Relationship between Gene Polymorphism and Type 2 Diabetes in Males Palestinian Population: a Study of Five Gene Polymorphisms.

العلاقة بين الأشكال المتعددة للجينات ومرض السكري من النوع الثاني في السكان الفلسطينيين الذكور: دراسة لأشكال متعددة في خمس جينات

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نتيجة الحكم على أطروحة ماجستير

بناءً على موافقة عمادة البحث العلمي والدراسات العليا بالجامعة الإسلامية بغزة على تشكيل لجنة الحكم على أطروحة الباحث/ محمود أحمد رشاد النجار لنيل درجة الماجستير في كلية العلوم الصحية قسم العلوم الطبية المخبرية موضوعها:

الكلاسيك المتعددة للجينات ومرض السكري من النوع الثاني في السكان الفلسطينييين الذكور: دراسة لأشكال متعددة في خمس جينات

Relationship between Gene Polymorphism and Type 2 Diabetes in Males
Palestinian Population: a Study of Five Gene Polymorphisms

وبدأت المناقشة التي تلت اليوم السبت الموافق 05 ربيع الثاني 1439 هـ، الموافق 23/12/2017م، الساعة الحادية عشرة صباحاً، اجتمعت لجنة الحكم على الأطروحة والموكلة من:

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وإذ تمنح هذه الدرجة فإنها توحي بتمويه الله تعالى ونوره وطاعته وأن يسخر علمه في خدمة دينه ووطنه.

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Abstract

**Background:** Type 2 Diabetes Mellitus (T2DM) is a multifactorial disease that results from the interaction between multiple genetic and environmental factors. Its prevalence rate in Gaza Strip is alarming. Human genome studies revealed many T2DM-associated genetic polymorphisms in various populations. Among the genes polymorphisms that were strongly associated with diabetes are (KCNQ1 rs2237892), (KLF14 rs972283), (ZBED3 rs4457053), (COL8A1 rs792837), and (FTO rs8050136).

**Objective:** To investigate the association between (KCNQ1 rs2237892), (KLF14 rs972283), (ZBED3 rs4457053), (COL8A1 rs792837), and (FTO rs8050136), genes polymorphisms and T2DM in Males Palestinian Population.

**Methods:** In this case-control study, 100 T2DM male patients and 100 control men were examined. The two groups were genotyped for the five genes polymorphisms using restriction fragment length polymorphism-PCR (RFLP-PCR) and Allele Specific (AS-PCR) techniques. Body mass index (BMI), glycated hemoglobin (HbA1c), insulin (C-peptide), total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-c), and low density lipoprotein cholesterol (LDL-c) were measured for all the study participants. The relation between the five genes polymorphisms, T2DM and the measured clinical parameters were statistically analyzed using appropriate tests.

**Results:** Among the tested polymorphisms, significant association were evident between KLF14 "GG" genotype (P-value = 0.014), FTO "CC" genotype (P-value = 0.043), and COL8A1 TC genotype (P-values= 0.015) and increased risk of T2DM. The KLF14 G-containing genotypes exerted significant effect on lowering HDL-c (P-value = 0.026) and on elevating LDL-c (P-value = 0.045) and cholesterol level (P-value = 0.042) in the control group. The FTO "CC" genotype showed a significant effect on raising HbA1c level in the patients (P-value = 0.007). KCNQ1 (rs2237892 T>C), ZBED3 (rs4457053 A>G) and COL8A1 (rs792837 T>C) genotypes did not reveal significant effects on the tested parameters.

**Conclusion:** KLF14 "GG", FTO "CC" and COL8A1 C allele and "TC" genotypes are significantly associated with T2DM in the investigated population. KLF14 "GG" and "AG" have an association with the levels of HDL-c, LDL-c and Cholesterol in control subjects. The HbA1c level was significantly higher in patients with the FTO "CC" genotype. The study recommends confirming the obtained results on a larger sample and examining the association of other genes polymorphisms with T2DM in Palestinian Population.

**Keywords:** (KCNQ1), (KLF14), (ZBED3), (COL8A1),( FTO), polymorphism , type 2 diabetes mellitus, Palestine.
المعرض

المقدمة: مرض السكري من النوع الثاني هو مرض متعدد العوامل ويشمل عن التفاعل بين عوامل وراثية وبيئية متعددة. ومع ذلك، تشير دراسات العالقة بين العوامل الوراثية على نطاق الجينوم ومرض السكري في مجموعات مختلفة عديد، ووجود علاقة بين الاتصالات المختلفة لجينات محددة وخطر الإصابة بمرض السكري. وكان من بين الاتصالات الوراثية التي ارتبطت بشكل قوي مع حدوث مرض السكري في الجينات التالية: (KCNQ1 rs2237892), (KLF14 rs972283), (ZBED3 rs4457053), (COL8A1 rs792837), (FTO rs850163)

الهدف: التحقق من الارتباط بين أنماط خمس جينات وهي (KLF14 rs972283), (KCNQ1 rs2237892), (FTO rs850136) (COL8A1 rs792837), (ZBED3 rs4457053) وخطر حدوث مرض السكري من النوع الثاني في السكان الفلسطينيين الذكور.

الطرق المستخدمة: منهج الدراسة (مجموعة مرضية - مجموعة ضابطة) المجموعة المرضية تحتوي على 100 رجل مريض بدء السكري من النوع الثاني، والمجموعة الضابطة تحتوي على 100 رجل من الأصحاء. تم فحص الطراز الجيني للمجموعتين للخمس جينات باستخدام تقنية (RFLP-PCR) والاضافية إلى قياس مخزون السكر، الأنسولين (c-پپتيد)، الكولسترول، الدهون الثلاثية، الكولسترول عالي الكثافة، الكولسترول منخفض الكثافة، وتحليل البيانات والتلاشي باستخدام الاختبارات الإحصائية المناسبة.

النتائج: من بين الأشكال المتعددة التي تم اختبارها، كانت الارتباطات المهمة واضحة وذلك بوجود علاقة ذات دلالة إحصائية بين الطراز الجيني "GG" (KLF14 rs972283) وبين الطراز الجيني "CC" (FTO rs850136) وبين الطراز الجيني "G" (COL8A1 rs792837) وبين الطراز الجيني "C" (ZBED3 rs4457053) وبين الطراز الجيني "C" (KCNQ1 rs2237892) بين جميع الجينات. أظهرت النتائج عدم وجود علاقة ذات دلالة إحصائية لكل من الطرز الجينية "GG", "AG", "TT" (fto rs850136) وبين الطراز الجيني "C" (ZBED3 rs4457053) وبين الطراز الجيني "G" (COL8A1 rs792837) وبين الطراز الجيني "C" (KCNQ1 rs2237892) وبين الطراز الجيني "G" (KLF14 rs972283) وبين الطراز الجيني "C" (COL8A1 rs792837) وبين الطراز الجيني "C" (ZBED3 rs4457053) وبين الطراز الجيني "G" (KCNQ1 rs2237892) وبين الطراز الجيني "C" (KLF14 rs972283) وبين الطراز الجيني "G" (COL8A1 rs792837) وبين الطراز الجيني "C" (ZBED3 rs4457053) وبين الطراز الجيني "C" (KCNQ1 rs2237892) وبين الطراز الجيني "C" (KLF14 rs972283) وبين الطراز الجيني "G" (COL8A1 rs792837) وبين الطراز الجيني "C" (ZBED3 rs4457053) وبين الطراز الجيني "C" (KCNQ1 rs2237892) وبين الطراز الجيني "C" (KLF14 rs972283) وبين الطراز الجيني "G" (COL8A1 rs792837) وبين الطراز الجيني "C" (ZBED3 rs4457053).

الخلاصة: وجود علاقة ذات دلالة إحصائية لكل من الطرز الجينية "GG", "AG", "TT" (fto rs850136) وبين الطراز الجيني "C" (ZBED3 rs4457053) وبين الطراز الجيني "C" (KCNQ1 rs2237892) وبين الطراز الجيني "C" (KLF14 rs972283) وبين الطراز الجيني "G" (COL8A1 rs792837) وبين الطراز الجيني "C" (ZBED3 rs4457053) وبين الطراز الجيني "C" (KCNQ1 rs2237892) وبين الطراز الجيني "C" (KLF14 rs972283) وبين الطراز الجيني "G" (COL8A1 rs792837) وبين الطراز الجيني "C" (ZBED3 rs4457053) وبين الطراز الجيني "C" (KCNQ1 rs2237892) وبين الطراز الجيني "C" (KLF14 rs972283) وبين الطراز الجيني "G" (COL8A1 rs792837) وبين الطراز الجيني "C" (ZBED3 rs4457053) وبين الطراز الجيني "C" (KCNQ1 rs2237892) وبين الطراز الجيني "C" (KLF14 rs972283) وبين الطراز الجيني "G" (COL8A1 rs792837) وبين الطراز الجيني "C" (ZBED3 rs4457053) وبين الطراز الجيني "C" (KCNQ1 rs2237892) وبين الطراز الجيني "C" (KLF14 rs972283) وبين الطراز الجيني "G" (COL8A1 rs792837) وبين الطراز الجيني 

كلمات افتتاحية: (KCNQ1), (KLF14), (ZBED3), (COL8A1), (FTO) الثاني، فلسطين.
Dedication

I would like to dedicate my work to my parents, without their caring & support it would not have been possible,

Spirit of My brother Rashad,

My beloved brothers: Dr.Bashar, Mr. Mohammed, Dr.Ali, Dr.Yehia and sisters: Ms. Amal and Ms. Mai,

The Islamic University; my second magnificent home,

My friends who encourage and support me,

All the people in my life who touch my heart,

I dedicate this research
Acknowledgement

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

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List of Abbreviations

ADA       American Diabetes Association
AS-PCR   Allele Specific- PCR
BMI      Body Mass Index
bp       Base Pairs
CHD      Coronary Heart Disease
CI       Confidence Intervals
CKD      Chronic Kidney Disease
COL8A1   Collagen Type VIII Alpha 1 Chain
CVD      Cardiovascular Disease
DM       Diabetes Mellitus
EDTA     Ethylene Diamine Tetra Acetic Acid
FTO      Fat mass and Obesity-Associated Protein
GDM      Gestational Diabetes Mellitus
GL       Glycemic Load
GLUT4    Glucose Transporter Type 4
GWAS     Genome-Wide Association Studies
HbA1c    Hemoglobin A1c
HDL-c    High Density Lipoprotein cholesterol
HLA      Human Leukocyte Antigen
HWE      Hardy-Weinberg Equilibrium
IDF      International Diabetes Federation
IFG      Impaired Fasting Glucose
IGT      Impaired Glucose Tolerance
KASPar  Kompetitive Allele-Specific PCR
KCNQ1    Potassium voltage-gated channel, KQT-like subfamily, member 1
KLF14    Kruppel-like factor 14
LADA     Latent Autoimmune Diabetes in the adult
LDL-c    Low Density Lipoprotein cholesterol
MAF      Minor allele frequency
MODY     Maturity-onset diabetes of the young
NCBI  National Center for Biotechnology Information
OR  Odds Ratios
PCR  Polymerase Chain Reaction
RE  Restriction Enzymes
RFLP-PCR  Restriction Fragment Length Polymorphism-PCR
SD  Standard Deviation
SNPs  Single Nucleotide Polymorphisms
T1DM  Type 1 Diabetes Mellitus
T2DM  Type 2 Diabetes Mellitus
UV  Ultra Violet
VDR  Vitamin D receptor
WHO  World Health Organization
ZBED3  Zinc finger, BED-type containing 3
Chapter 1

Introduction
Chapter 1
Introduction

1.1 Overview

Diabetes mellitus (DM) is a common health problem worldwide; it is one of the world’s most important causes of healthcare expenditure, mortality, morbidity and lost economic growth.

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by hyperglycemia, variable degrees of insulin resistance, impaired insulin secretion and increased hepatic glucose production. There are two major types of DM: T1DM, which is primarily a result of pancreatic β-cell destruction due to an immune-mediated process that is likely incited by environmental factors in genetically predisposed individuals (Harjutsalo, Reunanen, & Tuomilehto, 2006). The more prevalent form, T2DM, usually begins as insulin resistance, a disorder in which the cells do not use insulin properly. As the need for insulin rises, the pancreas gradually loses its ability to produce it (Cohen, 2006).

In complex diseases like T2DM, obesity and cardiovascular diseases, multiple genetic and environmental factors as well as the interaction between these factors determine the phenotype. The worldwide rise in prevalence of T2DM and other cardiometabolic disorders has led to an intense search for genetic factors influencing the susceptibility for these common disorders. Although environmental influences, such as high-caloric fat- and carbohydrate-enriched diets and a sedentary lifestyle with markedly reduced physical activity, certainly accelerate disease development in those with genetic predisposition, it is nonetheless of great clinical importance, and indeed a formidable challenge, to elucidate the genetic variants that increase the risk of diseases like T2DM (Permutt, Wasson, & Cox, 2005). Even though much research has been conducted, the knowledge of the specific causes of common complex diseases at the genetic level is still somewhat at its infancy. More detailed insight into the genetic risk factors and the underlying molecular mechanisms involved in T2DM and related traits is expected to improve clinical investigations, advance the prevention of disease development, elucidate the diseases mechanisms and hopefully high light new pathways relevant for therapeutic intervention.
The International Diabetes Federation (IDF) has estimated that the number of people with diabetes is expected to rise from 366 million in 2011 to 552 million by 2030 if no urgent action is taken. Furthermore, many people are unaware that they have diabetes, with a number around 183 million. T2DM represents > 90% of the cases (Lyssenko & Laakso, 2013). In Palestine, as in other countries, T2DM seems to be a serious health problem among the population with a prevalence rate of around 9% - 12% (Husseini et al., 2009).

T2DM is a complex multifactorial disease in which multiple genetic variants interact with environmental factors to trigger the disease (Lyssenko et al., 2008). There is sufficient evidence that T2DM has a strong genetic basis. The concordance of T2DM in monozygotic twins is ~76% (Medici, Hawa, Ianari, Pyke, & Leslie, 1999). The lifetime risk (at age 80 years) for T2DM has been calculated to be 38% if one parent had T2DM. If both parents are affected, the incidence of T2DM in the offspring is estimated to approach 60% by the age of 60 years (Stumvoll, Goldstein & van Haeften, 2005). Advances in genotyping technology have facilitated rapid progress in large-scale genetic studies. Since 2007, (GWAS) have identified >65 genetic variants that increase the risk of T2DM by 10–30% (Morris et al., 2012). Recent technological developments have allowed the successful identification of common (SNPs) contributing to diabetes susceptibility (Takeuchi et al., 2009).

In Gaza strip, studies on SNPs in different proposed risk genes and increased risk of T2DM are limited and restricted to Calpain-10 gene (Zaharna, Abed, & Sharif, 2010) and KCNJ11 gene (Abed, Ayesh, & Hamdona, 2017). However, no previous studies examined SNPs in other documented risk genes (e.g., Kruppel-like factor 14 (KLF14); Potassium voltage-gated channel, KQT-like subfamily, member 1 (KCNQ1); Zinc finger, BED-type containing 3 (ZBED3); Fat mass and obesity-associated protein (FTO); Collagen Type VIII Alpha 1 Chain (COL8A1) with increased risk of T2DM in Gaza Strip.
1.2 Significance

In Gaza strip, no previous studies investigated the relation between single nucleotide polymorphisms in reported risk genes \((KLF14), (KCNQ1), (ZBED3), (FTO)\) and \((COL8A1)\) and the risk of having T2DM.

It is therefore of utmost importance to identify individuals at high risk of developing diabetes for both investigators and health care providers which may aid in delay of disease onset, prevention of disease or its associated complications.

1.3 General objective

The overall aim of this work is to investigate the relation between SNP in five candidate genes and risk of having T2DM in Males Palestinian Population.

