوا معرضين لخطر أعلى بمقدار 10.479 مرة لتطوير ضعف في التحكم بسكر الدم مقارنة بالحاملين للنمط البري($p = 0.008$) .
وفي النهاية، أظهر المرضى الذين لا يستجيبون جيدًا للإنسولين الخارجي ويحملون التعدد الشكلي INSR (rs2229429) G>Aمستويات أعلى بشكل ملحوظ من مستقبلات الإنسولين في البلازما($p < 0.001$) .
وبالمثل، أظهر الأفراد الحاملون للأليل C في التعدد INSR (rs2245649) مستويات أعلى من مستقبلات الإنسولين القابلة للذوبان مقارنة بالحاملين للأليلT ($p = 0.02$) .

الاستنتاج: تم الكشف عن وجود كلا التعددين الشكليين INSR (rs2245649) T>C و INSR (rs2229429) G>A في عينة من السكان العراقيين وترتبط الأليلات الطفيفة للتعددات المدروسة بضعف التحكم في سكر الدم وارتفاع مستويات مستقبلات الإنسولين القابلة للذوبان في البلازما، مما قد يرتبط بمقاومة الإنسولين.

العراق جمهورية

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء

كلية الصيدلة

فرع الادوية والسموم

دراسة تأثير تعدد الأشكال الجينية في جين مستقبل الأنسولين (INSR) على الاستجابة للعلاج بالأنسولين الخارجي في عينة من مرضى السكري من النوع الأول العراقيين

رسالة

مقدمة الى مجلس كلية الصيدلة / جامعة كربلاء
كجزء من متطلبات نيل درجة الماجستير في علم الادوية والسموم

بواسطة

فرح علي القريشي

صيدلة بكالوريوس (كليه بغداد للعلوم الطبيه-٢٠١٤)

بإشراف

أ.م.د. محمد ابراهيم رسول
دكتوراه ادويه وعلاجيات

ميلادي ٢٠٢٥ **هجري ١٤٤٧**