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Association of *FTO rs9939609* Variant with Macrovascular Complications in Type 2 Diabetes Mellitus among Palestinian Population

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Dedication

To my mother and father…

To my family…

To my friends…

To my teachers…

To all the people who supported, and encouraged me.

Anas Rezq Hassan Sabarnah

Declaration:

I certify that this thesis submitted for the degree of Master, is the result of my own research, except there otherwise acknowledged, and that this study (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signature: ----------

Anas Rezq Hassan Sabarnah

Date: 5/5/2018

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Abstract

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes and accounts for over 90 % of all diabetes cases worldwide. Type 2 diabetes is characterized by insulin resistance and relative insulin deficiency, either of which may be present at the time that diabetes becomes clinically manifest. Genetic background was perceived to be linked with the development of T2DM and its related complications including retinopathy, nephropathy, diabetic foot and cardiovascular disease. Several variants in the *FTO* gene were analyzed and found to be associated with T2DM and obesity in different population. Therefore, our study was designed to investigate the association of *FTO* (rs9939609) gene polymorphisms with T2DM and its related complications.

A case-control study was conducted during the period of 2016-2017. A total of 281 diabetic patients (181 obese and 15 non-obese) and 118 controls (52 obese and 39 non-obese) were recruited. All of them were unrelated and aged ≥40 years. The anthropometric, clinical and biochemical data was collected on a structured questionnaire. The single nucleotide polymorphism (SNP) in the *FTO* gene was identified by PCR-RFLP. Comparison of allele frequencies and genotype distributions between the diabetic and non-diabetic groups were done using the Pearson's Chi-square test. R statistics (v2.8.0). software was used to measure OR for T2DM adjusted for age, gender and BMI.

Our results showed a strong association between the minor allele A at rs9939609 of the *FTO* and increase T2DM risk with an allelic odd ratio (OR) of 1.84, (95%CI [1.04-3.05], P=0.034) after adjustment by age, gender and BMI. Stratified data by glycemic status and across *FTO* genotype, revealed a marginally association between the *FTO* A variant and body mass index (BMI) in the diabetic group (P=0.057), while no association was found in non-diabetic control group (P=0.688). Furthermore, no significant association was observed between *FTO* genotypes and covariates of age, gender, T2DM complications or any tested metabolic trait in both diabetic and non-diabetic individuals (P>0.05).

In conclusion, our results demonstrated *FTO* rs9939609 variant was associated with T2DM. However, further large-scale study is required to elucidate the role of this variant on the predisposition of increased BMI in Palestinian population.

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List of abbreviations

Abbreviation	Term
ACR	albumin/creatinine ratio
ADA	American Diabetes Association
AGE	Advanced glycation end product
AIDS	Acquired immunodeficiency syndrome
AKt	Protein kinase B
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BMI	Body mass index
bp	Base pair
CAPN10	calpain 10
CI	Confidence interval
cSNP	Coding single nucleotide polymorphism
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DM	Diabetes mellitus
DN	Diabetic nephropathy
DNA	Deoxyribonucleic acid
DR	Diabetic retinopathy
EDTA	Ethylenediamine tetraacetic acid
ESRD	End stage renal disease
FFAs	Free fatty acids
FPG	Fasting plasma glucose

FTO	Fat mass and obesity-associated
GDM	Gestational diabetes mellitus
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
GLUT2	Glucose transporter 2
GWAs	Genome wide association studies
HbA1c	Glycohemoglobin A1 c
HDL	High density lipoprotein
HIV	Human Immunodeficiency Virus
HWE	Hardy–Weinberg equilibrium
IDDM	Insulin-dependent
IDF	International diabetes federation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IR	Insulin receptor
IRS	Insulin receptor substrate
LD	Linkage disequilibrium
LDL	Low density lipoprotein
M ± SD	Means ± Standard deviation
MAF	Minor allele frequency
MI	Myocardial infraction
MODY	Maturity-onset diabetes of the young
NCBI	National Center for Biotechnology Information
NIDDM	Non-insulin-dependent
OGTT	Oral glucose tolerance test
OMIM	Online Mendelian Inheritance in Man
OR	Odds ratio
PCR	Polymerase chain reaction
PDK1	Phosphoinositide-dependent protein kinase-1
PG	plasma glucose
PI3K	Phosphatidylinositol 3-kinase
PKB	Protein kinase B
PKC	Protein kinase C

PPARG	Peroxisome proliferator-activated receptor gamma
RAAS	Renin–angiotensin–aldosterone
RFLP	Restriction fragment length polymorphism
SBP	Systolic blood pressure
SD	Standard deviation
SNP	Single nucleotide polymorphism
SREBP	Sterol regulatory element binding protein
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TC	Total cholesterol
TCF7L2	Transcription factor 7-like 2
TE	Tris-EDTA
TG	Total Triglycerides
TGF- β1	Tissue growth factor β1
Tm	Melting temperature
UAE	Urinary albumin excretion
UCP2	Uncoupling protein 2
UV	Ultraviolet light
VLDL	Very low density lipoprotein
WFS1	Wolfram syndrome 1
WHO	World Health Organization
$\chi 2$	Chi square

Chapter One

1. Introduction

1.1 Diabetes Mellitus

Diabetes mellitus (DM) is a group of metabolic diseases that characterized by persistently elevated blood glucose levels (hyperglycemia) with disturbances in fat, carbohydrate and protein metabolism as a result of defects in insulin secretion, insulin action (insulin resistance), or both. If diabetes is undiagnosed or poorly controlled, it can lead to a state of persistent chronic hyperglycemia. The chronic hyperglycemia of diabetes is associated with a different complications and irreversible damage in a wide range of important organs, like the kidney glomeruli, eyes, neural tissue, heart and blood vessels (Tibaldi, 2012).

1.2 Classification of Diabetes mellitus

Recently, the old and confusing terms of non-insulin-dependent (NIDDM) or insulin-dependent (IDDM) which were suggested by World Health Organization (WHO) in1980 and 1985, have disappeared and the terms of new classification system identifies four types of DM as follow:

a) Type 1 diabetes mellitus (T1DM)

This type of diabetes was previously known by different names; juvenile diabetes, insulin dependent diabetes or immune-mediated diabetes. It accounts for only 5–10% of all diabetes cases, and is thought to be caused by destruction of insulin producing cells of the pancreas, primarily to an autoimmune-mediated reaction, resulting in absolute insulin deficiency (AAD, 2010). The reason why this occurs is not fully understood. In general, T1DM is diagnosed at any age, but most frequently it develops during childhood and adulthood. People with T1DM are usually insulin-dependent and require daily injections of insulin to regulate their glucose levels and to prevent progression of the disease.

b) Type 2 diabetes mellitus (T2DM)

T2DM is considered the most common form of diabetes and account for 90-95% of all diabetes cases worldwide and was previously referred to non–insulin dependent diabetes or adult-onset diabetes (González, Johansson, Wallander, & Rodríguez, 2009). T2DM characterized by reduced responsiveness of cells to insulin (known as insulin resistance) and patients usually have relative insulin deficiency (insulin produced but not enough to prevent hyperglycemia). T2DM usually occurs after the age of 35-40 years but may be diagnosed earlier, especially in populations with high diabetes prevalence. T2DM frequently goes undiagnosed (asymptomatic) for many years and the diagnosis is often appear from associated complications or incidentally through an abnormal blood glucose level or urine glucose test (Lu et al., 2012). T2DM is often, but not always, associated with metabolic abnormalities such as obesity and most patients with this form of diabetes may have an increased percentage of body fat distributed predominantly in the abdominal region, which itself can cause insulin resistance and lead to elevated blood glucose levels. In contrast to T1DM, patients with T2DM do not require exogenous insulin, but may require insulin to control glucose levels if this is not achieved by recommended diet alone or with hypoglycemic drugs (Thévenod, 2008) .

c) Gestational diabetes mellitus (GDM)

This type of diabetes has been defined as any degree of glucose intolerance with onset or first detection during pregnancy and usually diagnosed in the second or third trimester of pregnancy (Cypryk, Szymczak, Czupryniak, Sobczak, & Lewiński, 2008). GDM account for about 2% - 10% of pregnant women. The risk of GDM increased in older mothers especially over the age of 30 years of age, women with a family history of type 2 diabetes, who are overweight, who have had a previous pregnancy with gestational diabetes, who have had large babies or obstetric complications and woman who have had polycystic ovarian syndrome. Maternal hyperglycemia may lead to complications in the baby, including birth trauma, large size at birth, hypoglycemia and infant respiratory syndrome (H. S. C. R. Group, 2008; Mpondo, Ernest, & Dee, 2015).

d) Other specific type (Monogenic diabetes)

There are specific types of diabetes due to many causes including monogenic diabetes syndrome resulting from genetic defects of β-cells function, neonatal diabetes and maturity-onset diabetes of the young [MODY], diseases of the exocrine pancreas (e.g. cystic fibrosis), and diabetes induced by drugs or chemicals (in cases of the treatment of HIV/AIDS or after organ transplantation) (Association, 2010).

1.3 Risk factors for T2DM

T2DM is a very complex disease, and its development includes several risk factors that may include genetic factors, family history (first-degree relative) and pre and post-natal environmental factors. Genetic factors results in certain patterns of DNA sequence whereas pre and post-natal environmental factors include a wide range of elements; suboptimal intrauterine environment, low birth weight, obesity, physical inactivity, history of gestational diabetes, hypertension or dyslipidemia, polycystic ovarian syndrome leading to insulin resistance and finally, decline in insulin secretion due to advancing age (Jin & Patti, 2009). Figure (1.1) summarize the risk factors and their potential role for T2DM.

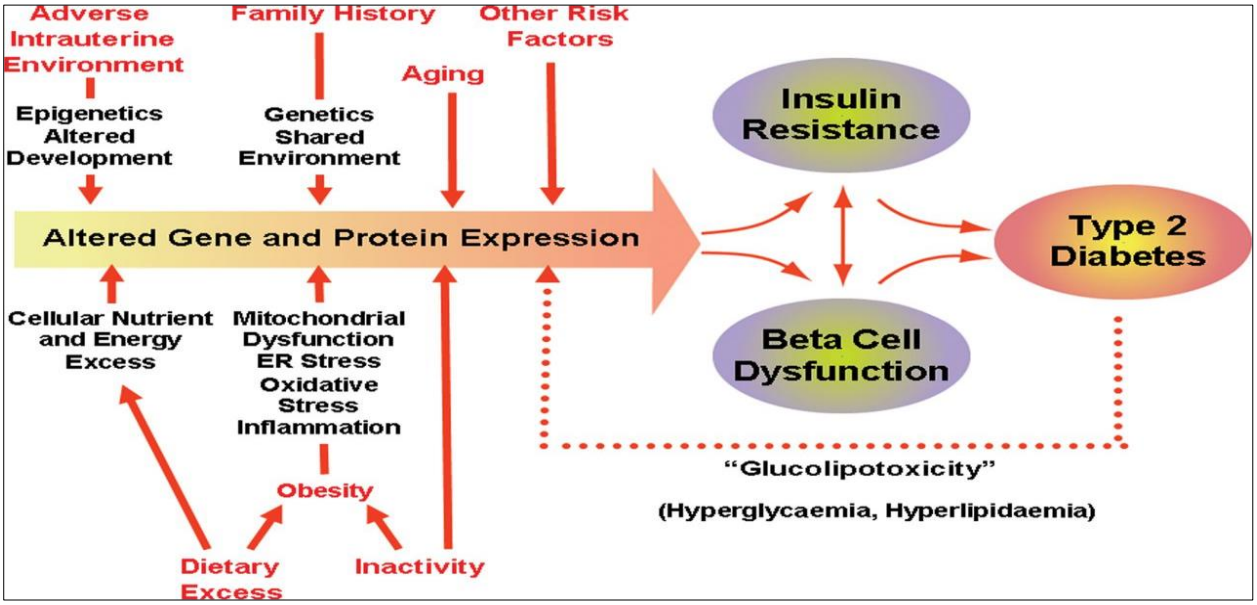

Figure 1.1: Multiple risk factors for T2DM, including classical genetic risk (family history) as well as a prominent contribution from multiple environmental risk factors (adopted from Jin & Patti, 2009).