1.4 Specific objectives

1. To determine the genotypic and allelic frequencies of \((KLF14), (KCNQ1), (ZBED3), (FTO)\) and \((COL8A1)\) SNPs in the study population.

2. To evaluate the glycemic status of T2DM patients and apparently healthy controls using whole blood hemoglobin A1c (\(HbA1c\)) and insulin (C-peptide).

3. To compare T2DM patients and apparently healthy controls in terms of their BMI and lipid profile (total cholesterol, triglycerides, high density lipoprotein cholesterol "HDL-c" and low density lipoprotein cholesterol "LDL-c").

4. To investigate the relation between the five SNPs and BMI, HbA1c, C-peptide, and the lipid profile (total cholesterol, triglycerides, HDL-c, and LDL-c) in the T2DM patients and the apparently healthy control subjects.
Chapter 2

Literature Review
Chapter 2
Literature Review

2.1 Diabetes mellitus type 2 and related traits

2.1.1 Definition and description of diabetes mellitus

T2DM is a chronic metabolic disorder characterized by hyperglycemia, variable degrees of insulin resistance, impaired insulin secretion and increased hepatic glucose production. The disease is classified as T1DM, T2DM, gestational diabetes and other types of diabetes, including monogenic diabetes. T1DM and T2DM are considered the two major types. T1DM normally develops before adulthood and is typically caused by an autoimmune destruction of the insulin-producing β-cells leading to an absolute insulin deficiency, whereas T2DM is normally associated with insulin resistance and relative insulin deficiency.

Diabetes is a major global health problem due to its dramatically increasing prevalence in both the western world and the developing countries. Rising health care costs impose a serious problem, and a significant portion of health care spending is incurred by people with diabetes. The number of people with diabetes is increasing due to aging (increase in the proportion of people >65 years of age), general population growth, urbanization, and increasing prevalence of obesity and physical inactivity. The total number of people worldwide with diabetes is projected to rise from 285 million in 2010 to 439 million in 2030 corresponding to a predicted increase in prevalence from 6.4% in 2010 to 7.7% in 2030 (Shaw, Sicree, & Zimmet, 2010).

Diagnosing diabetes, the diagnostic criteria for diabetes and pre-diabetes (intermediate hyperglycemia such as impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) have been debated for several years and modified numerous times). In 1985, World Health Organization (WHO) recommended fasting plasma glucose (FPG) $\geq$7.8 or 2-hour post-challenge plasma glucose (2-h PG) $\geq$11.1 mmol/L as
the criteria for diagnosing diabetes. Twelve years later, in 1997, American Diabetes Association (ADA) proposed to use only FPG and lowered the threshold from 7.8 to 7 mmol/L (Gavin III, Alberti, Davidson, & DeFronzo, 1997). The Revisions for the 2010 Clinical Practice Recommendations now include the use of glycated hemoglobin (HbA1c) as a diagnostic criterion for diabetes, with HbA1c values $\geq 6.5\%$ being diagnostic (ADA, 2014). People with impaired fasting glucose (100–125 mg/dl (5.6–6.9 mmol/L), and those with impaired glucose tolerance (at or above 140 mg/dl (7.8 mmol/L) but not over 200 mg/dl (11.1 mmol/L), and with an HbA1c levels ranging between 5.7–6.4% are considered prediabetic and are at considerable risk for developing diabetes mellitus as well as cardiovascular diseases (Santaguida et al., 2005). According to the WHO diagnostic criteria for diabetes mention in table (2.1).

Table (2.1): Diagnostic criteria for diabetes according to the WHO (WHO, 2006)

<table>
<thead>
<tr>
<th>Condition</th>
<th>2-hour glucose mg/dL (mmol/L)</th>
<th>Fasting glucose mg/dL (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>$&lt;$140 ($&lt;$7.8)</td>
<td>$&lt;$110 ($&lt;$6.1)</td>
</tr>
<tr>
<td>Impaired fasting glucose</td>
<td>$&lt;$140 ($&lt;$7.8)</td>
<td>$\geq$110 ($\geq$6.1) and $&lt;$126 ($&lt;$7)</td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
<td>$\geq$140 ($\geq$7.8)</td>
<td>$&lt;$126 ($&lt;$7)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>$\geq$200 ($\geq$11.1)</td>
<td>$\geq$126 ($\geq$7)</td>
</tr>
</tbody>
</table>

2.2 Classification of diabetes mellitus

The traditional view is that T1DM and T2DM characterize distinct conditions with widely different pathophysiology. On the other hand, there is a notable increase in the proportion of people having diabetes with the characteristics of both types (Pozzilli & Buzzetti, 2007; Tuomi, 2005). As an alternative, it has been suggested to think more in terms of a disease continuum, with the conventional autoimmune T1DM at one end and the conventional metabolic T2DM at the other (Lin, Zhou, Wang, Zhang, & Huang, 2008; Van Deutekom, Heine, & Simsek, 2008). However, current practical classification guidelines still apply the traditional view that T1DM and T2DM are two different conditions attributable to entirely distinct, but as yet somewhat uncertain mechanisms.
2.2.1 Type 1 diabetes mellitus

T1DM is caused by destruction of the insulin-producing cells of the pancreas, primarily due to an autoimmune-mediated reaction, resulting in absolute insulin deficiency. The reason why this occurs is not fully understood. In general, the disease is diagnosed at any age, but most frequently, it develops during childhood and puberty. T1DM is one of the most common endocrine and metabolic conditions in childhood and progresses rapidly. Latent autoimmune diabetes in the adult (LADA) is a slowly progressive form of autoimmune diabetes, characterized by diabetes-associated autoantibody positivity, and insulin independence at diagnosis, which distinguishes LADA from classic T1DM (Fourlanos et al., 2005). People with T1DM are usually insulin-dependent from diagnosis and require daily injections of insulin in order to control their blood glucose and to stay alive. Moreover, T1DM, especially the autoimmune process, is substantially determined by inherited variation (Pociot et al., 2010). There is a strong association between human leukocyte antigen (HLA) genes and T1DM. HLA variants confer either high risk of or protection against the disease. Currently, over 40 genetic loci have shown to affect risk of T1DM (Barrett et al., 2009). The incidence of T1DM is increasing, the reasons for which are unclear but may be due to changes in environmental risk factors that could initiate autoimmunity or accelerate already ongoing beta cell destruction.

2.2.2 Type 2 Diabetes Mellitus

Is the most common form of diabetes and accounts for over 90 % of all diabetes cases worldwide (González, Johansson, Wallander, & Rodríguez, 2009). T2DM is characterized by insulin resistance and relative insulin deficiency, either of which may be present at the time that diabetes becomes clinically manifest. T2DM usually occurs after the age of 35-40 years but may be diagnosed earlier, especially in populations with high diabetes prevalence. T2DM can remain undetected (asymptomatic), for many years and the diagnosis is often made from associated complications or incidentally through an abnormal blood or urine glucose test. T2DM is often, but not always, associated with metabolic abnormalities such as obesity, which itself can cause insulin resistance and lead to elevated blood glucose levels. In contrast to T1DM, people with T2DM are not absolutely dependent on exogenous insulin but may require insulin for control of
hyperglycemia if this is not achieved with diet alone or with oral hypoglycemic agents. T2DM has a strong familial component, and at least 50 genetic variants have been reported to influence susceptibility to type 2 diabetes (M. I. McCarthy, 2010).

2.2.3 Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) is one of the most common medical complications of pregnancy (ADA, 2015), and is defined as any degree of glucose intolerance with the onset or first recognition during pregnancy. This includes undiagnosed diabetes mellitus detected for the first time during pregnancy (Nankervis et al., 2014). Although GDM usually resolves following birth, it is associated with significant morbidities for the woman and her child both perinatal and in the long term (Tieu, Middleton, McPhee, & Crowther, 2010). This type is diagnosed during pregnancy mainly between 24 and 28 weeks of pregnancy. Women who have had GDM have an increased risk of developing diabetes later in life (Löbner et al., 2006). Moreover, children of women with GDM have an increased risk for childhood and adult obesity and an increased risk of glucose intolerance (Boney, Verma, Tucker, & Vohr, 2005).

2.2.4 Other rare types of DM

Several forms of the diabetic state may be associated with single gene (monogenic) defects in β-cells function. Maturity-onset diabetes of the young (MODY) is a group of monogenic disorders characterized by autosomal dominantly inherited non-insulin dependent form of diabetes classically presenting in adolescence or young adults before the age of 25 years. MODY is a rare cause of diabetes (1% of all cases) and is frequently misdiagnosed as T1DM or T2DM. People with MODY are generally not overweight and do not have other risk factors for T2DM, such as high blood pressure or abnormal blood fat levels (Anik, Çatlı, Abacı, & Böber, 2015). In addition, there are other specific types of diabetes with a known etiology, such as secondary to other diseases, trauma or surgery, or the effects of drugs. Examples include diabetes caused by hemochromatosis, exocrine pancreatic disease, or certain types of medications (e.g., long-term steroid use).
2.3 Type 2 Diabetes – etiology, pathophysiology and long-term complications

The etiology of T2DM is not fully understood, but presumably, T2DM develops when a diabetogenic lifestyle (i.e. excessive caloric intake, inadequate caloric expenditure, obesity) acts in conjugation with a susceptible genotype. The majority of patients who develop T2DM are obese (Eckel et al., 2011). Energy-dense diet as a risk factor has, however, shown to be independent of baseline obesity for the development of T2DM (Wang et al., 2008). Further, it has been suggested that T2DM in some cases are caused by environmental pollutants (Hectors et al., 2011). Even though there is some disparity regarding the reasons for the development of T2DM, most physicians and scientists agree that the major independent risk factors for developing T2DM are: obesity (Anderson, Kendall, & Jenkins, 2003; Schulze et al., 2006), family history of T2DM (first-degree relative) (Pierce, Keen, & Bradley, 1995), ethnicity (some ethnic groups have higher prevalence of diabetes) (Shai et al., 2006), history of previous IGT or IFG (Nichols, Hillier, & Brown, 2007), hypertension or dyslipidemia (Chehade, Gladysz, & Mooradian, 2013; Gress, Nieto, Shahar, Wofford, & Brancati, 2000), physical inactivity (Hu et al., 2001), history of gestational diabetes (C. Kim, Newton, & Knopp, 2002), low birth weight as a result of an in utero environment (Whincup et al., 2008), polycystic ovarian syndrome leading to insulin resistance (Legro, 2006), and finally, decline in insulin secretion due to advancing age (Dechenes, Verchere, Andrikopoulos, & Kahn, 1998; Group, 2003). Until recently, T2DM was considered to be a disease confined to adulthood, rarely observed in individuals under the age of 40, but clinically based reports and regional studies suggest that T2DM in children and adolescents is now more frequently being diagnosed (G. Alberti et al., 2004), this reflects the increasing number of children entering adulthood with unprecedented levels of obesity. T2DM is primarily caused by obesity, insulin resistance in liver, skeletal muscle and adipose tissue and a relative insulin secretion defect by the pancreatic β-cell (K. G. M. M. Alberti & Zimmet, 1998; Shaw et al., 2010). Insulin is a hormone produced by the pancreatic β-cells and is the key hormone for the regulation of blood glucose. The hormone stimulates uptake of glucose from the blood in the muscle and fat tissue, storage of glucose as glycogen in the liver and muscle cells, and uptake and esterification of fatty acids in adipocytes. In addition, insulin inhibits the
breakdown of proteins, the hydrolysis of triglycerides and the production of glucose from amino acids, lactate and glycerol. Glucagon, which is also secreted by the endocrine pancreas, has the opposite effects to that of insulin. The hormone causes the liver to convert stored glycogen into glucose, thereby increasing blood glucose. Besides, glucagon stimulates insulin secretion, so that glucose can be used by insulin-dependent tissues. Hence, glucagon and insulin are part of a feedback system that keeps blood glucose at the right level (Figure 2.1) (Guariguata, Nolan, & Beagley, 2014).

![Figure 2.1. Regulation of glucose by the hormones insulin & glucagon. Diabetes results from an imbalance between the insulin-producing capacity of the pancreatic β-cells and the requirement for insulin action in insulin target tissues such as liver, adipose tissue and skeletal muscle. Adopted from (Guariguata et al., 2014).](image)

For T2DM to occur the balanced relationship between insulin action and release have to be disrupted. In other words, T2DM develops mainly in those who cannot increase insulin secretion sufficiently to compensate for their insulin resistance. Whereas insulin resistance is an early phenomenon partly related to obesity, pancreas β-cell function
declines gradually over time already before the onset of clinical hyperglycemia. Several mechanisms have been proposed for these two defects (Guariguata et al., 2014).

Insulin resistance have been ascribed to elevated levels of free fatty acids (Boden, 1996), inflammatory cytokines (Wei et al., 2008), adipokines (Rabe, Lehrke, Parhofer, & Broedl, 2008) and mitochondrial dysfunction (J.-a. Kim, Wei, & Sowers, 2008), while glucose toxicity (Robertson, Harmon, Tran, Tanaka, & Takahashi, 2003), lipotoxicity (Robertson, Harmon, Tran, & Poitout, 2004), and amyloid formation (Hull, Westermark, Westermark, & Kahn, 2004) have been proposed as central aspects for β-cell dysfunction (Stumvoll, Goldstein, & van Haeften, 2005).

The medical and socioeconomic burden of T2DM is generally caused by the associated complications of the disease. The severe complications accompanying T2DM are mostly microvascular (e.g. retinopathy, neuropathy and nephropathy) and macrovascular diseases, leading to reduced quality of life and increased morbidity and mortality from end-stage renal failure and cardiovascular disease (CVD). Hyperglycemia plays a central role in the development and progression of the vascular complications, which often persist and progress despite improved glucose control, possibly as a result of prior occurrences of hyperglycemia. Increased cardiovascular risk, however, appears to begin before the development of frank hyperglycemia, presumably because of the effects of insulin resistance. This phenomenon has been described as the "ticking clock" hypothesis of complications (Haffner et al., 1999; Stern, 1996), where the clock starts ticking for microvascular risk at the onset of hyperglycemia, and for macrovascular risk at some antecedent point, i.e. with the onset of insulin resistance. It is generally accepted that the long-term complications of diabetes mellitus are far less common and less severe in people who have well controlled blood sugar levels (D. Nathan et al., 2009; D. M. Nathan, 2005). However, some recent trails that had great success in lowering blood sugar in T2DM patients, but no success in reducing deaths from cardiovascular disease, challenges the theory of hyperglycemia as the major cause of diabetic complications (Taubes, 2008).

The familial clustering of the degree and type of diabetic complications indicates that genetics may also play a role in causing diabetic complications (Monti et al., 2007). Although not fully understood, the complex mechanisms by which diabetes leads to these complications involves hyperglycemia and both functional and structural abnormalities
of small blood vessels along with accelerating factors such as smoking, elevated cholesterol levels, obesity, high blood pressure and lack of regular exercise (Milicevic et al., 2008).

2.4 Prevalence and mortality rate of diabetes mellitus

2.4.1 Mortality rate

Worldwide, one and a half million people died directly from diabetes in 2014. However, blood glucose levels that are higher than optimal but are below the diagnostic threshold for diabetes also increases the risk of death, particularly from CVDs, and have caused an additional 2.2 million deaths. The largest number of deaths due to high blood glucose has occurred in upper middle-income countries. Almost one-half of all deaths attributable to high blood glucose are premature - before the age of 70 years. In low-income countries, more than half of the deaths attributable to high blood glucose are premature, in contrast to high-income countries where about one-fifth of deaths are premature. This is probably the result of later detection and poorer management in low-income countries. WHO projects that diabetes will be the 7th leading cause of death in 2030 (Mathers & Loncar, 2006).

2.4.2 Prevalence and incidence

The number of people with diabetes has increased from 180 million in 1980 to 422 million in 2014. This increase is attributed to population growth and aging (40%), rise in age-specific prevalence (28%) and interaction of the two (32%). Age-standardized prevalence trends are shown in Figure (2.2). Age-standardized prevalence in adults 18-year-old and above has almost doubled, from 4.7% in 1980 to 8.5% in 2014. In 1980, the prevalence was highest in high-income countries (5.2%) and lowest in low-income
countries (3.3%). By 2014, the prevalence in low-income countries has become higher (7.4%) than in high-income (7.0%) countries. The prevalence of diabetes has risen faster in low- and middle-income countries and is currently highest in upper middle-income countries (9.3%). In the WHO regions, the prevalence is highest in the Eastern Mediterranean region (13.7%) and lowest in the African region (7.1%) (Atlas, 2015).

**Figure (2.2):** Trends in prevalence of diabetes, 1980-2014, by country income group (Atlas, 2015).

In 2015, the top 10 countries with higher number of people with diabetes are shown in Table (2.2) (Atlas, 2015).

**Table (2.2):** Top ten countries/territories for number of people with diabetes (20-79 years), 2015.

<table>
<thead>
<tr>
<th>#</th>
<th>Country/territory</th>
<th>Number of people with diabetes</th>
<th>Diabetes prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>China</td>
<td>109.6 million</td>
<td>8%</td>
</tr>
<tr>
<td>2</td>
<td>India</td>
<td>69.2 million</td>
<td>5.22%</td>
</tr>
<tr>
<td>3</td>
<td>United States of America</td>
<td>29.3 million</td>
<td>9.18%</td>
</tr>
<tr>
<td>4</td>
<td>Brazil</td>
<td>14.3 million</td>
<td>4.13%</td>
</tr>
<tr>
<td>5</td>
<td>Russian Federation</td>
<td>12.1 million</td>
<td>8.43%</td>
</tr>
<tr>
<td>6</td>
<td>Mexico</td>
<td>11.5 million</td>
<td>9.40%</td>
</tr>
<tr>
<td>7</td>
<td>Indonesia</td>
<td>10.0 million</td>
<td>4.00%</td>
</tr>
<tr>
<td>8</td>
<td>Egypt</td>
<td>7.8 million</td>
<td>9.50%</td>
</tr>
<tr>
<td>9</td>
<td>Japan</td>
<td>7.2 million</td>
<td>5.65%</td>
</tr>
<tr>
<td>10</td>
<td>Bangladesh</td>
<td>7.1 million</td>
<td>4.38%</td>
</tr>
</tbody>
</table>
2.4.3 Prevalence in Palestine

In Palestine in 2004, the reported prevalence in Palestinian Family Health Survey was 10.6% (8.7–12.5) versus an estimated 11.4% (9.7–13.4); in 2006, these values were 11.8% (9.8–13.8) and 12.3% (10.6–14.6), respectively and by 2010, the prevalence of type 2 diabetes had increased to 14.5% (12.2–16.7), in this period, prevalence in men rose from 11.7% (9.7–13.6) to 15.9% (13.4–18.1) and in women from 11.4% (9.3–13.3) to 13.2% (11.1–15.2). The forecasts for prevalence of diabetes are 20.8% (18.0–23.2) for 2020 and 23.4% (20.7–25.8) for 2030 (Abu-Rmeileh, Husseini, O'Flaherty, Shoaibi, & Capewell, 2012). The UN Relief reported the rate patient with diabetes is 11.8 % in Gaza strip and 10.5% in the west bank among the patients aged 40 years and older (UNRWA, 2007). However, in Palestine, there is under-reporting of the diseases and under diagnosis. This is due to lack of clinic recording and suitable hospital (Abu-El-Noor & Aljeesh, 2014).

2.5 Risk factors for T2DM

Many studies have elaborated the associations between several risk factors and occurrence of T2DM. Body mass index (BMI), lipids, smoking, physical inactivity, low education, dietary patterns, vitamin D deficiency, Ethnicity, Age, and recently Genetics are the most frequently documented risk factors for developing T2DM (Lyssenko et al., 2008; Valdés, Botas, Delgado, Álvarez, & Cadórniga, 2007)

2.5.1 BMI

Overweight and obesity are driving the global diabetes epidemic. They affect the majority of adults in most developed countries and are increasing rapidly in developing countries. If current worldwide trends continue, the number of overweight people (BMI ≥25 kg/m2) is projected to increase from 1.3 billion in 2005 to nearly 2.0 billion by 2030 (Mathers & Loncar, 2006).

Many longitudinal studies have reported that increased BMI is a strong risk factor for T2DM (Almdal, Scharling, Jensen, & Vestergaard, 2008; Meisinger et al., 2002). A strong positive association between obesity and T2DM is found in both men and women (Skarfors, Selinus, & Lithell, 1991). Obesity is associated with increased risk of
developing insulin resistance and T2DM. In obese individuals, adipose tissue releases increased amounts of non-esterified fatty acids, glycerol, hormones, pro-inflammatory cytokines and other factors involved in the development of insulin resistance. When insulin resistance is accompanied by dysfunction of the β-cells, the following fall in insulin secretion results in failure to control blood glucose level leading to T2DM (S. Kahn, 2003).

Many genes interact with the environment leading to obesity and in some also to diabetes. Many genes have been shown to be involved in determining the whole range of BMI in a population, with each gene only explaining a few hundred grams difference in body weight (Hebebrand & Hinney, 2009). Genes responsible for obesity and insulin resistance interact with environmental factors such as increased fat/calorie intake and decreased physical activity resulting in the development of obesity and insulin resistance followed ultimately by the development of T2DM (S. E. Kahn, Hull, & Utzschneider, 2006).

2.5.2 Lipids

Unfavorable blood lipids has been reported as a risk factor for T2DM by several prospective studies. An inverse relationship between HDL cholesterol and risk of T2DM have been documented (Jacobsen, Bønaa, & Njølstad, 2002). High plasma triglycerides and low plasma HDL cholesterol levels are both seen in the insulin resistance syndrome, which is a pre-diabetic state (Taskinen, 2003).

The mechanisms suggested are increased circulating levels of free fatty acids due to increased insulin levels and increased chylomicron-assembly and secretion in the gut, the latter process being a result of localized insulin resistance in the intestine. Cross sectional studies have shown that high BMI is associated with a higher level of total cholesterol and unfavorable lipids pattern, with low concentrations of HDL cholesterol and high triglycerides concentrations (Tsai, Yang, Lin, & Fang, 2004; Wild & Byrne, 2006).

2.5.3 Smoking

Several prospective studies reported that current smoking is a risk factor for developing T2DM (Yeh, Duncan, Schmidt, Wang, & Brancati, 2010). The association between smoking and T2DM was stronger for heavy smokers ≥ 20 cigarettes/day compared with
light smokers or former smokers (Nagaya, Yoshida, Takahashi, & Kawai, 2008). In addition some studies found an increased risk of T2DM the first 2-3 years after smoking cessation (Hur et al., 2007). Smoking leads to insulin resistance and inadequate compensatory insulin secretion response. This could be due to a direct effect of nicotine or other components of cigarette smoke on β-cells of the pancreas as suggested by the association of cigarette smoking with chronic pancreatitis and pancreatic cancer (Talamini et al., 1999).

2.5.4 Physical inactivity

Strong evidence in many studies shows that physical inactivity increases the risk of many adverse health conditions, including major non-communicable diseases such as T2DM. Longitudinal studies have found physical inactivity to be a strong risk factor for T2DM (Fretts et al., 2009). Prolonged television watching as a surrogate marker of sedentary lifestyle was reported to be positively associated with diabetes risk in both men and women. Moderate and vigorous physical activity was associated with a lower risk of T2DM (Krishnan, Rosenberg, & Palmer, 2008). Physical activity plays an important role in delaying or prevention of development of T2DM in those at risk both directly by improving insulin sensitivity and reducing insulin resistance, and indirectly by beneficial changes in body mass and body composition (Hamman et al., 2006).

2.5.5 Dietary pattern

Excessive caloric intake is a major driving force behind escalating obesity and T2DM epidemics worldwide. Dietary habits are important life style factor associated with the development of T2DM. Positive association have been reported between the risk of T2DM and different patterns of food intake. Many studies found that the quality of fats and carbohydrates plays an important role in the development of diabetes, independent of BMI and other risk factors (Hu et al., 2001). Higher dietary glycemic load (GL) and trans fat are associated with increased diabetes risk, whereas greater consumption of cereal fiber and polyunsaturated fat is associated with decreased risk (de Munter, Hu, Spiegelman, Franz, & van Dam, 2007). Higher consumption of fruits and vegetables is associated with reduced risk of T2DM. The possible mechanisms suggested are that insoluble fiber intake was consistently associated with improved insulin sensitivity (Meyer et al., 2000). A prospective study found that regular consumption of white rice is
associated with an increased risk of T2DM whereas replacement of white rice by brown rice or other whole grains is associated with a lower risk (Sun et al., 2010).

2.5.6 Vitamin D deficiency

A recent research showed that vitamin D deficiency may have negative effects on glucose intolerance, insulin secretion and T2DM (Pittas, Sun, Manson, Dawson-Hughes, & Hu, 2010), either directly via vitamin D receptor (VDR) activation or indirectly via calcemic hormones and also via inflammation (Chagas, Borges, Martini, & Rogero, 2012). As both 1-α-hydroxylase and VDR are present in pancreatic β-cells, vitamin D has significant roles in the synthesis and release of insulin. Furthermore, vitamin D has influence on the insulin sensitivity by controlling calcium flux through the membrane in both β-cells and peripheral insulin-target tissues (Wolden-Kirk, Overbergh, Christesen, Brusgaard, & Mathieu, 2011). In addition, vitamin D supplementation is recognized as a promising and inexpensive therapy, which may decrease the risk of T2DM and improve glycemic parameters in T2DM patients. Therefore, it seems that the positive effects of vitamin D are correlated with its action on insulin secretion and sensitivity as well as on inflammation (Takiishi, Gysemans, Bouillon, & Mathieu, 2010).

2.5.7 Ethnicity

The risk of T2DM is greater in African-American, Hispanic/Latin American, American Indian and Alaska Native, Asian-American, or Pacific Islander ethnicity. In some studies, the researchers found that the prevalence of diabetes was 16.1% among Native Hawaiians, 15.8% in Latinos, 15% in African-Americans, 10.2% in Japanese-Americans, and 6.3% in whites. The reasons why people of these specific ethnicities are at greater risk of T2DM are not fully understood and may be attributed to genetic effect (Service, 2009).

2.5.8 Age

The chance of getting T2DM increases with age. That is probably because people tend to exercise less, and gain weight as they age. There are combined effects of increasing insulin resistance and impaired pancreatic islet function with advanced age (Kirkman et
However, there is age-related declines of pancreatic islet function and islet proliferative capacity (Maedler et al., 2006).

2.5.9 Genetics

There is sufficient evidence that T2DM has a strong genetic basis (Medici et al., 1999). The concordance of T2DM in monozygotic twins is ~76%. The lifetime risk (at age 80 years) for T2DM has been calculated to be 38% if one parent had T2DM. If both parents are affected, the incidence of T2DM in the offspring is estimated to approach 60% by the age of 60 years (Stumvoll et al., 2005).

Family history of Type 2 diabetes. A family history of diabetes has a stronger risk factor for T2DM than T1DM. Having one or more first-degree relatives with T2DM increases the odds of having the disease compared with someone without such relatives. The estimations vary, but the odds usually range from two to six times more likely (Harrison et al., 2003). Since genetic tests are not currently available for T2DM; the use of tools that include a family history of diabetes is potentially applicable way (Valdez, 2009).

2.6 Genetic susceptibility to T2DM

Technological developments in molecular biology have allowed the successful identification of common (SNPs) contributing to diabetes susceptibility. Genome-wide approaches, such as (GWAS), have been successful at finding statistically significant associations between specific genomic loci and T2DM susceptibility (Basile et al., 2014).

2.6.1 Candidate gene study

This is based on the knowledge of the gene or protein function in relation to the disease in question, and determines the association between certain polymorphisms and the disease-related trait(s). For T2DM, most candidate gene studies have focused on both genes related to pancreatic beta cell function and insulin resistance genes (Guja, Gagniuc, & Ionescu-Tirgoviste, 2012).
2.6.2 Genome–wide association studies (GWAs)

To 2012, (GWAs) have fixed approximately more than 70 gene loci associated with T2DM (M. C. Ng et al., 2014). The rise of GWAs in the midle-2000 has developed the gene finding in T2DM, with great number of loci determined to date. In spite of all of the loci that have been fixed to be correlated with T2DM, there are details for less than 10% of the genetic heritability of the T2DM (Basile, Johnson, Xia, & Grant, 2014).

The greater part of GWAs on the side of T2DM have been implied on European population. The GWAS that has been performed in non-European population, such as individuals rising from Indian subcontinent (Pakistan, India and Bangladesh) are still in need to improve to exhibit logical vision into the genetic view of the disease in these populations (Zia, Kiani, Bhatti, & John, 2013).

Although the majority of GWAS results were conducted in populations of European ancestry, recent GWAS in Asians have made important contributions to the identification of T2DM susceptibility loci. These studies not only confirmed T2DM susceptibility loci initially identified in European populations, but also identified novel susceptibility loci that provide new insights into the pathophysiology of diseases. Review GWAS results of T2DM conducted in East and South Asians and compare them to those of European populations (Qi, Wang, Strizich, & Wang, 2015).

2.6.3 Polymorphism

About 99.9% of the human DNA sequence between unrelated individuals is similar, only 0.1% of coded DNA varies between two chromosomal strands (Kruglyak & Nickerson, 2001). These differences responsible for the variety in human phenotypes and their responsiveness to environmental exposures (Stenson et al., 2009).

DNA variation occurs in several known forms. Mutations can be defined as sequence variations that occur in less than 1% of the population while the more widespread variation are known as polymorphisms. The most common genetic variations are (SNPs).
About 34.4 Million of SNPs have been known according to NCBI dbSNP. About 90% of the genetic differences among individuals is believed to be resulted from SNPs (Palmer & Cardon, 2005). The common form of SNPs are transition (pyrimidine-pyrimidine C↔T or purine-purine A↔G) or transversion (pyrimidine-purine or purine-pyrimidine) replacements (Barreiro, Laval, Quach, Patin, & Quintana-Murci, 2008). About 2/3 of SNPs are transition replacements, while 1/3 of SNPs are transversion replacements (Guo & Jamison, 2005).

The classification of SNPs depended on their genomic site. The coding SNPs (cSNP) are located in exon sequences (translated sequences) of DNA and non-coding SNPs are located in untranslated sequences (e.g., promoters, introns, 5’ UTR, 3’ UTR, and intergenic regions) of DNA. SNPs may be either synonymous or non-synonymous (Burton, Tobin, & Hopper, 2005).

The synonymous cSNPs are classically silent and change the sequence of DNA, but do not change the sequence of amino acid in protein while, the non-synonymous cSNPs change the sequence of DNA in a coding region and they change the sequence of amino acids in proteins. Due to the changes in the function and structure of the amino acids, these cSNPs are considered as genetic markers for many diseases (Stenson et al., 2009).

The majority of SNPs are situated in the non-coding region of genome, some of these intronic SNPs have no recognized role but they may participate in a regulation of the gene expression of coding or translated regions; these SNPs are known as regulatory SNPs (rSNPs). Regulatory SNPs situated in the promoter region may influence the sites of transcription factor (Carlson, Eberle, Kruglyak, & Nickerson, 2004; Stenson et al., 2009). Generally the non-synonymous SNPs in a coding sequence are more likely to affect the function or availability of a protein than other SNP classes (Carlson et al., 2004).