1.4 Epidemiological transition of diabetes mellitus

DM is now recognized as one of the main public health challenges of the 21st century and it's considered sixth leading cause of death. People suffering from DM have twice the risk of death as comparing with others of the same age without diabetes. The main cause of death in diabetic patients is not from diabetes itself but from the complications of the disease (Rich, Norris, & Rotter, 2008). WHO estimates that, globally, 422 million adults aged over 18 years were living with diabetes in 2014 and this number may be elevated in 2035 to 592 million (Organization, 2016). In every country the whole number of people with T2DM is raising. The highest number of people detected with diabetes is 40 and 59 years of age.

Statistical reports show that about 5.1 million deaths due to diabetes were reported in 2013, meaning that every six seconds a person dies from diabetes (Guariguata et al., 2014). This epidemic relates particularly to T2DM which accounts for the most diagnosed cases of DM. Also, the highest prevalence of DM was in high-income countries (7.8 %) resulted from changes in the lifestyles and decrease physical activity and increase obesity (Shaw, Sicree, & Zimmet, 2010). In the Middle East, the prevalence of T2DM in these countries could reach 60 million diabetes patients in 2030 (Klautzer, Becker, & Mattke, 2014).

In Palestine, Studies enlightening the epidemiology of diabetes mellitus among Palestinians populations are scarce. In 2000, a study on the prevalence of DM in Palestine was conducted in Kober village located north east of the city of Ramallah in the center of the West Bank by Birzeit University, the prevalence of diabetes was 9.6% and 10.0% in females and males respectively (Husseini, 2000). Another two cross-sectional studies was conducted in Ramallah governorate showed a higher rate of this disease in an urban community (12.0%) than in a rural community (9.8%) at age 30–65 years (Abdul-Rahim et al., 2001; Husseini, 2000).

1.5 Diagnostic criteria of Type 2 diabetes mellitus

Diagnosis of T2DM is based on fasting plasma glucose (FPG) concentration and/or 2-hour plasma glucose (2hPG) concentration during an oral glucose tolerance test (OGTT). T2DM is preceded by a pre-diabetic state, which are characterized by mild increasing of fasting or postprandial glucose levels. This state may last for years, and about one third of individuals with pre-diabetes finally develop T2DM (Unwin, Shaw, Zimmet, & Alberti, 2002). Pre-diabetes status is defined by an OGTT, which includes impaired glucose tolerance (IGT, characterized by an elevated 2hPG), impaired fasting glucose (IFG, characterized by an elevated FPG), or both. The diagnostic criteria for IFG and IGT according to the American Diabetes Association (ADA) (Genuth et al., 2003) and World Health Organization (WHO) (Organization, 1999) are shown in table (1.1).

Table 1.1: Diagnostic criteria of glucose tolerance categories according to the WHO (Organization, 1999) and ADA (Genuth et al., 2003) criteria.

Measure	American Diabetes Association		World Health Organization	
	Diabetes	**pre-diabetes**	**Diabetes**	**Impaired Glucose Regulation**
Fasting plasma glucose (FPG)	≥126 mg/dl	100–125 mg/dl (IFG)	≥126 mg/dl	110–125 mg/dl (IFG)
2-Hr plasma glucose (during an OGTT with a loading dose of 75 g)	≥200 mg/dl	140–199 mg/dl (IGT)	≥200 mg/dl	140–199 mg/dl (IGT)
Casual (or random) plasma glucose (in a patient with classic hyperglycemic symptoms)	≥200 mg/dl		≥200 mg/dl	
Glycated hemoglobin	≥6.5%	5.7–6.4%	≥6.5%	

1.6 Pathogenesis of Type 2 diabetes mellitus

Under normal physiological conditions, plasma glucose levels are maintained within a narrow range and well balance to regulate interaction between tissue sensitivity to insulin (especially in liver) and insulin secretion, known as glucose homeostasis (Boden, 1996). In T2DM these mechanisms break down due to two main pathological defects:

a) Impaired insulin secretion due to a dysfunction of the pancreatic β-cells leading to a relative insulin deficiency (Chiu et al., 2007).
b) Impaired insulin action due to insulin resistance caused by defects in the signaling pathways that process the insulin signal in its target tissues (Wolfs, Hofker, Wijmenga, & Van Haeften, 2009).

However, several studies reported that T2DM is developed when the pancreas is unable to produce more insulin to compensate for existing insulin resistance. According to this, insulin resistance will be present early in the natural history of this disease, whereas marked β-cell dysfunction is a rather late event (Martin et al., 1992). In addition, both impaired insulin secretion and insulin resistance are influenced by genetic and environmental factors.

1.6.1. Impaired insulin secretion

Insulin is released from the β-cells of pancreatic islets in response to changes in blood glucose level. In brief, the process begins by transferring glucose to β-cells through diffusion facilitated by GLUT2 transporter. In the β-cell, glucose is metabolized to generate ATP and thus the ATP/ADP ratio increases leading to closure of cell-surface ATP-sensitive K+ channels (K_{ATP} channel) and these channels are capable of modulating insulin secretion in response to fluctuations in plasma glucose levels, and thus are an important regulators of glucose homeostasis. Accordingly, depolarization of the cell-membrane occurs and subsequently the Ca^{2+} dependent channels are opened, facilitating extracellular flow of Ca^{2+} in the β-cell which triggers the exocytosis of insulin as shown in figure (1.2).

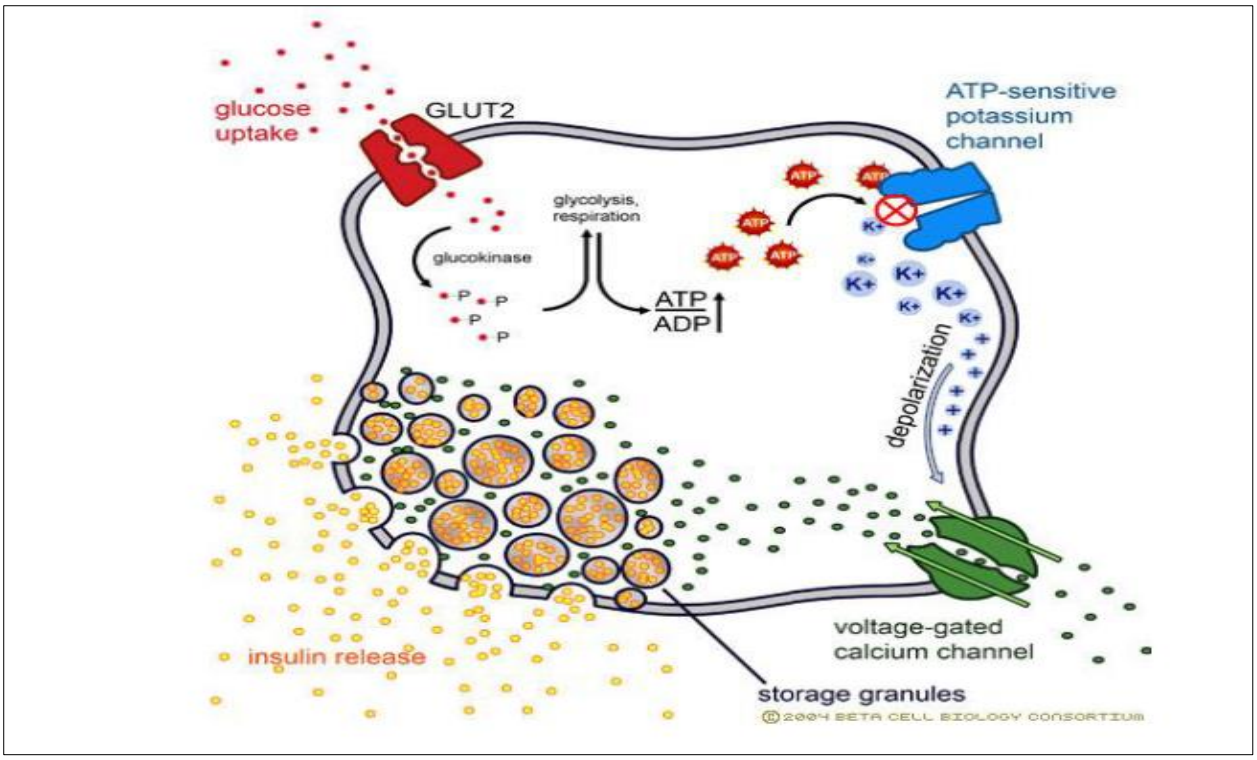

Figure 1.2: Insulin secretion by the beta-cell of the pancreatic islets (adopted from(Henquin, Ishiyama, Nenquin, Ravier, & Jonas, 2002).

The Insulin release process from the pancreatic β-cells takes place in two phases in response to a rapid increase in blood glucose level. The first phase, which is a short-term period (occurs within a few minutes) and characterized by an increase in insulin secretion, is followed by a more slowly evolving second phase, which lasts as long as the glucose level is elevated. On the other hand, a slow increase in plasma glucose level induces a gradually larger secretion without the first phase (Henquin, Ishiyama, Nenquin, Ravier, & Jonas, 2002).

In type 2 diabetic patients, the first phase of insulin secretion is lower than in healthy subjects, and is often absent, the second phase also much lower than in non-diabetic subject. Moreover, the impairment of both first and second phase insulin release occurs early in the natural history of diabetes (Gerich, 2002).

Pancreatic β-cell failure in T2DM can be due to several potential causes:

- Reversible metabolic abnormalities (glucotoxicity and lipotoxicity).
- Hormonal changes (inadequate secretion and action).
- Moderate reduction in total mass of pancreatic islet tissue due to apoptosis, and genetic abnormalities.

Chronic hyperglycemia has been shown to induce β-cell dysfunction and apoptosis in human cells leading to mitochondrial weakness, production of reactive oxygen species and increased levels of intracellular calcium, which leads to glucotoxicity (Wajchenberg, 2007).