2.6.4 Genes and Type 2 Diabetes

It has been known that T2DM is a multifactorial disease with a strong genetic component interacting with environmental factors (Sanghera & Blackett, 2012). Inheritance is a strong factor implicating inheritable genetics in T2DM: in monozygotic twins (96%) supports a substantial contribution of genetic factors to T2DM (Medici et al., 1999) also 40% having relatives (especially first degree) with T2DM the risks
substantially (M. McCarthy & Menzel, 2001; Wolford & de Courten, 2004). Many genes have been significantly associated with developing T2DM, until 2011 more than 36 genes have been found that contribute to the risk of T2DM, most of the discovered gene variants have been linked to beta-cell dysfunction, impaired glucose homeostasis and insulin secretion rather than insulin resistance (Christian Herder & Roden, 2011).

2.6.5 Genetic subgroups

2.6.5.1 Genes related to β-cell failure

It can be proposed that some of the candidate genes for T2DM related to β-cell failure can be grouped into four subgroups based on what is known about their molecular function (Figure 2.3). First, *KCNJ11, KCNQ1, ABCC8, KCNK16, SLC30A8, SRR, ADAMTS9, MTNR1B, CAMK1D, CENTD2, DUSP9, BCL11A, PRC1* and *CHCHD9* gene products are probably involved in cellular ion homeostasis and insulin secretion. A second group of genes – *TCF7L2, TCF1, TCF2, HHEX / IDE, IGF2BP2, CDKAL1, GLIS3, and NOTCH2* – is likely to be involved in the growth and development of the pancreas, which likely influences the overall capacity of the pancreas to secrete insulin. Of this group *TCF7L2, TCF1, and TCF2* are important in Wnt signaling (Jin, 2008). In line with the presence of this group of T2DM candidate genes, some genes whose deficiency can induce MODY, such as *NEUROD1, PDX1, HNF4A, and PAX4*, are also related to pancreas growth and development. A third group is related with cell cycle events: *JAZF1* and *TCF2* have both been associated with prostate cancer and these genes might play a role in the regulation of the cell cycle (Eeles et al., 2008; Gudmundsson et al., 2007; Thomas et al., 2008). *CDKN2A, TP53INP1, and CDC123* also play a role in the cell cycle. Finally, *THADA* and *WFS1* are thought to be involved in the apoptosis of β-cells. Genes involved in the cell cycle and apoptosis of β-cells might play a role in diabetes by dysregulating the response of β-cells when a higher insulin output is called for when insulin resistance is present.
2.6.5.2 Genes related to insulin resistance

Several genes associated to T2DM in GWAS seem to be related to insulin resistance (Figure 2.4), which can be classified into four subgroups. First, FTO, can be classified as a separate group amongst the T2DM candidate genes since it is the only gene that is associated with both obesity and T2DM (Hofker & Wijmenga, 2009; Tung & Yeo, 2011). In line with this, FTO deficient mice (Fto-/-) have a significant reduction in adipose tissue and lean body mass. Thus, the association between FTO and T2DM might be driven by obesity. PPARG may be the only member of the second subgroup, which is believed to influence insulin sensitivity by affecting both adiposity and post-receptor pathway of insulin. A third group includes IRS1, GRB14, PTPRD, DUSP9 and SPRY2, are closely associated with the insulin receptor signaling pathway, and are thought to be related to T2DM by this mechanism. For example, the protein product encoded by IRS1 is...
phosphorylated by insulin receptor tyrosine kinase directly. Finally, KLF14, ADAMTS9 and HMGA2 may be also related to insulin resistance (Voight et al., 2010); however, knowledge about their mechanism is still limited.

Figure (2.4): Classification of candidate genes for T2DM with a potential role in insulin resistance (Wolfs et al., 2013)

2.7 Candidate genes

2.7.1 Kruppel-like factor 14 (KLF14) rs972283

2.7.1.1 Location of gene

The Kruppel-like factors (KLFs) belong to the family of zinc finger-containing transcription factors that regulates a diverse array of cellular processes, including cell proliferation, differentiation, and apoptosis in normal as well as in pathological situations. The gene KLF14 is located on the long (q) arm of chromosome 7 at position q32.2 as mention in figure (2.5).
(Figure 2.5): Location of KLF14 gene on the human genome (Adopted from ncbi.nlm.nih.gov)

2.7.1.2 Gene Function

KLF14 proteins function as epigenetic reprogramming factors during cell differentiation in many cell populations and in engineered induced pluripotent stem cells. In 2009, Truty et al., in their work on pancreatic cancer cells, identified the first function of KLF14, as being the repressor of the transforming growth factor β receptor II gene (TGFβRII) (Truty, Lomberk, Fernandez-Zapico, & Urrutia, 2009). This TGFβRII belongs to the transforming growth factor β (TGFβ) signaling pathway. This pathway regulates a number of cellular activities, including cell differentiation, proliferation and apoptosis, with a negative influence on cellular growth (Shi & Massagué, 2003). The 18 KLFs described in mammals possess highly conserved cysteine and histidine zinc fingers, critical for recognition and binding to CACCC or CGCCC DNA motifs (McConnell & Yang, 2010).

2.7.1.3 Role in T2DM

Members of the KLF family have been found to be involved in adipogenesis (Banerjee et al., 2003) and the expression of GLUT4 in adipocytes (Gray et al., 2002). KLF14 is a master trans-regulator of multiple genes that are associated with metabolic phenotypes in adipose tissue. Because of this new role in fat metabolism, the KLF genes are thought to be candidates for conferring susceptibility to T2DM. KLF14 may acts in to regulate the expression of a network of genes controlling several metabolic traits (e.g., LDL-c, HDL-c, TG and BMI) (Small et al., 2011). Variants near the maternally-expressed transcription factor KLF14 are robustly associated with both T2DM and HDL-c concentration in GWAS.

In Pakistan, Rees et al., reported KLF14 (SNPs): 972283 was genotyped 1,678 T2DM and 1,584 normal participants from two populations; one resident in the UK and one indigenous to the District of Mirpur. All samples (3262) were genotyped for SNPs using either the Kompetitive Allele-Specific PCR "KASPar" (KBioscience, Hoddesdon, UK) or TaqMan (Applied Biosystems, Warrington, UK) method. The authors reported risk
allele of the rs972283 (G) associated with T2DM (OR = 1.14, \( P = 9.66 \times 10^{-3} \)) (Rees et al., 2011).

In Saudi Arabian, which has one of the world’s highest prevalence of T2DM, Al-Daghri et al, investigated the association of 38 T2DM risk loci in the Saudi Arabian population (1166 patients with T2DM and 1235 healthy controls). All DNA samples from cases and controls were genotyped for 38 SNPs using the KASPar method (K Bioscience, HoddesdonUK), KLF14 (rs972283) risk allele (G), however, was not among the significantly associated SNPs (\( p = 0.25 \); and OR 1.09, 95% CI = 0.94-1.26), (Al-Daghri et al., 2014).

2.7.2 Potassium voltage-gated channel, KQT-like subfamily, member 1 (KCNQ1) rs2237892

2.7.2.1 Location of gene

The KCNQ1 gene has a total of 17 exons, spans 404 kb of chromosome sequence and is located on chromosome 11p15.5 as mention in figure (2.6) (Neyroud et al., 1999).

(Figure 2.6): Location of KCNQ1 gene on the human genome (Adopted from ncbi.nlm.nih.gov).
2.7.2.2 Gene Function

*KCNQ1* codes for the pore-forming alpha subunit of the voltage-gated K+ channel (KvLQT1) which is expressed mainly in the heart and pancreas. This channel plays an important role in controlling repolarization of the ventricles (Barhanin et al., 1996). Specific function of a potassium channel depends on its protein components and its location in the body. Channels made with the *KCNQ1* protein are active in the inner ear and in heart (cardiac) muscle. In the inner ear, these channels help maintain the proper ion balance needed for normal hearing. In the heart, the channels are involved in recharging the cardiac muscle after each heartbeat to maintain a regular rhythm. The *KCNQ1* protein is also produced in the kidney, lung, stomach, and intestine, where it is involved in transporting molecules across cell membranes (Yang et al., 1997).

2.7.2.3 Role in T2DM

*KCNQ1* is ubiquitously expressed in epithelial cells, including the endocrine and exocrine pancreatic cells (Thévenod, 2002). *KCNQ1* was reported to be expressed in insulin-secreting cells, and inhibition of this potassium channel has been shown to significantly increase insulin secretion (Ullrich et al., 2005), suggesting that *KCNQ1* channels may play a role in regulation of insulin secretion. GWAS has been applied to complex diseases, including T2DM and has resulted in the identification of a growing number of trait susceptibility loci for T2DM (Prokopenko, McCarthy, & Lindgren, 2008).

**In Korea,** Lee et al., investigate the association among the polymorphisms of *SLC30A8, HHEX, CDKN2A/B, IGF2BP2, FTO, WFS1, CDKAL1* and *KCNQ1* and the risk of T2DM in the Korean population. This study was based on a multicenter case-control study, including 908 patients with T2DM and 502 non-diabetic controls. All DNA samples from cases and controls were genotyped by TaqMan PCR. The C allele of rs2237892 (OR = 1.31, 95% CI = 1.10-1.56, p = 0.003) in *KCNQ1* showed significant associations with T2DM (Lee et al., 2008).
In Japan, Yasuda et al., carried out a multistage GWAs of T2DM in Japanese individuals, with a total of 1,612 cases and 1,424 controls and 100,000 SNPs. The most significant association was obtained with SNPs in *KCNQ1*, and dense mapping within the gene revealed that rs2237892 in intron 15 yielded a P value of $6.7 \times 10^{-13}$ (OR = 1.49), (Yasuda et al., 2008).

2.7.3 Zinc-finger BED domain-containing 3 (*ZBED3*) rs4457053

2.7.3.1 Location of gene

The gene is *ZBED3* located on the long (q) arm of chromosome 5 at position q13.3 as mention in figure (2.7). It has three exons.

(Figure 2.7): Location of *ZBED3* gene on the human genome (Adopted from ncbi.nlm.nih.gov).
2.7.3.2 Gene Function

The *ZBED* gene family is a group of closely related genes that encode proteins involved in the regulation of diverse functions in vertebrates. *ZBED3* is an axin-interacting protein important for Wnt/β-catenin signal modulation, involved in embryogenesis and carcinogenesis in mammals (Chen et al., 2009). *ZBED3* acts as a positive regulator in the activation of the canonical Wnt/beta-catenin signaling pathway by stabilizing cytoplasmic beta-catenin. Involved in transcription activation of Wnt target gene expression. *ZBED3* Interacts with AXIN1; the interaction is direct, enhanced by protein kinase GSK3B and casein kinase CSNK1E activities and decreases GSK3B-induced beta-catenin serine and threonine phosphorylations (Chen et al., 2009).

2.7.3.3 Role in T2D

In 2011, (GWAS) have shown that the protein zinc-finger BED domain-containing 3 (*ZBED3*) is associated with T2DM (Ohshige et al., 2011). A more recent publication has proposed that changes in *ZBED3* are associated with increased fasting and postprandial glucagon levels in humans (Jonsson et al., 2013). *ZBED3* was expressed in almost all mouse tissues, with the highest levels detected in muscle. Muscle is often connected to glucose metabolism and insulin sensitivity, suggesting that this tissue may be an important contributor to systemic levels of *ZBED3*. ZBED3 mRNA and protein are expressed in both human skeletal muscle and adipose tissues and has been associated with insulin resistance. In a manner consistent with circulating *ZBED3* levels, Jia et al., further demonstrated that expression of ZBED3 mRNA was significantly increased in both the muscle and adipose tissue of T2DM patients (Jia et al., 2014).

In Pakistan, Rees et al., genotyped 1,678 participants with T2DM and 1,584 normoglycaemic control participants from two populations; one resident in the UK and one indigenous to the District of Mirpur. All samples (3262) were genotyped for SNPs using either the KASPar (KBioscience, Hoddesdon, UK) or TaqMan (AppliedBiosystems, Warrington, UK) method. No association was found between *ZBED3* gene variants (rs4457053) and T2DM risk. risk allele (G/A) (p = 0.405; and OR = 1.05, 95% CI = 0.94–1.18) (Rees et al., 2011).
In Saudi Arabian, Al-Daghri et al., examined the association of rs4457053 variant in ZBED3 with T2DM in 2401 subjects (1166 were type 2 diabetic patients and 1235 normoglycemic subjects). All DNA samples from patients and controls were genotyped for 38 SNPs using the KASPar method (KBioscience, HoddesdonUK) The rs rs4457053 variant of ZBED3 not associated with T2DM risk with an allelic odds ratio of 1.09 (95% CI [0.94-1.26], , p = 0.28), (Al-Daghri et al., 2014).

2.7.4 Fat mass and Obesity-associated protein (FTO) rs8050136

2.7.4.1 Location of gene

FTO is located on chromosome 16q12.2 as mention in figure (2.8), possesses a total length of about 430 kb and contains 9 exons and 8 introns. The human protein consists of 505 amino acids and is localized to the cell nucleus. (Gerken et al., 2007).

(Figure 2.8): Location of FTO gene on the human genome (Adopted from ncbi.nlm.nih.gov)
2.7.4.2 Gene Function

*FTO* gene encodes 2-oxoglutarate dependent nucleic acid demethylase that involved in metabolism of fatty acid, repairing DNA and post-translational modifications (Clifton et al., 2006). Also it may play an important role in the management of energy homeostasis (Fredriksson et al., 2008; Stratigopoulos et al., 2008), demethylation of the nucleic acid, and regulation fat masses of body via lipolysis (Wåhlén, Sjölin, & Hoffstedt, 2008). The *FTO* expression in the hypothalamus suggests a potential role in the regulation and control of food intake and whole body metabolism (Dina et al., 2007).

2.7.4.3 Role in T2DM

*FTO* polymorphisms have also been associated with T2DM, but it could be due to the increased BMI, which are known to impact on insulin responsiveness (C Herder et al., 2008; Legry et al., 2009; Ng et al., 2008; Sanghera et al., 2008; Yajnik et al., 2009). Several reports suggested that *FTO* expression could be regulated by environmental factors (Fredriksson et al., 2008; Gerken et al., 2007; Stratigopoulos et al., 2008). Study reported that the age-dependent decline of *FTO* expression is associated with peripheral defects of glucose and lipid metabolism in adipose tissue and skeletal muscle (Grunnet et al., 2009). However, to date, little is known regarding the factors and the mechanisms controlling *FTO* expression, as well as its cellular functions, particularly in human skeletal muscle.

In Oman, using case-control design, a total of 992 diabetic patients and 294 normoglycemic were genotyped for *FTO* rs8050136, by an allelic discrimination assay-by-design TaqMan method. NO association was found between *FTO* gene variant (rs8050136) and T2DM risk. Risk allele (A/C) (P = 0.770, OR = 1.03) (Al-Sinani et al., 2015).
In Qatar, O’Beirne et al., conducted a case-control study on association between T2DM and FTO (rs8050136). The study employed 1,124 T2DM patients and 590 controls. Subjects underwent genotyping for 37 (SNPs) in 29 genes known to be associated with T2DM in Europeans and/or Asian populations, and an additional 27 tag SNPs related to these susceptibility loci. All genotyping was performed using TaqMan SNP Genotyping Assays (Life Technologies, Carlsbad, CA). FTO (rs8050136) was not significantly associated with T2DM. Risk allele (A/C) \( (p = 8.1 \times 10^{-01}; \text{and OR} \ 1.04, \ 95\% \ CI = 0.881-1.218) \) (O’Beirne et al., 2016).

GWAs have dramatically increased the number of common genetic variants that are robustly associated with T2DM. Assessing the Combined Impact of 18 Common Genetic Variants of Modest Effect Sizes on T2DM risk. Assessed index (SNPs) for the 18 independent loci in 2,598 control subjects and 2,309 case subjects from the Genetics of Diabetes Audit and Research Tayside Study revealed that FTO rs8050136 is significantly associated with T2DM \( (\text{OR} = 1.11, \ 95\% \ CI = 1.02–1.20, p = 1.43 \times 10^{-02}) \) (Lango et al., 2008).