Elevation of free fatty acid levels (FFA) can also contribute to β-cell dysfunction through promote pro-apoptotic effect as result of endoplasmic reticulum stress, or through the intracellular accumulation of triglycerides as a response to the activation of the sterol regulatory element binding proteins (SREBP), that regulate cellular ATP production and leads to lipotoxicity (Joseph et al., 2004). The deleterious effects of FFAs are observed predominantly in the presence of high glucose. On the other hand, there are several hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) which are important gut hormones that released after food intake, and have been shown to increase β-cell proliferation and decrease β-cell apoptosis (Drucker, 2006).

1.6.2. Insulin signaling pathway

When blood glucose level rises, insulin released by the pancreatic β-cell and then begin to exercise biological functions through interaction with membrane-spanning insulin receptor (IR) on muscle cells and/or adipocytes and facilitated dimerization of the receptor. As a result of this dimerization the receptor is autophosphorylated at specific residues, leading to binding of various scaffold proteins such as insulin receptor substrate (IRS) proteins. IRS proteins are activated by phosphorylation of by their association with the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K). Followed by actions of phopshoinositide-dependent protein kinase-1 (PDK1) and protein kinase B (PKB or Akt). AKt binds to domain of PKB, and thus promotes glucose uptake by the GLUT4 transporter and glycogen synthesis (Changeux & Edelstein, 2005). These mechanism of insulin signaling pathway was described in figure (1.3). Other effects of insulin, such as its effect on protein synthesis, glycogen synthesis, lipogenesis and suppression of hepatic glycogenesis are also mediated by PKB.

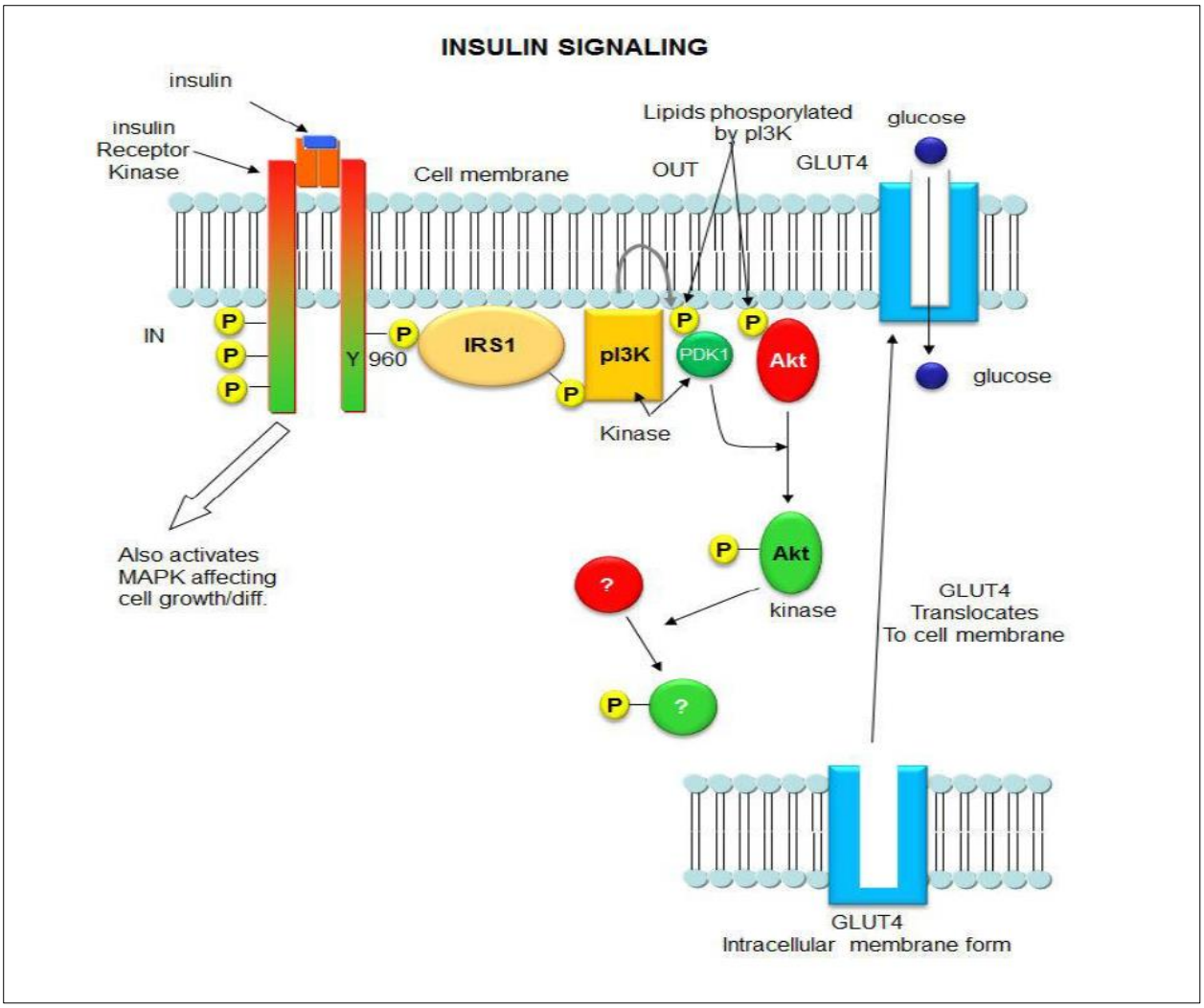

Figure 1.3: The mechanism of insulin signaling pathway (adopted from (Changeux & Edelstein, 2005))

Insulin resistance

Insulin resistance can be defined as the inability of tissues to respond properly to normal circulating insulin. To maintain a normal concentration of blood sugar, the pancreas compensates by secreting increased amount of insulin. However, high levels of insulin can compensate for poor insulin action only for a limited time. After a period of insulin resistance, pre-diabetes usually develops, especially in co-existence with impaired of β-cell functions. An early onset of insulin resistance in the natural history of diabetes was demonstrated by Vaag et al., in 1992 as they found that young healthy offspring of diabetes parents usually show insulin resistance and impaired skeletal muscle insulin signaling many decades before the onset of T2DM (Vaag, Henriksen, & Beck-Nielsen, 1992).

Although the exact mechanism resulting in insulin resistance is not clear, multiple abnormalities in the insulin signaling pathway have been identified. According to insulin signaling pathway, the most important site is IRS (IRS1 and IRS2), PI3K and PKB. Therefore, any mutation in one of these sites lead to enhanced insulin resistance, e.g. a mutation in the *IRS1* gene in human are associated with insulin resistance (Whitehead, Humphreys, Krook, & Jackson, 1998) and reduced activation of the PI3-kinase/Akt signaling pathway which lead to reduction of glucose transport and utilization in the skeletal muscle and adipocytes (Mulder, Ahren, Stridsberg, & Sundler, 1995). Moreover, there are still several other causes of insulin resistance which are summarized in table (1.2).

Table 1.2: Some causes of insulin resistance

Cause No.	Cause
1	Obesity/overweight (especially excess visceral adiposity).
2	Pregnancy, gestational diabetes.
3	Excess glucocorticoids (Cushing's syndrome or steroid therapy).
4	Excess growth hormone (acromegaly).
5	Lipodystrophy (acquired or genetic, associated with lipid accumulation in liver).
6	Mutations of the peroxisome proliferators' activator receptor γ (PPAR).
7	Hemochromatosis (a hereditary disease that causes tissue iron accumulation).
8	Polycystic ovary disease.
9	Autoantibodies to the insulin receptor.

1.7 Complications of type 2 diabetes mellitus

Improper management and treatment of T2DM can lead to very high levels of blood glucose and a number of serious health problems such as macrovascular and microvascular complications. Microvascular complications include retinopathy, neuropathy, nephropathy (which may lead to renal failure), blindness and lower limb amputation (diabetic foot). Macrovascular complications include cardiovascular disease (CVD) and stroke.

1.7.1. Microvascular complications of T2DM

Microvascular complication is developed through aldose reductase enzyme mechanism. This enzyme is considered the first enzyme in the intracellular polyol pathway. This pathway is working on converting of glucose to sorbitol (glucose alcohol). In hyperglycemic status, the flux of sugar molecules through the polyol pathway will be increasing, which causes sorbitol accumulation in cells and promotes osmotic stress (Gabbay, 1975). It has been suggested that osmotic stress is the mechanism by which diabetic microvascular complications were developed.

a) Diabetic retinopathy (DR)

DR is considered the most common microvascular complication that can affect the peripheral retina, the macula, or both and is a leading cause of visual disability and blindness in people with T2DM. The prevalence of DR in patient with T2DM is between 40-50% which can be increased with prolonged duration of diabetes (Cheung, Mitchell, & Wong, 2010). The major risk factors for rapid progression of DR in patients with T2DM has been found to be related to several factors including; increasing age, duration of diabetes, poor control of blood glucose hypertension, obesity, alcohol consumption and genetic factors (e.g. Aldose reductase gene) (Rivera et al., 2017) .

The severity of DR ranges from mild non-proliferative and pre-proliferative to more severely proliferative DR. Mild non-proliferative DR is characterized by the microaneurysms and hard exudates. In pre-proliferative DR, there might be intraretinal hemorrhages, soft exudates, venous beading and intra-retinal microvascular abnormalities (Elwali et al., 2017).

b) Diabetic neuropathy

Diabetic neuropathies are neurological disorders caused by diabetes, so people with T2DM can, over time, may develop nerve damage throughout the body. Some of these people have no symptoms, while others may have symptoms such as problems with urination, numbness, tingling, wasting of the muscle of the feet or hands, indigestion, nausea, or vomiting.

The risk of development of diabetic neuropathy is directly proportional to several factors (Fowler, 2008) such as:

- Metabolic factors such as high blood glucose, abnormal blood fat levels, long duration of diabetes and possibly low levels of insulin.
- Neurovascular factors which lead to damage the blood vessels carrying oxygen and nerve nutrients.
- Autoimmune factors (inflammation in nerves).
- Inherited traits that increase susceptibility to nerve disease.
- Lifestyle factors such as tobacco or alcohol use.
- Mechanical injury to nerves such as carpal tunnel syndrome.

c) Diabetic nephropathy (DN)

DN is characterized by an increased urinary albumin excretion (UAE) in the absence of other renal diseases. It is considered the most common cause of end stage renal disease (ESRD) (Bakris, 2011). Clinical evidence associated with DN refers to the presence of abnormal level of albumin in the urine (>30 mg/day or 20μg/min; urinary albumin/creatinine ratio [ACR] >3.0 mg/mmol), known as microalbuminuria. In T2DM, the prevalence of DN varies between 5-20%, 38% developed to microalbuminuria and 29% developed renal impairment after 15 years of follow up (Retnakaran, Cull, Thorne, Adler, & Holman, 2006).