In Lebanon, a case-control association study comprising 995 T2DM patients and 1,195 normoglycaemic control participants. They genotyped by allelic discrimination method 18 SNPs in or near ADAM30, NOTCH2, THADA, TMEFF2, COL8A1, ADAMTS9-AS2, WFS1, JAZF1, SLC30A8, KCNQ1, LOC387761, ALX4, TSPAN8, FTO, and HNF1, which were previously associated with T2DM in European populations. The risk-allele frequencies of the tested SNPs were comparable with those reported for Caucasians. Demonstrated significant associations of FTO rs8050136 \( [P = 0.039; \text{OR}(95\% \ CI) = 1.16 (1.01-1.34)] \) (Almawi, Nemr, Echtay, Saldanha, & Keleshian, 2013).

In Greek-Cypriot, Votsi et al., evaluated of T2DM susceptibility loci in the Greek-Cypriot population by performing a replication case-control study. One thousand and eighteen individuals (528 T2DM patients, 490 controls) were genotyped at 21 T2DM susceptibility loci, using the allelic discrimination method. Samples were genotyped using pre-designed TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA,USA). FTO rs8050136 was among the significantly associated variants \( (\text{Non Risk/Risk Allele})(C/A) (\text{OR} = 1.33\% \ CI = 1.08–1.63, p = 0.006) \) (Votsi et al., 2017).
In UK, starting from genome-wide genotype data for 1,924 diabetic cases and 2,938 population controls generated by the Wellcome Trust Case Control Consortium, Zeggini analyzed 3,757 additional cases and 5,346 controls. They found that *FTO* rs8050136 is strongly associated with T2DM (Non Risk/Risk Allele) (C/A) (OR = 1.23% CI = 1.18-1.32, p = 7.3x10^{-14}) (Zeggini et al., 2007).

### 2.7.5 Collagen Type VIII Alpha 1 Chain (*COL8A1*) rs792837

#### 2.7.5.1 Location of gene

The gene is *COL8A1* located on the long (q) arm of chromosome 3 at position q12.1 as mention in figure (2.9). It has a total of five exons.

![Location of COL8A1 gene on the human genome](Adopted from ncbi.nlm.nih.gov)

#### 2.7.5.2 Gene Function

The gene product is a macromolecular component of the subendothelium. Major component of the Descemet's membrane (basement membrane) of corneal endothelial cells. Also component of the endothelia of blood vessels. *COL8A1* necessary for migration and proliferation of vascular smooth muscle cells and thus, has a potential role in the maintenance of vessel wall integrity and structure, in particular in atherogenesis (Xu et al., 2001).

#### 2.7.5.3 Role in T2DM

In Lebanon, a case-control association study comprising 995 T2DM patients and 1,195 normoglycaemic control participants was conducted by Almawi et al. The authors genotyped 18 SNPs in or near *ADAM30, NOTCH2, THADA, TMEFF2, COL8A1, ADAMTS9-AS2, WFS1, JAZF1, SLC30A8, KCNQ1, LOC387761, ALX4, TSPAN8, FTO, and HNF1*, which were previously associated with T2DM in European populations, by allelic discrimination method. The risk-allele frequencies of the tested SNPs were
comparable with those reported for Caucasians. The authors demonstrated significant associations of $COL8A1$ rs792837 [$P <0.001$; OR(95% CI) = 1.45 (1.24-1.68)] (Almawi et al., 2013).

Table (2.3): Information of the selected type 2 diabetes mellitus (T2DM)-related (SNPs).

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Chromosome loci</th>
<th>SNP Type</th>
<th>Disease Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLF14</td>
<td>rs972283</td>
<td>7q32.3</td>
<td>47 kb upstream</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>KCNQ1</td>
<td>rs2237892</td>
<td>11p15.5</td>
<td>Intrinsic</td>
<td>Beta-cell dysfunction</td>
</tr>
<tr>
<td>ZBED3</td>
<td>rs4457053</td>
<td>5q13.3</td>
<td>41 kb upstream</td>
<td>Beta-cell dysfunction</td>
</tr>
<tr>
<td>FTO</td>
<td>rs8050136</td>
<td>16q12.2</td>
<td>Intrinsic</td>
<td>Increased BMI-dependent insulin resistance</td>
</tr>
<tr>
<td>COL8A1</td>
<td>rs792837</td>
<td>3q12.1</td>
<td>Intergenic</td>
<td>unknown</td>
</tr>
</tbody>
</table>

2.8 Pharmacogenomics of Antidiabetic Drugs

The treatment options of T2DM target the two main defects in the pathogenesis of the disease. As mentioned earlier, the initial phase of the disease is characterized by an insulin resistance, compensated by an increase in insulin secretion by pancreatic β cells. Later, these cells will become dysfunctional and will limit their secretion of insulin. Both of these conditions lead to hyperglycemia (Bailey, 2000).

The treatment options e.g., insulin injections or different classes of oral hypoglycemic agents, such as Thiazolidinediones, increase the insulin action through its influence on the insulin resistance that will subsequently cause a decrease in fasting and postprandial glucose. On the other hand, sulfonylureas contribution to diabetes management is materialized by directly stimulating the pancreas to produce and secrete more insulin (Bailey, 2000). Following an expected decrease in pancreatic β cell
functions, these oral medications fail to adequately regulate the plasma glucose level; at this point, insulin injections are introduced as a complementary element to the treatment protocol (Bailey, 2000). These injections will elevate plasma insulin level, and that amplifies the peripheral glucose utilization (Bailey, 2000).

Response of different individuals to pharmacotherapy may vary due to many factors such as age, gender, liver and/or kidney function and co-medications. It could also be partially attributable to polymorphisms in genes encoding drug-metabolizing enzymes, transporters, receptors and molecules involved in signal transduction. These variants may contribute to the variability in pharmacokinetics (drug absorption, distribution, metabolism and excretion) and pharmacodynamics (drug target, mechanism of drug action and drug response) of a specific drug, and thus lead to varied efficacious and toxic effects (Eichelbaum, Ingelman-Sundberg, & Evans, 2006). Studies exploring such inherited differences have updated our knowledge, and the focus has been recently shifted from candidate genes (pharmacogenetics) to GWAs (pharmacogenomics). Pharmacogenetics/pharmacogenomics approaches are considered to be of importance in the promotion of personalized management of glycemia, helping physicians with the practice of a specific pharmacologic treatment for a genetically defined patient subset, by providing information for the decisions of drug selection, dose titration, treatment duration and avoidance of adverse drug reactions. In addition, they may also shed light on the mechanism of drug action and provide potential therapeutic targets. Most of the pharmacogenetic studies of T2DM have focused on oral anti-diabetic drugs including sulfonylureas, glinides, metformin and thiazolidinediones. Numerous genetic markers have been identified so far, particularly those affecting drug disposition (pharmacokinetics).

Meglitinides (Repaglinide and Nateglinide) are a new class of insulin secretagogues. They decrease blood glucose by stimulating release of insulin. Repaglinide, similar to sulfonylureas, closes the β cell K+-ATP channel. Inhibition of the K+-ATP channel depolarizes the cells and causes cell calcium channel open, as a result, the calcium influx induces insulin secretion (Holstein & Egberts, 2003). KCNQ1 rs2237892 polymorphism was found to be associated with the therapeutic efficacy of Repaglinide in Chinese T2DM patients. Following Repaglinide treatment, those T2DM patients with the rs2237892 T allele and rs2237895 C allele were more likely to have a
positive response to Repaglinide in terms of PPG levels ($P < 0.05$) than T2DM patients with the rs2237892 CC and rs2237895 AA genotypes (Dai et al., 2012).

In this study of Mastouri, the treatment option with either insulin injections or an oral hypoglycemic agents was used to sub classify the diabetic subjects. The genotype GG of \textit{KLF14-rs972283} was found to be linked to diabetic patient not treated with insulin ($P = 0.02$). This may suggest this mutation may be protective against a severe $\beta$ cell failure, since these diabetic patients were still able to control the glucose level in the plasma with oral agents only (Mastouri, 2011).
Chapter 3
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3.1 Study design

This is a case control study, in which men suffering from T2DM were compared to age- and sex-matched apparently healthy controls.

3.2 Study Sample

The target population of this study consisted of 200 Palestinian men residing in Gaza strip, 100 with T2DM and 100 apparently healthy controls. All participants were between 35 and 50 years old.

3.3 Study location

The study was done in the Genetics Lab. at the Islamic University of Gaza and in the Palestinian Medical Relief Society laboratory.

3.4 Exclusion criteria

- Cases and controls who are under 35 and over 50 years old.
- Type 1 diabetic patients or any other types of diabetes.
- Patients with renal disease, liver disease, thyroid disorders or other endocrine or chronic diseases.
- Cases and controls who are on hormone replacement therapy or corticosteroid therapy.

3.5 Ethical considerations

Informed consent was taken from all the subjects who agreed to participate in the study. The objective of the study was fully explained to all participants. The study was approved by the Helsinki ethics committee in Gaza Strip, copy of Helsinki ethics committee is attached in appendix.
3.6 Data collection

3.6.1 Body mass index

Body mass index (BMI) was calculated as the ratio of body weight in Kg/height in square meter. Participants were asked to remove heavy clothes and shoes before measurement of weight and height. Medical balance (Seca Model 762, Germany) was used for weight measurement. People with BMI=18.5-24.9 were considered to have normal weight, people with BMI=25.0-29.9 were classified overweight and people with BMI≥30.0 were considered obese (WHO, 2000).

3.6.2 Blood samples collection and processing

About 6 ml venous blood were drawn from all study participants by venipuncture, under quality control and safety procedures. Two milliliters of the collected blood were placed into sterile ethylene diamine tetra acetic acid (EDTA) tubes for DNA extraction and consequent SNPs genotyping. Two milliliters were delivered in plain tubes and left for a while without anticoagulant to allow blood to clot. The tubes were then centrifuged at 3,000 rpm for 10 minutes and the serum was collected into fresh tubes. The obtained sera were used for the determination of C-peptide, cholesterol, triglycerides, HDL-C, LDL-C. About 2 ml blood were placed into separate EDTA vacutainer tubes to be used for the determination of hemoglobin A1c (HbA1c).

3.7 Materials

3.7.1. Equipment

The present work was carried out in the Genetics lab at the Islamic University of Gaza and in the Palestinian Medical Relief Society laboratory.

The major equipments used in the study are listed in Table 3.1.
Table (3.1): The major equipment's used in the study

<table>
<thead>
<tr>
<th>#</th>
<th>Item</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thermal Cycler</td>
<td>BioRad, USA/Biometra</td>
</tr>
<tr>
<td>2</td>
<td>Horizontal electrophoresis chambers/tanks</td>
<td>BioRad, Germany</td>
</tr>
<tr>
<td>3</td>
<td>Electrophoresis power supply</td>
<td>BioRad, Germany</td>
</tr>
<tr>
<td>4</td>
<td>Digital balance</td>
<td>AE adam, USA</td>
</tr>
<tr>
<td>5</td>
<td>Vortex mixer</td>
<td>BioRad, Germany</td>
</tr>
<tr>
<td>6</td>
<td>UV transilluminator Gel documentation system</td>
<td>Vision, Scie-Plas Ltd, UK</td>
</tr>
<tr>
<td>7</td>
<td>Safety cabinet</td>
<td>Heraeus, Germany</td>
</tr>
<tr>
<td>8</td>
<td>Microcentrifuge</td>
<td>BioRad, Germany</td>
</tr>
<tr>
<td>9</td>
<td>Freezer, refrigerator</td>
<td>ORSO, pharml-spain</td>
</tr>
<tr>
<td>10</td>
<td>Micropipettes 0.1-2.5 μl/ 0.5-10 μl/ 5-50 μl/ 20-200 μl/ 100-1000 μl</td>
<td>Dragon-lab, USA</td>
</tr>
<tr>
<td>11</td>
<td>Microwave Oven</td>
<td>L.G, Korea</td>
</tr>
<tr>
<td>12</td>
<td>Immulite1000</td>
<td>Siemens, Germany</td>
</tr>
<tr>
<td>13</td>
<td>Bio-Rad D-10</td>
<td>USA</td>
</tr>
<tr>
<td>14</td>
<td>Respons®91</td>
<td>DiaSys, Germany</td>
</tr>
</tbody>
</table>

3.7.2. Chemicals, Kits and Disposables

Chemicals, kits and disposables that were used in this study are listed in Table 3-2.

Table (3.2): Chemicals, kits and disposables

<table>
<thead>
<tr>
<th>#</th>
<th>Item</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wizard ® Genomic DNA Purification Kit</td>
<td>Promega (Madison, USA)</td>
</tr>
<tr>
<td>2</td>
<td>PCR Go Taq® Green Master Mix</td>
<td>Promega (Madison, USA)</td>
</tr>
<tr>
<td>3</td>
<td>Agarose</td>
<td>Promega (Madison, USA)</td>
</tr>
<tr>
<td>#</td>
<td>Item</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>----</td>
<td>----------------------------------------------------------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>4</td>
<td>PCR primers</td>
<td>Promega (Madison, USA)</td>
</tr>
<tr>
<td>5</td>
<td>Nuclease-free water</td>
<td>Promega (Madison, USA)</td>
</tr>
<tr>
<td>6</td>
<td>Ethidium Bromide (EtBr) 10mg/ml</td>
<td>Promega (Madison, USA)</td>
</tr>
<tr>
<td>7</td>
<td>DNA molecular size marker (100 bp ladder)</td>
<td>BioLab, New England</td>
</tr>
<tr>
<td>8</td>
<td>Ethanol 70%</td>
<td>Sigma, USA</td>
</tr>
<tr>
<td>9</td>
<td>Absolute Isopropanol</td>
<td>Sigma, USA</td>
</tr>
<tr>
<td>10</td>
<td>Filter tips: 0.1-10 μl/ 5-50 μl/ 20-200 μl/ 100-1000 μl</td>
<td>Labcon, USA</td>
</tr>
<tr>
<td>11</td>
<td>Microfuge tubes for PCR - thin wall 0.2 mL capacity</td>
<td>Sigma, USA</td>
</tr>
<tr>
<td>12</td>
<td>Microfuge tubes - 1.5 mL capacity</td>
<td>Sigma, USA</td>
</tr>
<tr>
<td>13</td>
<td>EDTA tubes</td>
<td>Hy. Labs. Israel</td>
</tr>
<tr>
<td>14</td>
<td>Disposable tips</td>
<td>Labcon, USA</td>
</tr>
<tr>
<td>15</td>
<td>C-peptide kit</td>
<td>Siemens, Germany</td>
</tr>
<tr>
<td>16</td>
<td>Cholesterol kit</td>
<td>DiaSys, Germany</td>
</tr>
<tr>
<td>17</td>
<td>Triglycerides kit</td>
<td>DiaSys, Germany</td>
</tr>
<tr>
<td>18</td>
<td>HDL-C kit</td>
<td>DiaSys, Germany</td>
</tr>
<tr>
<td>19</td>
<td>HbA1c kit</td>
<td>Stanbio Kit, Texas-USA</td>
</tr>
</tbody>
</table>

3.7.3 PCR primers and restriction enzymes

Genotyping of the four genes SNPs was carried out by PCR-RFLP and one by PCR-AS. The primer sequences were obtained from published studies and are provided in Table 3.3.