The pathogenesis of DN is similar to other microvascular complications and caused by the association of both metabolic alterations (possibly hyperlipidemia and hyperglycemia) and haemodynamic alterations (systemic and glomerular hypertension). Hyperglycemia plays role in induced oxidative stress and the activation of several metabolic pathway such as advanced glycation end-product (AGE), protein kinase C (PKC) and polyol pathway which lead to evolution of DN (Cade, 2008; Dronavalli, Duka, & Bakris, 2008). Haemodynamic alterations occur as a result of the activation of different vasoactive systems, such as endothelin systems and renin–angiotensin–aldosterone (RAAS), which arise in response to the secretion of pro-fibrotic factors, such as transforming growth factor β1 (TGF-β1), resulting in increased systemic and intraglomerular pressure (Dronavalli et al., 2008).

The risk of development of microalbuminuria in T2DM was associated with several factors such as:

- Elevated systolic blood pressure.
- Waist circumference.
- Previous cardiovascular disease.
- Smoking history.
- Elevated plasma triglycerides.
- Gender especially male.

1.7.2. Macrovascular complications of T2DM

Cardiovascular diseases (CVD) are the principal cause of mortality (~70%). Patients with T2DM are considered to have a 4-fold-greater risk for developing a CVD than people without T2DM after controlling for traditional risk factors for CVD, such as, age, smoking, high blood pressures, high serum cholesterol and obesity (Bugger & Abel, 2014). These cardiovascular risk factors are common in diabetes, but recent studies and data suggest that diabetes is an independent risk factor for cardiovascular disease (Forbes & Cooper, 2013).

Patients with T2DM also exposed to the risk of premature atherosclerosis and development of Myocardial Infarction, impaired cardiac function and stroke. Recent data suggest that type 2 diabetic patient also have a 5-fold-greater risk for a first Myocardial Infarction and a 2-fold-greater risk for a recurrent Myocardial infarction than people who previously had a Myocardial Infarction but do not have diabetes (Forbes & Cooper, 2013; Kalofoutis et al., 2007).

1.8 Risk Factors for the development of T2DM Complications

Several factors play multiple roles in duration and control of the disease. They include:

a) Elevation of blood pressure has a significant effect on CVD in people with T2DM compared with non-diabetic individuals. Thus the control of blood pressure (<130/80 mmHg in adults) will have an effective reduction of cardiovascular morbidity and mortality in T2DM patients (Donaghue, Chiarelli, Trotta, Allgrove, & Dahl-Jorgensen, 2009).

b) Familial history of complications worsens the risk for nephropathy (Prasad et al., 2006) and retinopathy (Dorchy, Claes, & Verougstraete, 2002).
c) Higher body mass index (BMI) is a documented risk factor for neuropathy, retinopathy (Dorchy et al., 2002), cardiovascular disease and microalbuminuria (Fowler, 2008).
d) Life style: low physical activity individual with T2DM had a higher mortality rate than those with high physical activity (Moy et al., 1993).
e) Microalbuminuria predicts strictly cardiovascular morbidity and mortality in individuals with T2DM (Tagle, Acevedo, & Vidt, 2003). A cohort study conducted by (Valmadrid, Klein, Moss, & Klein, 2000) showed that the risk for CVD was doubled in patients with microalbuminuria compared to those without.
f) Dyslipidemia: high levels of triglycerides, low level of HDL-C and high level of LDL-C, is a risk factor for macrovascular diabetic complications. Lowering LDL-C with diet or lipid-lowering medication has an important effect in reducing the cardiovascular risk of patients with T2DM (Howard et al., 2000).

1.9. Literature review

1.9.1. Genetics of type 2 diabetes mellitus

It is generally agreed that T2DM has a strong genetic component. There are several evidences supporting this view. First, the prevalence of T2DM varies across different ethnic groups and it is modified by genetic admixture (Florez, Hirschhorn, & Altshuler, 2003). Secondly, T2DM often exhibits familial aggregation (Rotimi, Cooper, Cao, Sundarum, & McGee, 1994). The estimated risk of developing T2DM is 40% for individuals who have one parent with T2D and almost 70% if both parents are affected (Kobberling, 1982). Furthermore, the relative risk for first degree relatives has approximately 3.5-fold increased risk of developing the disease for those with single diabetic parent, and ~6-fold if both parents are affected compared with offspring without parental diabetes (Meigs, Cupples, & Wilson, 2000). Third, twin studies showed a high concordance of type 2 diabetes in monozygotic twins (concordance rates of over 90%), whereas in dizygotic twins the concordance is about 50% lower than in monozygotic twins (concordance rates of 9-16%) (Wolfs et al., 2009). Finally, there is a strong heritability of intermediate phenotypes, since both insulin secretion and

insulin sensitivity have an important genetic component (P. Poulsen, Kyvik, Vaag, & Beck-Nielsen, 1999).

Genetic contribution to T2DM arises from genetic differences in many genes, each conferring a small increase in the risk. These genes variations do not cause diabetes but increase their risk through interaction with other genes for diabetes (diabetes-susceptibility genes), metabolic environment of the body (e.g. lipotoxicity and glucotoxicity) and life-style factors (e.g. smoking, excess calories, stress and sedentary life style) (Hansen & Pedersen, 2005). The most studied of genetic differences that determine individual predisposition to T2DM are single nucleotide polymorphism (SNPs), which cover ~90% of the sequence variation within the human genome.

1.9.2. Strategies in the search for the genetic basis of T2DM

The section below briefly describes the three strategies that have been used in many studies to elucidate the role of polymorphisms in T2DM.

1.9.2.1. Linkage studies

This traditional method of identifying disease-causing genes has been used to search for linkage between a chromosomal region and a disease by genotyping of a large number of polymorphic markers in affected families (Elbers et al., 2007).

Although linkage-based studies in affected families have shown success in providing consistent results on linkage but they may lack the statistical power and mapping resolution to identifying susceptibility alleles of complex diseases such as T2DM (Ahlqvist, Ahluwalia, & Groop, 2011). The Figure (1.4) below illustrates the chromosomal regions (q32, 6q22-6q24, 11q24, 12q24, and 20q12 – 20q13) which encompasses about 612 genes which were found to be linked to both T2DM and obesity susceptibility (Elbers et al., 2007).

1.9.2.2. Candidate gene studies

These studies focus on the knowledge of the gene or protein function in relation to the disease in question to identify the relationship between certain polymorphisms and the disease-related trait (s). Polymorphisms and association testing identified by the candidate gene approach can also be conducted in populations and not limited in families only as is done in

linkage studies. Also in these studies, a random sample of unrelated T2DM patients is compared with matched controls, thus identifying a polymorphic allele that occurs at a higher frequency in the patient group.

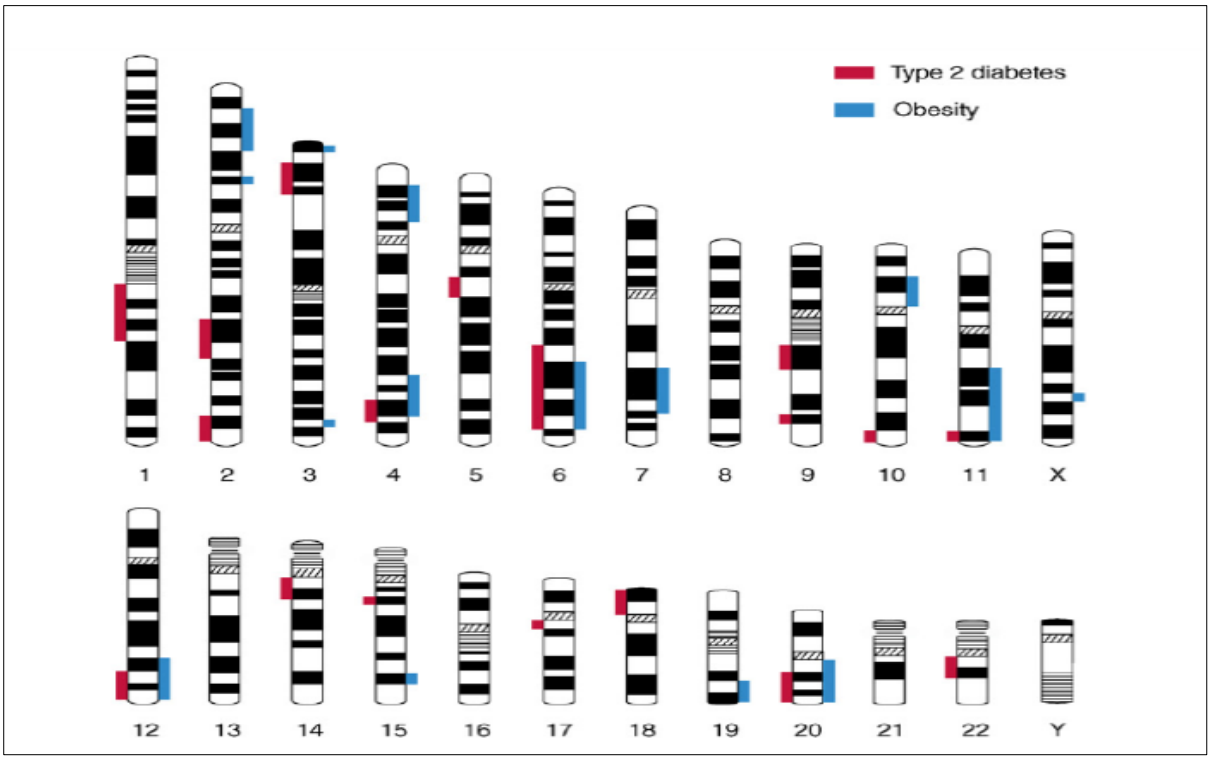

Figure 1.4: Genetic linkage map for T2DM and obesity. The red bars indicate the susceptible loci for T2DM and the blue bars for obesity (Adopted from Elbers et al., 2007).

The usual strategy of these studies concentrated on genes already known to be involved in insulin secretion, glucose metabolism, insulin receptors, post-receptor signaling and lipid metabolism. For T2DM, most candidate gene studies have focused on both genes related to pancreatic β-cell function (insulin secretion) and insulin action (resistance) genes (Guja, Gagniuc, & Ionescu-Tîrgovişte, 2012).

A large number of studies have reported several functional candidate genes with T2DM, however association could be replicated for only 6 genes includes insulin receptor substrate (*IRS1*) and *IRS-2*, peroxisome proliferator-activated receptor gamma (*PPARG*), potassium inwardly-rectifying channel, subfamily J, member 11 (*KCNJ11*), HNF1 homeobox A (*HNF1A*), HNF1 homeobox B (*HNF1B*) and Wolfram syndrome 1 (wolframin) (*WFS1*) (Cheekurthy, Rambabu, & Kumar, 2016) (Kato, 2013).

1.9.2.3. Genome –wide association studies (GWAs)

In contrast to candidate gene and linkage studies, genome wide association studies (GWAs) conducted to investigate genetic variation across the entire whole human genome regardless of their functionality and to identify its genetic link in the presence or absence of the disease (HUANG, CHENG, & Sen-Lin, 2006). Until today the GWA studies have identified nearly more than 70 gene loci associated with T2DM (Haddad et al., 2017) . The greater part of GWAs have been focused on European population. However, further studies on Asian, African and other populations were also done in order to improve the genetic view of the disease in these population (Ng et al., 2014; Zia, Kiani, Bhatti, & John, 2013). The first GWA scans were published in 2007, six novel diabetes-susceptibility genes have been identified: *SLC30A8*, *HHEX-IDE*, *CDKN2A/2B*, *IGF2BP2*, *CDKAL1* and *FTO* (Sladek et al., 2007).

1.9.3. Polymorphism

About 99.9% of the human DNA sequence is the same between unrelated individuals, whereas only 0.1% of the coded DNA varies between two chromosomal strand (Kruglyak & Nickerson, 2001). 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 form of polymorphisms is the single nucleotide polymorphisms (SNPs), which are a change in a single base pair in the genomic DNA and may affect the gene function. Other forms of polymorphisms are inversions, insertions, deletions and variable number of tandem repeats (VNTRs).

About 12 million of SNPs have been identified and genotyped by a number of genotyping platforms and most commonly used as marker in association studies (I. S. M. W. Group, 2001). 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 replacements (pyramidine-pyramidine C↔T or purine-purine A↔G) and it constitutes about 2/3 of SNPs,

while the other form is transversion replacements (pyramidine-purine or purine-pyramidine) and it constitutes about 1/3 of SNPs (Guo & Jamison, 2005).

The classification of SNPs is depended on their genomic site. The coding SNPs (cSNP) are located in exon sequences of DNA which may be either synonymous (no change in amino acid previously termed "silent" but Can alter mRNA stability) or non-synonymous (changes amino acid conservative and radical) (Burton, Tobin, & Hopper, 2005). These SNPs have the potential to alter the function or availability of a protein. However, a non-synonymous SNP in a coding sequence can also affect the function or availability of a protein and considered as genetic markers for many diseases (Carlson, Eberle, Kruglyak, & Nickerson, 2004).

1.9.4. Fat mass and obesity-associated (*FTO*) gene

The epidemic nature and the prevalence of diabetes are expected to increase in Palestine. Since there are a little of genetic studies describing the impact of gene variants that associate with T2DM and related risk factors amongst Palestinians. In this study, the potential association of the A allele were investigated at rs9939609 of *FTO* gene with T2DM. To the best of our knowledge, this gene has not been previously tested in the Palestinian population.

1.9.4.1. Mechanism of action of *FTO* gene

FTO gene in human is very large gene with nine exons with more than 400 Kb on chromosome 16q12.2 and is well conserved in vertebrates and algae as shown in figure (1.5).

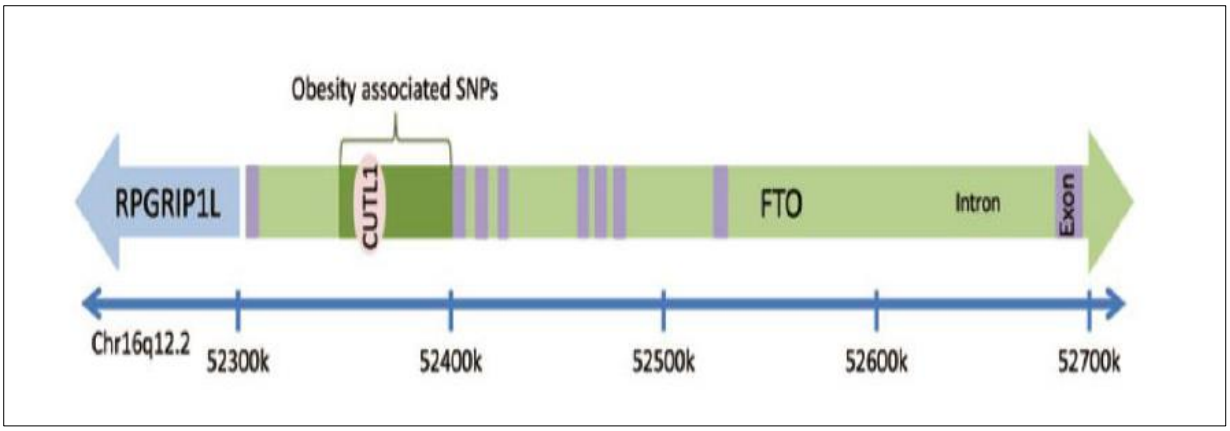

Figure 1.5: Schematic representation of the *FTO* gene chromosome 16. The obesity associated SNPs are located in the first intron. CUTL1 is a transcriptional activator, located in the associated region. (Adopted from(Gerken et al., 2007)).

FTO gene is primarily expressed in the hypothalamus and encodes a 2- oxoglutarate-dependent nucleic acid demethylase. It has homology with the AlkB family with a strong preference for 3-methylthymidine and 3-methyluracil in single-stranded DNA and RNA, respectively, owing to unique structural features (Han et al., 2012). The biochemical studies in vitro revealed that *FTO* to be a member of the Fe (II) and 2-oxoglutarate (2OG) dependent oxygenase superfamily (Gerken et al., 2007).

In metazoans, these enzymes are involved in a variety of processes including DNA repair, oxygen sensing, metabolism of fatty acid and post-translational modifications (Clifton et al., 2006). The mechanism action of *FTO* gene encodes for a protein 2-oxoglutarate dependent nucleic acid demethylase involved in repairing DNA, post- translational modifications, metabolism of fatty acid, management of energy homeostasis, regulation fat masses of body via lipolysis and demethylation of the nucleic acid (Church et al., 2010).

The *FTO* expression in the hypothalamus suggests a potential role in the regulation of the global metabolic rate, energy expenditure and energy homeostasis, regulation of body size and body fat accumulation and it is involved in the regulation of thermogenesis and the control of adipocyte differentiation into brown or white fat cells (Dina et al., 2007; Speakman, 2015). The full name, chromosomal location, exon number and size of the *FTO* gene is summarized in table (1.3) below.

1.9.4.2. Human studies on *FTO* gene variants

Several independent studies identified a number of genetic variables in the *FTO* as it is associated with BMI and obesity. In 2007, Frayling and colleagues studied the association of *FTO* genetic variant rs9939609 and T2DM in European population the association was observed (OR=1.27) but once adjusted for body mass index (BMI), the association of *FTO* SNP and diabetes disappeared (OR=1.03) suggesting that this *FTO* variant was primarily affecting BMI rather than T2D risk directly (Frayling et al., 2007). Another study conducted at the same time by (Dina et al., 2007) identified other genetic variants in *FTO* gene polymorphism (rs17817449, rs1421085) that were strongly associated with early-onset obesity among both children and adults. In addition, another study conducted by Scuteri et al., 2007 (GWA study) found the strong association between several variants within *FTO* and obesity (Scuteri et al., 2007).

Since then, the association of *FTO* gene polymorphisms with obesity and BMI have been replicated in a number of studies and they have been expanded among Asians (Tan et al., 2008), Europeans (Price, Li, & Zhao, 2008) as well as Africans (Adeyemo et al., 2010).

Several studies also been conducted on other human measurements of obesity and show the *FTO* risk allele is associated with increased these measurements or parameters. For example, the *FTO* genetic variant rs9930506 is associated with increased waist to hip ratio (Scuteri et al., 2007), waist circumference, the amount of body fat (Kring et al., 2008) and fat mass (Andreasen et al., 2008).

Few studies have investigated the *FTO* mRNA expression level in human adipose tissue. In one study, the expression level of *FTO* mRNA was threefold higher in subcutaneous adipose tissue than in visceral adipose tissue (Wåhlén, Sjölin, & Hoffstedt, 2008). Moreover, several studies conducted on metabolic traits related to glucose metabolism and homeostasis such as serum insulin, plasma glucose, insulin sensitivity and insulin resistance have shown the *FTO* genetic variant rs9939609 risk allele is associated with decreased insulin sensitivity and increased insulin response (Adeyemo et al., 2010). However, no significant effect was observed on plasma glucose levels (Müller et al., 2008), serum insulin levels or insulin resistance (Andreasen et al., 2008).

1.9.4.3. Animal models

Several *in vivo* studies in animal models demonstrated the effect of null and loss-of-function mutations on expression of *FTO* protein, compared to in vitro experiments. The first murine model of global germline reduction of *FTO* expression was reported by Fischer et al., 2009. Mice with low expression of *FTO* suffered from a high perinatal lethality as well as postnatal growth characterized by reduction in body weight and length as a result of reduction in fat mass and resistance against diet-induced obesity as a consequence of increased energy expenditure.

The result provided by Fisher et al., 2009 was supported by another study conducted by (Church et al., 2010) showed that a dominant point mutation in the *FTO* gene in mice resulted in reduced fat mass and increased energy expenditure. Contrary to these results, a study by (Gao et al., 2010) showed that deletion of *FTO* in mice leads to increased body and fat mass. Food intake was significantly increased, but energy expenditure and physical activity were unaltered in these mice. In addition, several studies have been conducted to study the effect of additional copies of *FTO* gene and showed how these extra copies of this gene lead to a dose-dependent increase in fat mass as a result of increased food intake (Church et al., 2010).

1.9.4.4. Gene report of *FTO* gene

Table 1.3: Summary of *FTO* gene

Official symbol	*FTO* (Eynon et al., 2013)
Full gene name	Fat mass and obesity-associated gene (Eynon et al., 2013)
Chromosomal location	16q12.2 (Gerken et al., 2007)
Gene ID	79068 (Hubacek et al., 2012)
Exon number	9 exones (Gerken et al., 2007)
size	400,000bp (Gerken et al., 2007)
Gene type	Protein-coding (Hubacek et al., 2012)
OMIM accession number	610966 (Hubacek et al., 2012)
Known as	Fat mass and obesity- associated protein (Frayling et al., 2007).

	Alpha-ketoglutarate-dependent dioxygenase (Frayling et al., 2007).
Amino acid no.	505 (Gerken et al., 2007)

1.10 Study objectives

In Palestine, no studies have been conducted to determine the genetic association of *FTO* variants with T2DM. Our study aimed to investigate the association of rs9939609 variant (T>A) in the *FTO* gene with T2DM among Palestinian Population. This study may provide valuable information for comparison with other ethnic groups.

Specific objectives:

1. To investigate the potential association of the A allele at rs9939609 of *FTO* gene with T2DM.
2. To study the relationship between A allele at rs9939609 of *FTO* gene and blood pressure, BMI and other related metabolic parameters.
3. To clarify the association of rs9939609 variant in the *FTO* gene with CVD risk in type 2 diabetic Palestinians.

Chapter Two

2. Materials and Method

2.1. Study population

Our study recruited 399 Palestinian participants. Among them, 281 were type2 diabetic patients (cases) and 118 were non-diabetic individuals (controls). Both groups were above 40 years' age who attending United Nations Relief and Works Agency (UNRWA) clinics and residing in different regions of the West Bank (South and Middle West Bank). For diabetic cases, 186patients were from Hebron, 34 from Bethlehem, 55 from Ramallah and 6 from Jericho. For non-diabetic participants, all of them (n=118) were from Hebron. Figure (2.6) indicates the sampling area.