The restriction enzymes required for the PCR-RFLP identification of each allele were selected from new England Biolabs database. The length of digested fragments (bp) are shown in Table 3.3.
Table (3.3): PCR primers and their characteristics with restriction enzymes, and the length of digestion products.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Method</th>
<th>Primer</th>
<th>Primer sequence</th>
<th>PCR Product (bp)</th>
<th>RE</th>
<th>Annealing (°C)</th>
<th>Length of digested fragments (bp)</th>
<th>Primer reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCNQ1</td>
<td>rs2237892</td>
<td>PCR-RFLP</td>
<td>Forward</td>
<td>5'- CTTGTGCCCCTTGCAACCA     -3'</td>
<td>354</td>
<td>MspI</td>
<td>61</td>
<td>T allele: 354 bp</td>
<td>(Gao et al., 2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reverse</td>
<td>5'- GGCTTCAGCCTCAAGCTG     -3'</td>
<td></td>
<td></td>
<td></td>
<td>C allele: 269+85 bp</td>
<td></td>
</tr>
<tr>
<td>KLF14</td>
<td>rs972283</td>
<td>PCR-RFLP</td>
<td>Forward</td>
<td>5'- ATCAGTGCAAGGTCTATAGC      -3'</td>
<td>245</td>
<td>SexAI</td>
<td>58</td>
<td>A allele: 245 bp</td>
<td>(Jing, 2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reverse</td>
<td>5'- AGGAGGGAGGAAGATCTGT      -3'</td>
<td></td>
<td></td>
<td></td>
<td>G allele: 140+105 bp</td>
<td></td>
</tr>
<tr>
<td>ZBED3</td>
<td>rs4457053</td>
<td>PCR-RFLP</td>
<td>Forward</td>
<td>5'- TAAATCAATGCCCTTGGCTAC    -3'</td>
<td>701</td>
<td>AcII</td>
<td>59</td>
<td>A allele: 701 bp</td>
<td>(Voight et al., 2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reverse</td>
<td>5'- CCCACCAGAGGGGAAGTAAT    -3'</td>
<td></td>
<td></td>
<td></td>
<td>G allele: 403 + 298 bp</td>
<td></td>
</tr>
<tr>
<td>COL8A1</td>
<td>rs792837</td>
<td>PCR-RFLP</td>
<td>Forward</td>
<td>5'- CAGCCTGATCAGCATGAATCT    -3'</td>
<td>704</td>
<td>MmeI</td>
<td>60</td>
<td>T allele: 704 bp</td>
<td>This article</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reverse</td>
<td>5'- CAGTCCTAATGAACAGCTTGTA   -3'</td>
<td></td>
<td></td>
<td></td>
<td>C allele: 472 + 232 bp</td>
<td>This article</td>
</tr>
<tr>
<td>FTO</td>
<td>rs8050136</td>
<td>PCR-AS</td>
<td>Forward Mutated</td>
<td>TGCCCACTGTGGGCAATC</td>
<td>246</td>
<td>------</td>
<td>57</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Forward Normal</td>
<td>TGCCCACTGTGGCAATA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reverse</td>
<td>AGACTTCTAGCCCTGAGATTGT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.8 Biochemical analysis

3.8.1 Determination of serum C-peptide

The level of C-peptide was measured using enzyme-linked immunosorbent assay (ELISA) (Andersen, Dinesen, Jørgensen, Poulsen, & Røder, 1993).

3.8.2 Determination of serum cholesterol

Enzymatic colorimetric method was used for the quantitative determination of total cholesterol in serum (Meiattini, Prencipe, Bardelli, Giannini, & Tarli, 1978).

3.8.3 Determination of serum triglycerides

Enzymatic colorimetric method was used for the quantitative determination of triglycerides in serum (Bucolo & David, 1973).

3.8.4 Determination of serum high density lipoprotein cholesterol

Liquid high density lipoprotein cholesterol (HDL-c) precipitant was used for the determination of HDL-C (Grove, 1979).

3.8.5 Determination of serum low density lipoproteins cholesterol

Serum low density lipoproteins cholesterol (LDL-c) was calculated by using the empirical relationship of Friedewald (Grove, 1979).

\[ \text{LDL-C} = \text{Total Cholesterol} - \text{HDL-C} - \frac{\text{TG}}{5} \]

3.8.6 Determination of HbA1c

Glycated hemoglobin was determined by the colorimetric determination of glycated hemoglobin in whole blood using Stanbio Kit, Texas-USA (Trivelli, Ranney, & Lai, 1971).

3.9 Genotyping

3.9.1 DNA extraction and polymorphism determination

Genomic DNA was isolated from blood using Wizard Genomic DNA Purification Kit (Promega, USA) following the manufacturer instructions. The isolated DNA was stored at -20°C until analysis.
3.9.2 PCR primers reconstitution

Primers were received in a lyophilized state. Primer containers were first centrifuged at 14,500 rpm for 3 minutes, and then reconstituted with ultrapure nuclease-free water to create a stock solution of each primer with a final concentration of 100 pmol/µl. The stock primer solution was then vortex mixed. Thirty microliter aliquot was taken from stock primer and diluted with 270 µl nuclease free water to make 10 pmol/µl working solution.

3.9.3 Determination of genes polymorphisms

Polymorphisms of the four genes SNPs was carried out by PCR-RFLP and one by PCR-AS. In PCR-RFLP, two primers -reverse and forward - were used to amplify the gene fragments encompassing the desired SNP. The primers used for PCR amplification published in these studies KCNQ1 (rs2237892), KLF14 (rs972283) and ZBED3 (rs4457053) (Gao et al., 2014; Jing, 2011; Voight et al., 2010) respectively. FTO (rs8050136) and COL8A1 (rs792837) specific primers were designed for SNP using BatchPrimer3 v1.0 (probes.pw.usda.gov/batchprimer3). Primers sequences and the expected size of PCR products are listed in Table 3.3. The reactions were carried out in one tube per each SNP for PCR-RFLP but PCR-AS requires two reaction tubes/sample. The final volume for each PCR reaction was 20µl, and the reaction components were as described in Tables (3.4 and 3.5). Microfuge tubes were then placed in a thermal cycler and PCR amplification was started according to the programs provided in Table (3.6). The sizes of PCR products were visualized under ultraviolet light with a 100bp ladder DNA after agarose gel electrophoresis and staining with ethidium bromide. PCR products were then digested with the appropriate restriction enzymes. Reaction conditions were set as recommended by the manufacturer. Restriction fragments were resolved on 3% agarose gels along with 100bp ladder DNA and the results were interpreted as depicted in Table 3.3.
Table (3.4): PCR components for amplification of the four genes polymorphisms (KLF14, KCNQ1, ZBED3, and COL8A1).

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (μl)</th>
<th>Final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward Primer</td>
<td>2</td>
<td>10 pmol</td>
</tr>
<tr>
<td>Reverse Primer</td>
<td>2</td>
<td>10 pmol</td>
</tr>
<tr>
<td>Nuclease free water</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>PCR Go Taq® Green Master Mix</td>
<td>10</td>
<td>1X</td>
</tr>
<tr>
<td>DNA sample</td>
<td>2</td>
<td>100 ng</td>
</tr>
</tbody>
</table>

Table (3.5): PCR components for amplification of the FTO (rs8050136) gene Polymorphisms.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (μl)</th>
<th>Final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward Normal</td>
<td>2</td>
<td>10 pmol</td>
</tr>
<tr>
<td>Forward Mutated</td>
<td>2</td>
<td>10 pmol</td>
</tr>
<tr>
<td>Reverse (Common) Primer</td>
<td>2</td>
<td>10 pmol</td>
</tr>
<tr>
<td>Nuclease free water</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>PCR Go Taq® Green Master Mix</td>
<td>10</td>
<td>1X</td>
</tr>
<tr>
<td>DNA sample</td>
<td>2</td>
<td>100 ng</td>
</tr>
</tbody>
</table>
Table (3.6): PCR reaction program protocols for *KLF14* (rs972283), *FTO* (rs8050136), *KCNQ1* (rs2237892), *ZBED3* (rs4457053), *COL8A1* (rs792837) genes.

<table>
<thead>
<tr>
<th>Type of Cycle</th>
<th>Temperature</th>
<th>Time</th>
<th>No. of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>94°C</td>
<td>3 minutes</td>
<td>1 cycle</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94°C</td>
<td>30 seconds</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>KLF14</em> rs972283</td>
<td>58°C</td>
<td>30 seconds</td>
<td>35 cycles</td>
</tr>
<tr>
<td><em>FTO</em> rs8050136</td>
<td>57°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>KCNQ1</em> rs2237892</td>
<td>61°C</td>
<td>30 seconds</td>
<td></td>
</tr>
<tr>
<td><em>ZBED3</em> rs4457053</td>
<td>59°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>COL8A1</em> rs792837</td>
<td>60°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>40 seconds</td>
<td></td>
</tr>
<tr>
<td>Final Extension</td>
<td>72°C</td>
<td>5 minutes</td>
<td>1 cycle</td>
</tr>
<tr>
<td>Cooling</td>
<td>4°C</td>
<td>∞</td>
<td>1 cycle</td>
</tr>
</tbody>
</table>
3.9.4 Agarose gel electrophoresis (3.0%)

1. Dried agarose gel (2.4 gm) was dissolved in 80 ml 1x Tris-Aacetate-EDTA buffer (2M Tris base 1M Glacial Acetic Acid, 0.05 M EDTA) by heating in microwave.
2. Then 3.0 μl Ethidium Bromide (10 mg/ml) was added and mixed, the gel was casted into a mold, which was fitted with a well-forming comb.
3. The agarose gel was submerged in electrophoresis buffer within a horizontal electrophoresis apparatus.
4. After amplification and digestion by RE, the PCR products and a DNA ladder size marker (Promega, Madison, WI, USA) were loaded into the sample wells to aid in fragment size determination.
5. PCR fragments were detected by size in the agarose gel. Electrophoresis was performed by using Electrophoresis power supply (BioRad, USA) at 70 volts for 40 min at room temperature, and the DNA bands were visualized and documented using a UV trans-illuminator documentation system.

3.9.5 Statistical analysis

Data were analyzed with the SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). Results are presented as mean ± SD or percentages. The clinical and laboratory characteristics of the T2DM patients and the controls were compared with the unpaired Student’s t test or One-Way ANOVA test as appropriate. The test for Hardy–Weinberg equilibrium and comparison of genotype and allele frequencies in the controls were performed using the Chi square test. Odds ratios (OR) and their 95% confidence intervals (CI) were calculated, using the Calculator for Confidence Intervals of Odds ratio. P-values of less than 0.05 were considered significant.
Chapter 4

Results
4.1 Polymorphism Genotyping

4.1.1 Results of amplification reactions (PCR-RFLP & PCR-AS)

The following figures (4.1 through 4.5) illustrate in a respective manner, genotyping examples for the "KLF14, KCNQ1, ZBED3, COL8A1 and FTO" genes polymorphisms investigated in this study.

**Figure (4.1):** PCR product (245bp) of *KLF14* gene (rs972283) digested with SexAI restriction enzyme. The products of digestion were electrophoresed on 3% agarose and directly visualized with ethidium bromide under UV light. Lane M: 100 bp DNA ladder, lanes 1&4 indicate samples with heterozygous genotype AG (105+140+245bp), lanes 2&3 indicate homozygous genotype GG (105+140bp), and lane 5 indicates a sample of homozygous genotype AA (245 bp).
Figure (4.2): PCR product (354bp) of *KCNQ1* gene (*rs2237892*) digested with MspI restriction enzyme. The products of digestion were electrophoresed on 3% agarose and directly visualized with ethidium bromide under UV light. Lane M: 100 bp DNA ladder, lanes 1&4 indicate samples with heterozygous genotype CT (85+269+354 bp), and lanes 2&3 indicate samples with homozygous genotype CC (85+269 bp).

Figure (4.3): PCR product (701bp) of *ZBED3* gene (*rs4457053*) digested with AcII restriction enzyme. The products of digestion were electrophoresed on 3% agarose and directly visualized with ethidium bromide under UV light. Lane M: 100 bp DNA ladder, lane 1 indicates a homozygous genotype AA (701 bp), and lanes 2&3 indicate samples with heterozygous genotype AG (298+403+701 bp).
Figure (4.4): PCR product (704bp) of *COL8A1* gene (rs792837) digested with MmeI restriction enzyme. The products of digestion were electrophoresed on 3% agarose and directly visualized with ethidium bromide under UV light. Lane M: 100 bp DNA ladder, lane 1 indicates a homozygous TT genotype (704 bp), and lanes 2&3 indicate heterozygous TC genotype (232+472+704 bp) samples.

Figure (4.5): A photograph of ethidium bromide stained 3% agarose gel showing the PCR-AS results of *FTO* gene polymorphism (rs8050136). Lane M: 100 bp DNA ladder, lane 1&2 indicates a homozygous CC (246 bp in mutant tube), lane 3&4 indicates a heterozygous CA (246bp in both tubes), lanes 5&6 indicates a homozygous AA sample (246 bp in normal tube), lanes 7&8 indicates negative controls.
4.2 Genotype and allele frequencies of "KLF14, KCNQ1, ZBED3, COL8A1 and FTO" genes' polymorphisms in patients and controls.

Table 4.1 illustrates genotypes and alleles frequencies, odds ratios, 95% confidence intervals and P values for the five "KLF14, KCNQ1, ZBED3, COL8A1 and FTO genes' polymorphisms among T2DM patients and controls. Statistical analyses of genotypic and allelic frequencies for the tested SNPs revealed no significant (all P values are > 0.05) difference between T2DM patients and controls in the tested genes polymorphisms ZBED3 and KCNQ1. KLF14 "rs972283" polymorphism revealed a significant (P value is < 0.05) difference between T2DM patients and controls, GG genotype was significantly (P = 0.014) more frequent in the patient group. FTO "rs8050136" polymorphism revealed a significant (P value is < 0.05) difference between T2DM patients and controls. The CC genotype was significantly (P = 0.043) more frequent in the patient group. COL8A1 "rs792837" polymorphism revealed a significant (P value is < 0.05) difference between T2DM patients and controls. The COL8A1 TC genotype and the C allele were significantly (P = 0.015) more frequent in the patient group.

4.3 The frequencies, odds ratios, and P-values of the KLF14 (rs972283) genotypes among T2DM patients and control subjects under recessive and dominant models.

Table 4.2 illustrates the frequencies, odds ratios, and P-values of the KLF14 'rs972283' genotypes among T2DM and control subjects under recessive and dominant models. The statistical analyses showed that there is a significant difference between the two groups under the dominant model.
**Table (4.1):** Genotypes and alleles frequencies of "*KLF14*, *KCNQ1*, *ZBED3*, *COL8A1* and *FTO*" genes polymorphism in diabetic and control subjects.

<table>
<thead>
<tr>
<th>Gene/SNP</th>
<th>Allele</th>
<th>Patients</th>
<th>Controls</th>
<th>Odd Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KLF14</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs972283</td>
<td>GG</td>
<td>46 (46%)</td>
<td>29 (29%)</td>
<td>2.086 (1.163 to 3.740)</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>38 (38%)</td>
<td>55 (55%)</td>
<td>0.501 (0.285 to 0.882)</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>16 (16%)</td>
<td>16 (16%)</td>
<td>1.00 (0.469 to 2.130)</td>
<td>1.0</td>
</tr>
<tr>
<td>Normal G</td>
<td>130 (65%)</td>
<td>113 (56.5%)</td>
<td></td>
<td>0.699 (0.467 to 1.047)</td>
<td>0.082</td>
</tr>
<tr>
<td>Mutant A</td>
<td>70 (35%)</td>
<td>87 (43.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MAF %</strong></td>
<td>35 %</td>
<td>43.5 %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FTO</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs8050136</td>
<td>AA</td>
<td>26 (26%)</td>
<td>27 (27%)</td>
<td>0.950 (0.507 to 1.780)</td>
<td>0.873</td>
</tr>
<tr>
<td></td>
<td>AC</td>
<td>39 (39%)</td>
<td>51 (51%)</td>
<td>0.614 (0.350 to 1.077)</td>
<td>0.089</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>35 (35%)</td>
<td>22 (22%)</td>
<td>1.909 (1.020 to 3.573)</td>
<td>0.043</td>
</tr>
<tr>
<td>Normal A</td>
<td>91 (45.5%)</td>
<td>105 (52.5%)</td>
<td></td>
<td>1.324 (0.894 to 1.961)</td>
<td>0.162</td>
</tr>
<tr>
<td>Mutant C</td>
<td>109 (54.5%)</td>
<td>95 (47.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MAF %</strong></td>
<td>54.5 %</td>
<td>47.5 %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>KCNQ1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2237892</td>
<td>CC</td>
<td>98 (98%)</td>
<td>90 (90%)</td>
<td>5.444 (1.161 to 25.522)</td>
<td>0.032</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>2 (2%)</td>
<td>10 (10%)</td>
<td>0.184 (0.040 to 0.861)</td>
<td>0.032</td>
</tr>
<tr>
<td>Mutant T</td>
<td>2 (1%)</td>
<td>10 (5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal C</td>
<td>198 (99%)</td>
<td>190 (95%)</td>
<td></td>
<td>5.210 (1.127 to 24.092)</td>
<td>0.035</td>
</tr>
<tr>
<td><strong>MAF %</strong></td>
<td>1 %</td>
<td>5 %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ZBED3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs4457053</td>
<td>AA</td>
<td>47 (47%)</td>
<td>58 (58%)</td>
<td>0.642 (0.367 to 1.122)</td>
<td>0.120</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>53 (53%)</td>
<td>42 (42%)</td>
<td>1.557 (0.891 to 2.722)</td>
<td>0.120</td>
</tr>
<tr>
<td>Normal A</td>
<td>147 (73.5%)</td>
<td>158 (79%)</td>
<td></td>
<td>1.356 (0.854 to 2.155)</td>
<td>0.197</td>
</tr>
<tr>
<td>Mutant G</td>
<td>53 (26.5%)</td>
<td>42 (21%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MAF %</strong></td>
<td>26.5 %</td>
<td>21 %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>COL8A1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs792837</td>
<td>TT</td>
<td>50 (50%)</td>
<td>67 (67%)</td>
<td>0.492 (0.278 to 0.873)</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>50 (50%)</td>
<td>33 (33%)</td>
<td>2.030 (1.146 to 3.598)</td>
<td>0.015</td>
</tr>
<tr>
<td>Normal T</td>
<td>150 (75%)</td>
<td>167 (83.5%)</td>
<td></td>
<td>1.687 (1.032 to 2.758)</td>
<td>0.037</td>
</tr>
<tr>
<td>Mutant C</td>
<td>50 (25%)</td>
<td>33 (16.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MAF %</strong></td>
<td>25 %</td>
<td>16.5 %</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*MAF* minor allele frequency
Table (4.2): The frequencies, odds ratios and P-values of the KLF14’rs972283’ gene polymorphism among T2DM patient and control subjects under recessive and dominant models.

<table>
<thead>
<tr>
<th>SNP</th>
<th>model</th>
<th>Allele</th>
<th>Patients</th>
<th>Controls</th>
<th>Odds Ratio (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLF14</td>
<td>dominant</td>
<td>AG+AA</td>
<td>54 (54%)</td>
<td>71 (71%)</td>
<td>0.479 (0.26 to 0.85)</td>
<td>0.014</td>
</tr>
<tr>
<td>rs972283</td>
<td>recessive</td>
<td>GG</td>
<td>46 (46%)</td>
<td>29 (29%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>16 (16%)</td>
<td>16 (16%)</td>
<td>1.00 (0.467 to 2.13)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG+AG</td>
<td>84 (84%)</td>
<td>84 (84%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.4 The frequencies, odds ratios, and P-values of the COL8A1 (rs792837) genotypes among T2DM patients and control subjects under recessive and dominant models.

Table 4.3 illustrates the frequencies, odds ratios, and P-values of the COL8A1’rs792837’ genotypes among T2DM and control subjects under recessive and dominant models. The statistical analyses showed that there is a significant difference between the two groups under the dominant model.

Table (4.3): The frequencies, odds ratios and P-values of the COL8A1’rs792837’ gene polymorphism among T2DM patient and control subjects under recessive and dominant models.

<table>
<thead>
<tr>
<th>SNP</th>
<th>model</th>
<th>Allele</th>
<th>Patients</th>
<th>Controls</th>
<th>Odds Ratio (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL8A1</td>
<td>recessive</td>
<td>TC+TT</td>
<td>100 (100%)</td>
<td>100 (100%)</td>
<td>1.00 (0.02 to 50.89)</td>
<td>1.00</td>
</tr>
<tr>
<td>rs792837</td>
<td>dominant</td>
<td>CC</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>50 (50%)</td>
<td>67 (67%)</td>
<td>0.49 (0.27 to 0.87)</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC+TC</td>
<td>50 (50%)</td>
<td>33 (33%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.5 The frequencies, odds ratios, and P-values of the *KCNQ1* (rs2237892) genotypes among T2DM patients and control subjects under recessive and dominant models.

Table 4.4 illustrates the frequencies, odds ratios, and P-values of the *KCNQ1* 'rs2237892' genotypes among T2DM and control subjects under recessive and dominant models. The statistical analyses showed that there is a significant difference between the two groups under the recessive model.

Table (4.4): The frequencies, odds ratios and P-values of the *KCNQ1* 'rs2237892' gene polymorphism among T2DM patient and control subjects under recessive and dominant models.

<table>
<thead>
<tr>
<th>SNP</th>
<th>model</th>
<th>Allele</th>
<th>Patients</th>
<th>Controls</th>
<th>Odds Ratio (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>KCNQ1</em></td>
<td>dominant model</td>
<td>CT+TT</td>
<td>2 (2%)</td>
<td>10 (10%)</td>
<td>0.18 (0.039 to 0.86)</td>
<td>0.032</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>98 (0%)</td>
<td>90 (0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>recessive model</td>
<td>TT</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1.00 (0.019 to 50.9)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC+CT</td>
<td>100 (100%)</td>
<td>100 (100%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.6 The frequencies, odds ratios, and P-values of the *FTO* (rs8050136) genotypes among T2DM patients and control subjects under recessive and dominant models.

Table 4.5 illustrates the frequencies, odds ratios, and P-values of the *FTO* 'rs8050136' genotypes among T2DM and control subjects under recessive and dominant models. The statistical analyses showed that there is a significant difference between the two groups under the recessive model.
Table (4.5): The frequencies, odds ratios and P-values of the FTO ‘rs8050136’ gene polymorphism among T2DM patient and control subjects under recessive and dominant models. However, ZBED3 gene polymorphisms was not significantly different between the two groups under recessive and dominant models.

<table>
<thead>
<tr>
<th>SNP</th>
<th>model</th>
<th>Allele</th>
<th>Patients</th>
<th>Controls</th>
<th>Odds Ratio (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTO</td>
<td>recessive</td>
<td>AA+CA</td>
<td>65 (65%)</td>
<td>78 (78%)</td>
<td>0.52 (0.27 to 0.98)</td>
<td>0.043</td>
</tr>
<tr>
<td>rs8050136</td>
<td>model</td>
<td>CC</td>
<td>35 (35%)</td>
<td>22 (22%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>dominant</td>
<td>AA</td>
<td>26 (26%)</td>
<td>27 (27%)</td>
<td>0.949 (0.50 to 1.78)</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>model</td>
<td>CC+CA</td>
<td>74 (74%)</td>
<td>73 (73%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.7 Hardy-Weinberg equilibrium in the "COL8A1, KLF14, KCNQ1, ZBED3 and FTO" genes' polymorphisms genotypes.

Deviation from Hardy-Weinberg equilibrium (HWE) was assessed as given in the following representative example for COL8A1 rs792837 genotypes in control group:

Frequency of major allele T (p) = (67*2 +33*1)/100*2 = 0.835
Frequency of minor allele C (q) = (0*2 +33*1)/100*2 = 0.165

**Expected genotype frequencies:**
- Genotype TT: (p)^2 *100 = (0.835)^2 * 100 = 69.7 individuals.
- Genotype TC: (2pq) *100 = 2 * 0.835* 0.165* 100 = 27.6 individuals.
- Genotype CC: (q)^2 *100 = (0.165)^2 * 100 = 2.7 individuals.

The difference between observed and expected genotype frequencies in the control group was determined by using Chi (X^2) square test.

Table 4.6 illustrates the observed and expected genotypes frequencies of "COL8A1, KLF14, KCNQ1, ZBED3 and FTO" genes' polymorphisms in the control group. KLF14, FTO, and KCNQ1 genotypes frequencies did not deviate from Hardy-Weinberg equilibrium expectations. However, ZBED3 and COL8A1 genotypes deviated significantly from Hardy-Weinberg equilibrium expectations.
Table (4.6): Observed and expected genotype frequencies of the "KLF14, FTO, KCNQ1, ZBED3 and COL8A1" genes polymorphisms in the controls group.

<table>
<thead>
<tr>
<th>Gene\SNP</th>
<th>Genotype</th>
<th>Controls</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Genotype</td>
<td>Observed Genotype</td>
<td>Expected Genotype</td>
</tr>
<tr>
<td>KLF14 rs972283</td>
<td>AG</td>
<td></td>
<td>55</td>
<td>49.15</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td></td>
<td>16</td>
<td>18.92</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td></td>
<td>46</td>
<td>31.92</td>
</tr>
<tr>
<td>FTO rs7903146</td>
<td>CA</td>
<td></td>
<td>51</td>
<td>49.9</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td></td>
<td>22</td>
<td>22.6</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td></td>
<td>27</td>
<td>27.6</td>
</tr>
<tr>
<td>KCNQ1 rs2237892</td>
<td>CT</td>
<td></td>
<td>10</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td></td>
<td>90</td>
<td>90.3</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td></td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>ZBED3 rs4457053</td>
<td>AG</td>
<td></td>
<td>42</td>
<td>33.2</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td></td>
<td>58</td>
<td>62.4</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td></td>
<td>0</td>
<td>4.4</td>
</tr>
<tr>
<td>COL8A1 rs792837</td>
<td>TC</td>
<td></td>
<td>33</td>
<td>27.6</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td></td>
<td>67</td>
<td>69.7</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td></td>
<td>0</td>
<td>2.7</td>
</tr>
</tbody>
</table>

4.8 Comparative analyses of the investigated parameters in patients and controls.

As indicated in Table 4.7, there was a significant increase in the means of BMI, triglycerides and HbA1c in patients as compared to controls (P values= <0.001, 0.02, and <0.001, respectively). No significant difference was evident between patients and controls in terms of the rest of the measured parameters.
Table (4.7): Characteristics of the studied population (n=200).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>n</th>
<th>Mean ± SD</th>
<th>t-test for Equality of Means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>t</td>
<td>Sig. (2-tailed)</td>
</tr>
<tr>
<td>BMI</td>
<td>Patient</td>
<td>100</td>
<td>30.27±4.60</td>
<td>3.903</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>100</td>
<td>27.89±3.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C-peptide</td>
<td>Patient</td>
<td>100</td>
<td>1.80±0.86</td>
<td>-1.29</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>100</td>
<td>1.95±0.75</td>
<td>0.20</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Patient</td>
<td>100</td>
<td>191.44±32.27</td>
<td>-0.339</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>100</td>
<td>193.10±36.75</td>
<td>0.74</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>Patient</td>
<td>100</td>
<td>181.39±95.54</td>
<td>2.366</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>100</td>
<td>148.50±100.98</td>
<td>0.02*</td>
</tr>
<tr>
<td>HDL-C</td>
<td>Patient</td>
<td>100</td>
<td>49.55±3.04</td>
<td>-0.286</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>100</td>
<td>49.68±3.39</td>
<td>0.78</td>
</tr>
<tr>
<td>LDL-C</td>
<td>Patient</td>
<td>100</td>
<td>109.80±32.24</td>
<td>-1.203</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>100</td>
<td>115.45±34.10</td>
<td>0.23</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Patient</td>
<td>100</td>
<td>8.46±1.73</td>
<td>17.447</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>100</td>
<td>5.40±0.31</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* P Value is significant at the ≤ 0.05 level.

4.9 The relation between *KLF14, KCNQ1, ZBED3, COL8A1 and FTO* polymorphisms and the tested parameters

The clinical parameters (BMI, C-peptide, lipid profile and HbA1c) were analyzed in the study subjects with respect to the different genotypes of the *KLF14, KCNQ1, ZBED3, COL8A1 and FTO* genes' polymorphisms. The analyses results are presented in Tables 4.8 to 4.12.

Analysis results of the relation between *KLF14* (rs972283 G>A) polymorphism genotypes and the measured clinical parameters are illustrated in Table 4.8 below. The *KLF14* G-containing genotypes exerts significant effect on HDL-c level in controls (P values = 0.026). Interestingly, the HDL-c levels were significantly lower in controls subjects with the G-containing genotype compared to controls with the AA genotypes.
Moreover, G-containing genotype exerts significant effect on LDL-c level in controls (P values = 0.045). Interestingly, the LDL-c levels were significantly higher in control subjects with the G-containing genotype compared to controls with the AA genotypes. The G-containing genotype exerts significant effect on Cholesterol level in controls (P values = 0.042). The Cholesterol levels were significantly higher in controls subjects with the G-containing genotype compared to controls with the AA genotypes.

Table (4.8): Relation between KLF14 (rs972283 G>A) gene polymorphism genotypes and the investigated parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Genotype</th>
<th>n</th>
<th>Mean± SD</th>
<th>P value</th>
<th>Genotype</th>
<th>n</th>
<th>Mean± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>GG</td>
<td>46</td>
<td>29.75±4.81</td>
<td>0.319</td>
<td>GG</td>
<td>29</td>
<td>28.52±3.83</td>
<td>0.314</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>16</td>
<td>31.77±5.46</td>
<td></td>
<td>AA</td>
<td>16</td>
<td>26.62±3.84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>38</td>
<td>30.26±3.88</td>
<td></td>
<td>AG</td>
<td>55</td>
<td>27.93±4.07</td>
<td></td>
</tr>
<tr>
<td>C-peptide</td>
<td>GG</td>
<td>46</td>
<td>1.8±0.929</td>
<td>0.693</td>
<td>GG</td>
<td>29</td>
<td>1.86±0.738</td>
<td>0.357</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>16</td>
<td>1.96±0.719</td>
<td></td>
<td>AA</td>
<td>16</td>
<td>2.187±0.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>38</td>
<td>1.74±0.844</td>
<td></td>
<td>AG</td>
<td>55</td>
<td>1.929±0.72</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>GG</td>
<td>46</td>
<td>186.5±31.52</td>
<td>0.335</td>
<td>GG</td>
<td>29</td>
<td>192.89±31.07</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>16</td>
<td>198.75±30.07</td>
<td></td>
<td>AA</td>
<td>16</td>
<td>172.94±15.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>38</td>
<td>194.34±33.89</td>
<td></td>
<td>AG</td>
<td>55</td>
<td>199.07±41.87</td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>GG</td>
<td>46</td>
<td>177.76±97.48</td>
<td>0.19</td>
<td>GG</td>
<td>29</td>
<td>151±59.55</td>
<td>0.258</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>16</td>
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<td>46</td>
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<td>GG</td>
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<td>AG</td>
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<td>5.46±0.312</td>
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</table>

Statistical analyses of the effect of FTO (rs8050136 A>C) polymorphism genotypes and the measured parameters are illustrated in Table 4.9. The FTO "CC" genotype showed a significant effect on raising HbA1c level in the patients (P-value = 0.007).

Interestingly, the HbA1c levels were significantly higher in patient subjects with the CC genotype compared to patients with the AA genotypes.
Table (4.9): Relation between FTO (rs8050136 A>C) gene polymorphism genotypes and the investigated parameters.