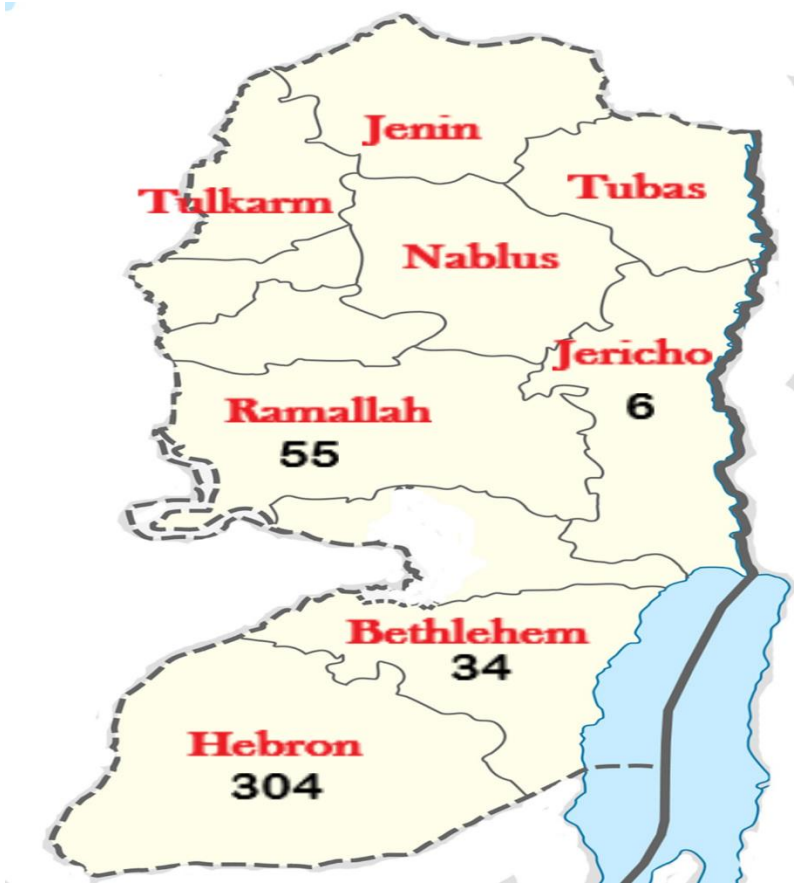

Figure 2.6: Residency of participants admitted to the UNRWA clinics in west bank.

2.2. Study design and Eligibility criteria

A case-control study was conducted to study the association of *FTO* gene polymorphism (rs9939609) with T2DM, the Participants were unrelated, recruited within the period 2016-2017 based on the following criteria.

- **Inclusion criteria:**

  Cases:

  - Males or females who were diagnosed by specialist physicians as having type 2 diabetes based on WHO guidelines: Diabetes is diagnosed if FBG is ≥ 126 mg/dl).
  - Patients receiving treatment at UNRWA diabetes clinics and aged ≥40 years.

  Controls:

  - Males or females not diagnosed with diabetes mellitus.

- Residents receiving periodic follow-up at UNRWA primary health care clinics.

- **Exclusion criteria:**
  - Patients diagnosed with Type 1 diabetes
  - Participants under the age of 40 years.
  - Participants with FBG ≥126 mg/dl for control subjects.

2.3. Data collection and ethics statements

The clinical data and medical history were recruited from the UNRWA clinic records. The study protocol was approved by the UNRWA officials and Al-Quds University ethics committee (see Appendix 1). Informed consent was obtained from all subjects before sampling (Appendix 2). All data was filled in a well-designed questionnaire (Appendix 3) containing the following data:

- Demographic information: age, gender and place of residence.
- Family history of diabetes mellitus in first degree relatives: father, mother, sister, brother.
- Complications for type 2 diabetic patients: CVD, nephropathy, retinopathy and amputation/diabetic foot.
- Type of medication: anti-diabetic agents (oral and insulin medication), anti-hypertensive and lipid lowering agents.
- Anthropometric parameters:
  - Blood pressure: SBP (systolic blood pressure) and DBP (diastolic blood pressure).
  - Body Mass Index (BMI): person's weight in kilograms divided by height in meters squared (BMI=kg/m2). Subjects were considered to be normal weight if their BMI was < 25 kg/m^2, overweight if their BMI was 25-29.9 kg/m^2 and obese if their BMI > 30 kg/m^2.
- Biochemical tests:
  - HbA1C: normal <5.5%.
  - Fasting blood sugar: normal (70 to 110 mg/dl).
  - Total cholesterol: normal < 200mg/dl.

- Triglyceride: normal <150mg/dl.
- HDL-cholesterol: normal between 40-50 mg/dl (1.0-1.3 mmol/l) for men and between 50-59 mg/dl (1.3-1.5 mmol/l) for women.
- LDL- cholesterol: normal<100mg/dl (2.59 mmol/l).

2.4. Data management and analysis

All collected data was entered and arranged in excel file. For data analysis, the Statistical Packages for Social Science (SPSS) version 24 was used. Continuous variables (age, BMI, SBP, DBP, HbA1C, FBG, TC, TG, HDL and LDL) are expressed as mean ± SD. Categorical variables (gender, family history of diabetes, and diabetic complications) are expressed as percentage. The genotype frequencies were tested for Hardy–Weinberg equilibrium using a chi-square test through the https:// ihg.gsf.de/ihg/index_engl.html website. ANOVA was used to assess the association between *FTO* genotypes and continuous variables. Logistic regression by R statistics (version 2.3v) software was used to measure OR for T2DM adjusted for age, gender and BMI. A probability value of < 0.05 was considered significant.

2.5. Methods

2.5.1. Blood Sampling

Whole blood samples (5 ml) were taken from all individuals by vein puncture in fasting status and collected in tubes containing 0.5 ml of ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. All samples were kept at 4C°; the DNA was extracted within 24 hours.

2.5.2. DNA extraction

DNA extraction was done from all blood samples (T2DM patients and non-diabetic control) using NucleoSpin® Tissue kit (Germany). The extraction steps were performed according to the manufacturer's instruction; the procedure was as followed:

1. 25 µL Proteinase K were pipetted into the bottom of a 1.5 ml microcentrifuge tube.
2. 200 µl samples were added to the microcentrifuge tube.
3. 200 µl Buffer B3 were added to the sample, mixed by pulse-vortexing for 10-20 s.
4. Mixture was incubated at 70 °C for 10–15 min.

5. Absolute ethanol (100%) in a volume of 210 µl was added to each sample and immediately mixed by vortex for 10 seconds.
6. Mixture from step 5 was applied carefully to the NucleoSpin® Tissue Column (in a 2ml collection tube). The cap was closed and column in the collection tube was centrifuged at 11,000 x g for 1 min. Discard collection tube with flow-through and place the column in a new Collection Tube
7. BW Buffer in a volume of 500 µl was added to the NucleoSpin® Tissue Column and centrifuged at 11,000 x g for 2 min. And discarded the filtrate.
8. The NucleoSpin® Tissue Column was replaced in a clean 2 ml collection tube, 600 µl Buffer B5 were added and the tubes were centrifuged at 14,000 x g for 3 min. then discarded the filtrate.
9. Finally, the NucleoSpin® Tissue Column was placed in a clean microcentrifuge tube, 100 µl elution buffers was added to the column and centrifuged at 11000 x g for 1 min.
10. The obtained DNA was stored at -20 ºC until further analysis.

2.5.3. Genetic detection of *FTO* (rs9939609) polymorphism

2.5.3.1. DNA amplification

A fragment of 187 bp of *FTO* gene targeting the rs9939609 polymorphism was amplified using 2 primers (forward /reverse) as described previously by Adeela Shahid et al., 2013 (Shahid et al., 2013). The primers sequences and the melting temperatures are shown in Table (2.4).

Table 2.4: The sequence of primers used for PCR amplification of FTO (rs9939609) gene. (Shahid et al., 2013)

Gene	primer	Sequence	Tm (°C)
FTO rs9939609	Forward	5'AACTGGCTCTTGAATGAAATAGGATTCAGA3'	67
	Reverse	5'AGAGTAACAGAGACTATCCAAGTGCAGTAC3'	69

2.5.3.2. PCR conditions

A total volume of 25 µl for each PCR reaction was performed using PCR- ready mix (Syntezza Bioscience-Jerusalem) containing 1.2 µl of each set of primers and 5µl of the extracted DNA. The amplification reactions were carried out using (Gene Amp, PCR System 9700). The PCR reaction started with initial denaturation for 5 min at 95°C followed by 35 cycles in the following order, 30 sec. at 95°C for denaturation, 35sec at 62 °C for annealing and 30 sec. at 72 °C for extension, additional extension step was performed for a further 5 min at 72 °C, assuring the complete extension of the amplified product. The PCR product was frozen at -20°C until further analysis. In all amplification reactions, negative controls (mix without DNA) were included.

2.5.3.3. Detection of PCR product

The amplified product was visualized on 2.5 % agarose gel electrophoresis and 50bp DNA ladder (GeneDireX, Cat No. DM012-R500) was added to confirm the correct band size.

I. Agarose gel preparation and electrophoresis

- 2.5 grams' agarose (2.5%) were weighed, placed into a glass beaker, and then 100 ml of 1X (TBE) buffer were added.
- The agarose was boiled until it was well dissolved and clear, after that, agarose suspension was allowed to cool to about 40°C before it pouring.
- Ethidium bromide (4.5 µl) was added to the solution, carefully mixed, poured into agarose gel casting system (Bio-Rad, SUB-CELL®GT) and a desired comb was inserted.
- The comb was gently removed, and then the gel was placed in an electrophoresis chamber and covered (just until wells are submerged) with electrophoresis buffer (the same buffer that used to prepare the agarose gel).
- At this step, the PCR product was loaded (5 µl) directly into the gel well. 3µl from 50bp marker (DNA ladder) was used.
- The cathode was connected to the well side of the unit and the anode to the other side.

- Finally, electrophoresis was done at 120 volts, for 90 minutes (using Bio-Rad power supply, POWER PAC 3000) or until DNA ladder have migrated an appropriate distance, the movement depending on the size of the DNA to be visualized.

II. Gel documentation

In this step, agarose gel has been placed above the UV transilluminator device and then exposed to UV light; the photos were taken using digital camera of the gel documentation system (Pharmacia Biotech).

2.5.3.4. Restriction Fragment Length Polymorphism (RFLP) and genotyping

The A allele (rs9939609) of *FTO* gene was detected by RFLP, the amplified product was digested using *ScaI* restriction enzyme as previously described (Adeela Shahid et al., 2013).

Briefly, 0.5 µl of *ScaI* restriction enzyme and 1.5µl restriction buffer were added to the amplified product for a total volume of 20 µl. The digestion reactions were incubated in water bath at 37°C for 1 h. The digested products were visualized on 2.5% gel as described above (section 2.5.3.3 I and II). Approximately, 5% of the samples was tested again to confirm the genotyping accuracy using the same method.

2.5.4. Sequence analysis

To validate the genotyping results, 5% of samples were randomly selected but representing the three genotypes and sent for sequencing. The PCR product was purified and sequenced in both directions by HyLab sequencing service (Rehovot, Israel). The obtained sequences were assembled using Bioedit software, used in a BLAST search (ncbi.nlm.nih.gov/blast) and aligned with each other.

Chapter Three

3. Results

3.1. Study Subjects

A total of 399 participants were recruited in this study (281 with T2DM and 118 non-diabetic individuals). The male to female ratio was 94:187 in diabetic group compared to 48:70 in control as shown in table (3.5). There was no significant difference in gender of T2DM patients compared to those of non-diabetic control group (P=0.164), while a significant difference in the mean age among the two groups was observed (P = 0.0001).

Table 3.5: Number of study subjects.

Variable	Type 2 diabetic Patients n=281(%)	Non-diabetic Controls n=118(%)	*P*-value
Gender			
Male	94 (33.5%)	48 (40.7%)	**0.164**
Female	187 (66.6%)	70 (59.3%)	
Total	281 (100%)	118 (100%)	
Age (means ±SD)	58.15±12.09	49.62±8.71	**0.0001**
Age at diagnosis (means ±SD)	50.39±11.05	N.A	

P<0.05 is considered to be significant, N.A; Not Available

3.2. Clinical and biochemical characteristics of the study subjects

The clinical and biochemical characteristics were compared between type 2 diabetic and non-diabetic groups. The results indicate that there are significant differences in all clinical and biochemical parameters except of TC (P = 0.251) among the two groups as shown in table (3.6).

Table 3.6: comparison of the Clinical and biochemical characteristics in diabetic and non-diabetic groups.

Clinical characteristics	Type 2diabetic patients n=281	Non-diabetic controls n=118	*P*-value
SBP (mmHg)	136.21(±17.42)	122.33(±11.44)	**0.0001**
DBP (mmHg)	80.21(±10.83)	76.72(±9.37)	**0.0024**
FBS (mg/dl)	163.31(±58.72)	87.14(±7.82)	**0.0001**
HbA1c (%)	8.03(±2.01)	N.A	
TG (mg/dl)	181.25(±123.23)	N.A	
TC (mmol/l)	187.91(±47.53)	182.15(±40.95)	**0.251**
HDL (mg/dl)	42.09(±10.66)	N.A	
LDL (mg/dl)	108.86(±26.71)	N.A	

Values are expressed as means ±SD, $P<0.05$ is considered to be significant, N.A; Not Available

3.3. Body mass index

Body mass index (BMI) values were calculated in the group of T2DM patients and non-diabetic control group according to the following equation:

$$\text{BMI} = (\text{weight in kg}) / (\text{height in meters})^{2}$$

Participants were classified according to World Health Organization (WHO) classification of obesity (BMI Classification, 2012). The results indicate that there are significant differences in the mean BMI among T2DM patients and non-diabetic control group (32.9±6.6 vs. 29.2 ±6.2; $P<0.0001$) respectively). Furthermore, the frequency of obesity (BMI of 30 or greater) was significantly higher among T2DM patients than non-diabetic controls (64.4% vs. 44.1%; $P=0.0002$) as shown in table (3.7).

Table 3.7: BMI distribution of the study subjects.

	Type 2 diabetic patients n (%)	**Non-diabetic control n (%)**	***P*-value**
BMI(kg/m^2) (means ±SD)	32.9 ± 6.6	29.2 ± 6.2	**<0.0001**
Normal (18.5-24.9 kg/m^2)	15 (5.3%)	39 (33.1%)	**<0.0001**
Over weight (25-29.9 kg/m^2)	85 (30.2%)	27 (22.9%)	0.23
Obese (≥ 30 kg/m^2)	181 (64.4%)	52 (44.1%)	**0.0002**
Total	281 (100%)	118 (100%)	

Values are expressed as frequencies (%), $P<0.05$ is considered to be significant.

3.4. Microvascular and Macrovascular complications in type 2 diabetic patients

Our results showed that 41% (114/281) of type 2 diabetic patients suffer from at least one of the following complications; retinopathy, nephropathy, diabetic foot and CVD. Our study revealed that the most prevalent complication was CVD (14.6%) followed by nephropathy (12.1%), diabetic foot (7.8%) and retinopathy (6%) as shown in table (3.8).

Table 3.8: prevalence of microvascular and macrovascular complications among type 2 diabetic patients.

complications	**No. of type 2 diabetic patients (%)**

Microvascular complications	
Retinopathy	17(6%)
Nephropathy	34(12.1%)
Diabetic Foot	22(7.8%)
Macrovascular complication	
CVD	41(14.6%)
Total	114

Our results revealed that (74.7%) of diabetic patients have first degree family history of diabetes. T2DM complications were compared among patients with and without family history of diabetes as shown in table (3.9). Our results showed that the prevalence of retinopathy was significantly higher among patients without family history of diabetes compared to those with family history of diabetes (*P*= 0.001). However, no significant difference was seen between and T2D complications (*P* >0.05).

Table 3.9: Frequencies of diabetic complications among type 2 diabetic patients with and without first degree family history of diabetes.

Complications	**Type 2 Diabetic Patients**		***P*-value**
	Family History n=210	**No Family History n=71**	
Retinopathy	7(3.3%)	10(14.08%)	**0.001**
Nephropathy	27(12.85%)	7(9.85%)	**0.503**
Diabetic Foot	15(7.14%)	7(9.85%)	**0.461**
CVD	31(14.76%)	10(14.08%)	**0.889**

Values are expressed as frequencies (%), *P*<0.05 is considered to be significant.

3.5. Anti-diabetic treatment at the time of sampling

The current study revealed that 6 (2.13%) of T2DM patients were treated with insulin, 204 (76.59%) were treated with oral hypoglycemic agents while 71 (25.26%) received a combination of insulin and oral hypoglycemic agent. The results were shown in Figure (3.7).

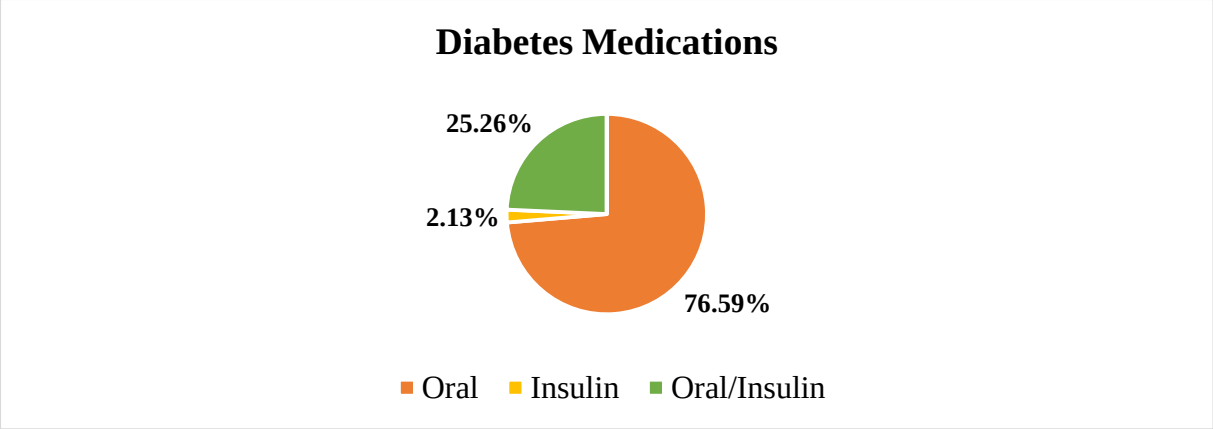

Figure 3.7: Percentages of type 2 diabetes route of treatment.

3.6. Genotyping

3.6.1. Measurement of DNA concentration and purity

DNA quantity and purity was estimated by measuring the concentration in ng/ul and A260/A280 ratio. The concentration was ranged from 50-200 ng/ul. The results of representative samples are shown in the table (3.10).

260/230 Ratios

Few contaminants have characteristic profiles, e.g. phenol, however many contaminants have similar characteristics. Absorbance at 230 nm or less. Abnormality 260/230 values may indicate there was a problem in the sample or in the extraction protocol, therefore it is important to consider both

260/280 Ratios

The abnormal 260/280 ratios frequently indicate that the sample was either contaminated by protein or with a reagent such as phenol or that there was an issue with the measurement.

Table 3.10: Results of DNA concentration and purity

DNA sample	ng/ul	A260	A280	260/280	260/230
1	105.49	2.11	1.17	1.8	1.34
2	117.4	2.349	1.225	1.92	1.52
3	70.77	1.415	0.759	1.87	1.39

3.6.2. DNA amplification

The amplification product of *FTO* gene polymorphism (rs9939609) was evident to have a size of 187 bp amplicon as described by Adeela Shahid et al., 2013. Sizes of amplicons were confirmed by agarose gel electrophoresis as shown in figure (3.8).

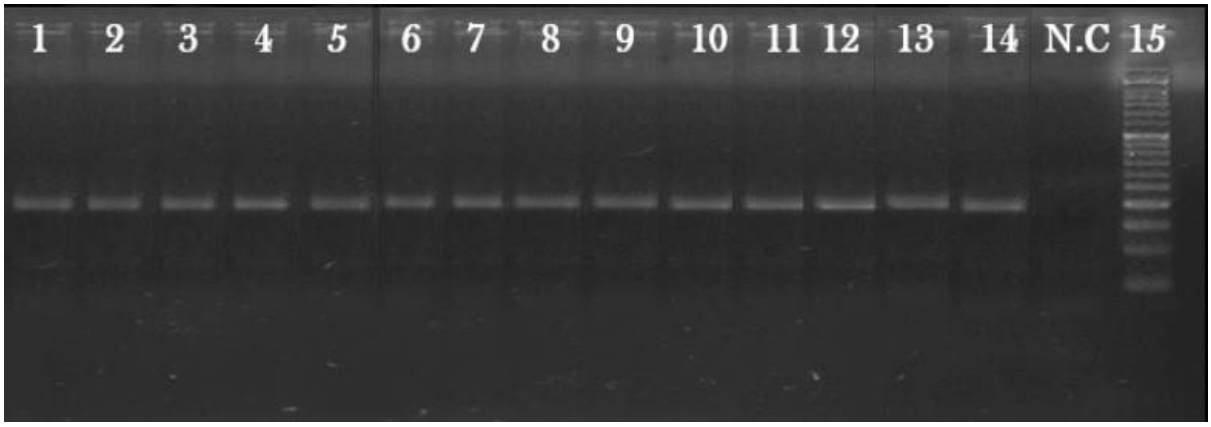

Figure 3.8: PCR product of *FTO* gene polymorphism (rs9939609) analyzed by agarose gel electrophoresis. DNA was extracted from blood; amplification was carried out for the *FTO* gene (rs9939609) using specific primers and template DNA. The PCR product was electrophoresed on 2.5% agarose (120V and 90 min.) and directly visualized with ethidium bromide under UV light.
Line 15: DNA markers (Ladder 50 bp) N.C: Negative control Line 1-14: PCR product, ampilcon size 187bp

3.6.3. RFLP analysis

To determine the genotype of the studied population, the PCR product of *FTO* gene polymorphism (rs9939609) was digested by *ScaI* restriction enzyme and visualized by agarose gel electrophoresis as described previously (Adeela Shahid et al., 2013). A band of (187 bp) was observed on 2.5% agarose gel for the wild type genotype (TT), two bands of (154, 33 bp) was observed for the homozygous recessive genotype (AA) while three bands of (187, 154, 33 bp) were observed for the heterozygous genotype (TA) and as shown in table (3.11) and figure (3.9).