<table>
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<th>Parameter</th>
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<th>P value</th>
<th>Genotype</th>
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<th>Mean± SD</th>
<th>P value</th>
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<td>0.346</td>
<td>CC</td>
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<td>CA</td>
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<td>CA</td>
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<td>1.92±0.75</td>
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<td>Cholesterol</td>
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</tr>
<tr>
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<td>CA</td>
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<td>CA</td>
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</tr>
<tr>
<td>Triglyceride</td>
<td>CC</td>
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<td>49.60±3.19</td>
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<td>CC</td>
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<td>51</td>
<td>118.63±32.91</td>
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</table>

Results of the relation between KCNQ1 (rs2237892 T>C), ZBED3 (rs4457053 A>G) and COL8A1 (rs792837 T>C) genotypes and the measured parameters are presented in Tables 4.10, 4.11 and 4.12 respectively. Statistical analyses revealed no significant relation between the genotypes and the tested parameters.
Table (4.10): Relation between KCNQ1 (rs2237892 T>C) gene polymorphism genotypes and the investigated parameters.

<table>
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<th>Parameter</th>
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<th>P value</th>
<th>Genotype</th>
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<th>P value</th>
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Table (4.11): Relation between ZBED3 (rs4457053 A>G) gene polymorphism genotypes and the investigated parameters.

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<th>P value</th>
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</tr>
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<td>AG</td>
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<td>AG</td>
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Table (4.12): Relation between COL8A1 (rs792837 T>C) gene polymorphism genotypes and the investigated parameters.

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<td>TC</td>
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<td>8.54±1.62</td>
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Chapter 5
Discussion
Chapter 5
Discussion

The continuous identification of new susceptibility genes associated to T2DM appears to be an active process and remains a vital challenge. It is considered to be a prominent route to elucidate the complex pathogenesis underlying the development of this ailment. T2DM is a multifactorial, complex disease, caused by a combination of a genetic predisposition and environmental factors. Positive family history, obesity and physical inactivity are considered essential risk factors for this disease.

The effectiveness of current T2DM management is greatly improved when it is started at an early stage of the disease. If genetic testing could be used to predict T2DM, preventive measures could be taken and diabetes could potentially be managed more easily.

The goal of this study was to investigate the polymorphisms: rs972283 in KLF14, rs8050136 in FTO, rs2237892 in KCNQ1, rs4457053 in ZBED3, and rs792837 in COL8A1 in the Palestinian T2DM patients with an emphasis to relate these polymorphisms with the risk of having T2DM and with T2DM-related clinical parameters.

5.1 Association between candidate genes polymorphisms and T2DM.

5.1.1 Association between KLF14 (rs972283 G>A) polymorphism and T2DM:

*KLF14* is a master trans-regulator of multiple genes that are associated with metabolic phenotypes in adipose tissue. Variants near the transcription factor *KLF14* are robustly associated with both T2DM and HDL-c level in GWAS. The results of this study (Rees et al., 2011) suggest significant association between the disease and variants in or near *KLF14* (rs972283).

In our study, statistical analyses of genotypic frequencies for the *KLF14* (rs972283) revealed significant difference between T2DM patients and controls in the examined population. The statistical analyses showed that there is a significant difference between the two groups under the recessive model.
Additionally, and as depicted in Table (4.8), the tested KLF14 polymorphism showed significant association with HDL-c (P values = 0.026), LDL-c (P values = 0.045) and cholesterol level (P values = 0.042). The HDL-c levels were significantly lower and LDL-c levels and total Cholesterol levels were significantly higher in control subjects harboring KLF14 G-containing genotypes.

Contradictory to our results, in Saudi Arabian population, it was reported that KLF14 (rs972283) polymorphism is not associated with susceptibility to T2DM (Al-Daghri et al., 2014). Therefore, the different genetic/ethnic backgrounds of the different populations seem to play determinative roles in candidate gene association studies.

According the Geneimprint website (www.geneimprint.com), KLF14 and KCNQ1 are maternally expressed in humans and show stronger effects on T2DM when the risk allele is transmitted from the mother than from the father (Kong et al., 2009; Small et al., 2011), and when examine these polymorphisms we must test the polymorphism for patient and their parents.

5.1.2 Association between FTO (rs8050136 A>C) polymorphism and T2DM:

FTO polymorphisms have also been associated with T2DM, but it could be due to their influence on BMI (Freathy et al., 2008), which is known to have impacts on insulin responsiveness. Study reported that the age-dependent decline of FTO expression is associated with peripheral defects of glucose and lipid metabolism in adipose tissue and skeletal muscle (Grunnet et al., 2009).

In our study, statistical analyses of genotypic frequencies for the FTO (rs8050136) revealed significant difference between T2DM patients and controls in the examined population. Furthermore, statistical analyses showed that there is a significant difference between the two groups under the recessive model. Concordantly, the results of several studies (Almawi et al., 2013; Lango et al., 2008; O’Beirne et al., 2016; Votsi et al., 2017; Zeggini et al., 2007) suggest significant association between the disease and variants in FTO (rs8050136).

Contradictory to our results, it was reported that FTO (rs8050136) polymorphism is not associated with susceptibility to T2DM (Al-Sinani et al., 2015).
In the present study, and as depicted in Table (4.9), FTO "CC" genotype exerts a significant effect on HbA1c level in patients (p = 0.007). Interestingly, the HbA1c levels were significantly higher in patient subjects with the CC genotype compared to patients with the AA genotypes. Because FTO play a role in BMI this gene may have important impact in diabetes and must be tested for predict risk for diabetes.

5.1.3 Association between KCNQ1 (rs2237892 C>T) polymorphism and T2DM:

KCNQ1 was reported to be expressed in insulin-secreting cells, and inhibition of this potassium channel has been shown to significantly increase insulin secretion. The results of several studies (Lee et al., 2008; Yasuda et al., 2008) suggest significant association between the disease and variants in KCNQ1 (rs2237892).

Analysis of our data indicated that the KCNQ1 gene polymorphism does not have significant effect on any of the measured clinical parameters (BMI, C-peptide, lipid profile, or HbA1c).

The major finding of the present study was that the KCNQ1 (rs2237892 C>T) polymorphism is not significantly associated with T2DM in the examined Palestinian population. Similar result was reported by other investigator (Votsi et al., 2017).

Moreover, the TT homozygote genotype was not encountered in any of our investigated subjects. The explanation for the absence of this genotype is mainly due to the uncommon occurrence of the T-allele (minor allele frequency "MAF" ~ 0.01) in our population.

5.1.4 Association between ZBED3 (rs4457053 A>G) polymorphism and T2DM:

GWAS have shown that the (ZBED3) is associated with T2DM. ZBED3 has been shown to be associated with increased fasting and postprandial glucagon levels in humans. The gene is expressed in almost all mouse tissues, with the highest levels detected in muscle (Jonsson et al., 2013).
In our study, statistical analyses of genotypic and allelic frequencies for the ZBED3 (rs4457053) revealed no significant difference between T2DM patients and controls in the studied tested population. Several studies also showed a lack of association between this variant and T2DM (Al-Daghri et al., 2014; Rees et al., 2011; Votsi et al., 2017).

Moreover, the GG homozygote genotype was not encountered in any of our investigated subjects. The explanation for the absence of this genotype could be related to the small sample size employed.

ZBED3 (rs4457053 A>G) polymorphism is one of the two genes whose genotypes frequencies deviated from Hardy-Weinberg equilibrium (the other gene is COL8A1). This deviation could be due to our small sample size (i.e., a mere chance). High consanguineous (non-random) mating in our population could be another possible cause for the observed deviation. Additionally, errors in genotyping cannot be excluded. Therefore, our findings have to be replicated with a larger sample size and genotyping has to be done using a different techniques e.g., Taqman probe assay or direct sequencing.

5.1.5 Association between COL8A1 (rs792837 T>C) polymorphism and T2DM:

The current study evaluated the potential association of COL8A1 (rs792837 T>C) polymorphisms in patients with T2DM. Analyses of genotypes revealed that COL8A1 (rs792837 T>C) polymorphism is significantly associated with T2DM in the study population. Similar result was reported by another investigator (Almawi et al., 2013) who also reported that COL8A1 (rs792837 T>C) polymorphism predispose to T2DM. The TT homozygote genotype was not encountered in any of our investigated subjects. The explanation for the absence of this genotype is mainly due to small size samples.

Survey of the literature regarding the association between particular gene polymorphism(s) and multifactorial diseases e.g., T2DM, always come out with controversy between studies conducted on different populations. This could be due to many reasons including population genetic variation (background/ethnicity) unrelated to the investigated alleles, presence of nucleotide polymorphism somewhere else in the
examined genes, epigenetic alterations and linkage disequilibrium to other sequence variants in the vicinity of the investigated loci.

5.2 Association between BMI, C-peptide, lipid profile, HbA1c and T2DM

5.2.1 Serum lipid profile

In patients with T2DM, triglycerides are often elevated, and HDL-C is often decreased or normal. High levels of plasma triglycerides (TGs) are a risk factor for cardiovascular diseases (CVDs). Additionally, TGs are frequently associated with impaired fasting glucose, impaired glucose tolerance, insulin resistance and metabolic syndrome (Grundy et al., 2005).

A TG/HDL-C ratio of $\geq 3.5$ was previously reported to be highly correlated with insulin resistance and atherogenic dyslipidemia in men. This threshold was also associated with metabolic syndrome (McLaughlin et al., 2005). The TG/HDL-c ratio is also considered a predictor of myocardial infarction and other CVDs (Salazar et al., 2013; Urbina et al., 2013).

Our study showed that the mean level of triglycerides is significantly higher in patients as compared to controls (p=0.02). However, differences in the mean levels of cholesterol, HDL-C and LDL-c between patients and controls were not significant (P>0.05). The patients TG/HDL-c ratio is 3.66 and this score represents an average risk for cardiovascular diseases, myocardial infarction and insulin resistance.

5.2.2 HbA1c

Glycated hemoglobin (HbA1c) is an important indicator of long-term glycemic control with the ability to reflect the cumulative glycemic history of the preceding (2 to 3) months. HbA1c has also been regarded as a future risk factor for coronary heart disease (CHD), cardiovascular diseases CVDs, chronic kidney disease (CKD), stroke, and all cause mortality (Di Angelantonio et al., 2014; Selvin et al., 2011; Selvin et al., 2010).

Furthermore, it has been suggested that improving glycemic control in T2DM patients may be more important than treating dyslipidaemia for the prevention of both microvascular and macrovascular complications, and can substantially reduce the risk of cardiovascular events. It has been estimated that reducing the HbA1c level by 0.2% could
lower the mortality by 10% (Vaag, 2006). The target HbA1c level for people with diabetes is usually less than 7%. The higher the HbA1c, the higher the risk of having complications related to diabetes. In this study, we also noted that the mean level of whole blood HbA1c was significantly higher in patients than in controls (8.46±1.73 versus 5.40±0.31; P<0.001). Moreover, about 84% of our T2DM patients are poor glycemic control (HbA1c ≥ 7%). This result is alarming and points to an increased future risk of CVD and other complications related to T2DM in the investigated population.

5.2.3 C-peptide

C-peptide, a cleavage product of insulin, exerts biological effects in patients with T1DM, but its role in T2DM mellitus is controversial. Furthermore, the role of C-peptide is not well defined in T2DM, which is considered to be a consequence of the reduced β-cell function superimposed on a condition of insulin resistance. In theory, testing C-peptide for very few years should also give some idea of whether or not beta cells are slowly failing.

If patients C-peptide is significantly very low their beta cells are likely to be dead or dying. If they are young or very recently diagnosed with diabetes of any type, a very low C-peptide value is a good way of diagnosing T1DM (autoimmune) rather than T2DM. However, if patients of T2DM have had the disease for decades, and have not kept their blood sugars at normal levels, they may also have a very low C-peptide test values because they may have killed off their insulin-producing beta cells. Conversely, A high fasting C-peptide level taken at the same time as a high fasting blood glucose test level means that patients are insulin resistant. If patients of T2DM have a high fasting C-peptide level, it is very likely that patients will be able to control their blood sugar by cutting down the amount of carbohydrates they consume (Jones & Hattersley, 2013).

In the current study, we noted that C-peptide level was not significantly different between T2DM patients and controls (1.80±0.86 versus 1.95±0.75 μIU/ml; p=0.20). We also found that all the enrolled T2DM patients have normal fasting C-peptide levels. This finding could be due to the relatively young age (35-50 year) of the study patients in whose major T2DM complications have not developed yet.
5.2.4 BMI

The majority of people with T2DM are overweight or obese. Worldwide, the proportion of T2DM patients with BMI ≥ 25 kg/m² is estimated to be 36.9% in men and 38.0% in women (M. Ng et al., 2014).

The results of our study clearly showed that there was a significant increase in the mean of BMI among patients as compared to controls (30.27±4.60 versus 27.89±3.98, P<0.001). The majority of our enrolled T2DM patients are overweight and obese. Patients with BMI ≥ 25 kg/m² presented (84%), and those with BMI > 30 kg/m² constituted (51%). Overweight or obese patients (BMI ≥ 25 kg/m²) have a higher rate of cardiac events (such as the acute coronary syndrome and heart failure) and other chronic diseases (Costanzo et al., 2015).
Chapter 6

Conclusion & Recommendations
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6.1 Conclusion

The present case-control study focused on the contribution of KLF14 (rs972283 G>A), FTO (rs8050136 A>C), KCNQ1 (rs2237892 T>C), ZBED3 (rs4457053 A>G), and COL8A1 (rs792837 T>C) polymorphisms to the risk of having T2DM in a Palestinian Population. The study also examined the relation between those polymorphisms and BMI and various biochemical parameters in the study sample. The results of the study can be summarized as follows:

1. This study suggests that gene polymorphisms rs8050136 of FTO is associated with a risk of T2DM in a Palestinian Population. The C/C genotype was significantly higher in the T2DM patients as compared to controls.
2. KLF14 "rs972283" polymorphism revealed a significant difference between T2DM patients and controls; GG genotype was significantly more frequent in the patient group.
3. COL8A1 "rs792837" polymorphism showed a significant difference between T2DM patients and controls. The COL8A1 TC genotype and the C allele were significantly more frequent in the patient group.
4. Genotypic and allelic frequencies of KCNQ1 (rs2237892 T>C), and ZBED3 (rs4457053 A>G) did not differ significantly between T2DM patients and controls.
5. The genotypes of the investigated loci were all in Hardy-Weinberg equilibrium except ZBED3 and COL8A1.
6. The KLF14 G-containing genotypes exerted significant effect on lowering HDL-c and elevating LDL-c and total cholesterol level in the control group.
7. The FTO "CC" genotype showed a significant effect on raising HbA1c level in the patients.
8. The mean BMI is significantly higher in patients as compared to controls. The majority of the investigated T2DM patients are overweight and obese.
9. The mean levels of HbA1c and triglycerides are significantly higher in T2DM patients as compared to controls.
6.2 Recommendations

1. Urging patients to control their HbA1c, LDL-c, triglyceride levels and body weight in order to avoid the complications associated with T2DM.

2. Our findings should be confirmed by examining a larger sample along with more accurate genotyping tools.

3. Employing FTO (rs8050136 A>C), KLF14 (rs972283 G>A) and COL8A1 (rs792837 T>C) polymorphism testing for predicting the risk of developing T2DM in asymptomatic individuals.

4. Performing further studies to investigate the role of other documented loci polymorphisms in T2DM in our population.

5. Carrying out a similar study on T2DM female patients in order to reveal combined gender/polymorphism effect, if any.
References
References


Mastouri, N. A. (2011). *New susceptibility loci associated with Type 2 Diabetes*. LEBANESE AMERICAN UNIVERSITY.


Appendix

Helsinki Committee
For Ethical Approval

Date: 04/04/2016
Name: Fadel A. Sharif

Number: PHRC/HC/89/16

We would like to inform you that the committee had discussed the proposal of your study about:

Association between single nucleotide polymorphisms in different proposed risk genes and increased risk of T2DM in Gaza Strip

The committee has decided to approve the above mentioned research. Approval number PHRC/HC/89/16 in its meeting on 04/04/2016

Member

Signature

General Conditions:-
1. Valid for 2 years from the date of approval.
2. It is necessary to notify the committee of any change in the approved study protocol.
3. The committee appreciates receiving a copy of your final research when completed.

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Gaza - Palestine

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