Table 3.11: Number and size of bands that observed on 2.5% agarose gel for each genotype.

Genotype		**No. of bands**	**Size of bands (bp)**
Wild type	TT	1	187
Heterozygous	TA	3	187, 154, 33

Homozygous	AA	2	154, 33

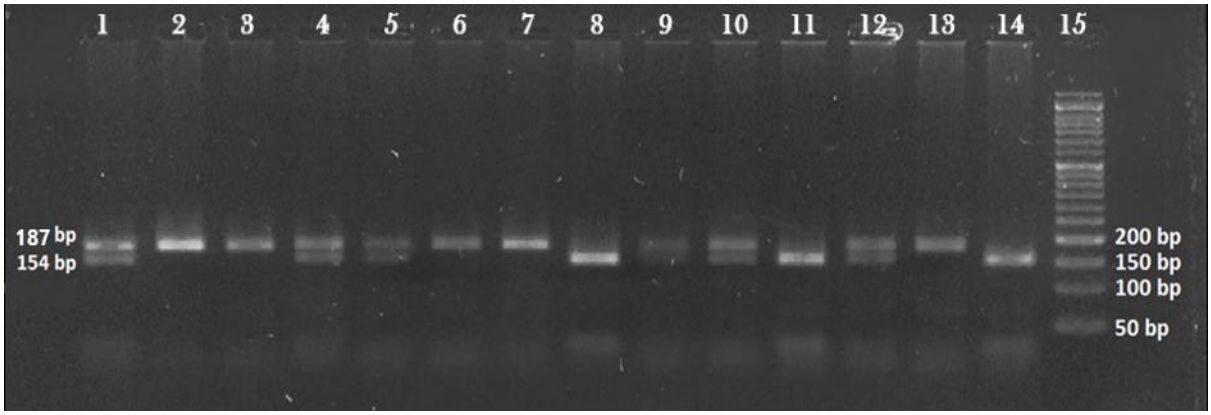

Figure 3.9: Results of *FTO* gene polymorphism (rs9939609) product on agarose gel electrophoresis. PCR product of *FTO* gene (rs9939609) was digested with *ScaI* restriction enzyme. The products of digestion were electrophoresed on 2.5% agarose (120V and 90 min) and directly visualized with ethidium bromide under UV light. Line 15: DNA Marker (Ladder 50 bp)
Line: 2, 3, 6, 7 and 13: TT genotype 187 bp
Line: 1, 4, 5, 9, 10 and 12: TA genotype 187, 154, 33 bp.
Line: 8, 11, and 14: AA genotype 154, 33 bp

3.6.4. Sequencing of *FTO* gene polymorphism (rs9939609)

The PCR product was purified and sequenced in both directions (forward /reverse). Examples on the sequencing results for the three different genotype are illustrated in figure (3.10 A, B and C).

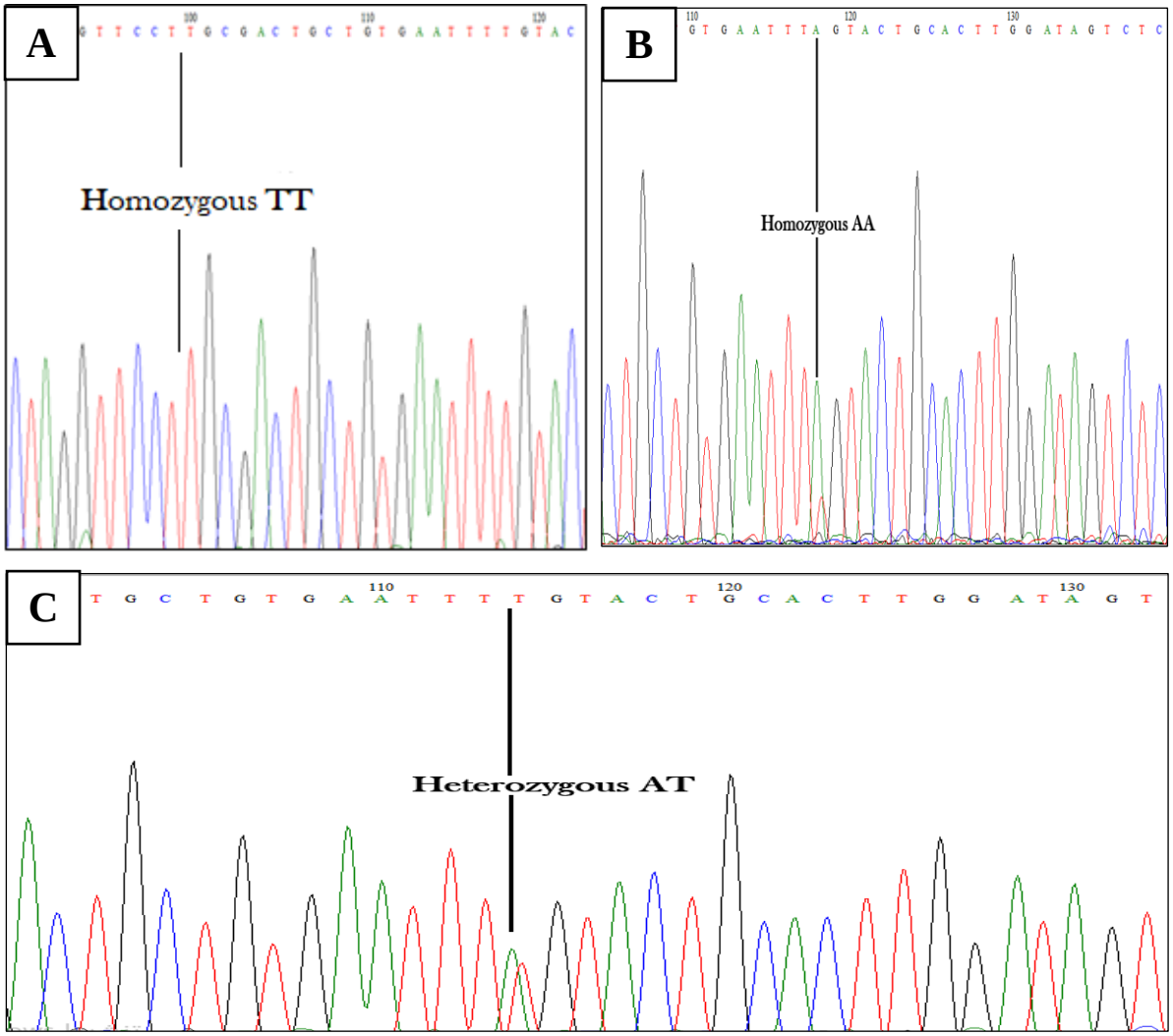

Figure 3.10: DNA sequencing results of *FTO* (rs9939609) variant in three different genotype. A) TT genotype of rs9939609 SNP T>A. B) AA genotype of rs9939609 SNP T>A. C) AT genotype of rs9939609 SNP T>A.

3.6.5. Genotype and allele frequencies

The genotype and allele frequencies of *FTO* gene polymorphism (rs9939609) in type 2 diabetic patients and non-diabetic control were analyzed and shown in table (3.12). The results indicated the frequency of homozygous genotype (AA) allele was significantly higher in T2DM patients when compared with those of non-diabetic control (36% vs. 16%). The minor allele frequency (A allele) was 58.8% in T2DM patients and 38.9% in non-diabetic controls. The genotyping distribution was in Hardy Weinberg equilibrium in both the control (P=0.679) and diabetic groups (P=0.384).

Table 3.12: genotypic and allelic frequency of *FTO* gene polymorphism (rs9939609) in type 2 diabetic patients and non-diabetic control.

Genotype	Type 2 diabetic patients n=281	Non-diabetic control n=118
TT	51	45
TA	129	54
AA	101	19
MAF (%)	**58.8%**	**38.9%**

3.6.6. Association of *FTO* gene polymorphism (rs9939609) with response to type 2 diabetes mellitus

The association of *FTO* genotyping with type 2 diabetic was examined by logistic regression analysis with the use of co-dominant and recessive models as shown in table (3.13). Strong association was found between the minor A allele (rs9939609) of the *FTO* and increased T2DM risk with an allelic odd ratio (OR) of 2.11 (95% CI [1.26-3.52], unadjusted *P* <0.0001); this association was partly attenuated after adjustment by age, gender and BMI with an allelic odd ratio (OR) of 1.84 (95% CI [1.04-3.05], *P*=0.034 as shown in table (3.13).

Table 3.13: Association of *FTO* gene polymorphism (rs9939609) with type 2 diabetes mellitus.

Genotype	OR (95% CI)	*P*-value	OR (95% CI)	**P*-value
AA vs TT	4.69 (2.49–8.83)	**<0.0001**	4.03 (2.01–8.06)	**<0.0001**
TA vs TT	2.11 (1.26-3.52)	**<0.0001**	1.84 (1.04-3.05)	**0.034**
AA vs (TA+TT)	2.78 (1.72-4.49)	**<0.0001**	2.71 (1.5-4.9)	**0.001**

P<0.05 is considered to be significant, **P*- value adjusted by age, gender and BMI.

3.6.7. Clinical characteristics of study subjects according to *FTO* gene polymorphism (rs9939609) genotype

The clinical and biochemical parameters (BMI, SBP, DBP, FBS, and TC) of study subject's relative to the *FTO* gene polymorphism (rs9939609) were analyzed by using ANOVA test as shown in table (3.14). Our results demonstrated a weak but not significant association between *FTO* (rs9939609) genotype and BMI in the group of type 2 diabetic patients (*P*=0.057), while no association was found in the group of non-diabetic control (*P*=0.688). Moreover, no significant association was seen in the other investigated parameters (SBP, DBP, FBS, and TC) across *FTO* (rs9939609) genotype (*P* >0.05) as seen in table (3.14). In addition, no association was observed between the levels of glycated hemoglobin (HBA1C) and *FTO* genotypes: AA (7.845 ± 1.88), TA (8.02 ± 2.03) and TT (8.43 ± 2.17).

Table 3.14: clinical characteristics of study subjects according to the *FTO* (rs9939609) genotype.

	Type 2 diabetic patients n=281				**Non-diabetic controls n=118**			
	TT (n=51)	**TA (n=129)**	**AA (n=101)**	***P*-value**	**TT (n=45)**	**TA (n=54)**	**AA (n=19)**	***P*-value**
Age	57.8±12.3	58.4±11.9	58.1±12.3	0.952	48.6±8.9	50.9±8.6	48.3±8.2	0.329
BMI (kg/m^2)	31.8±6.5	32.3±6.1	34.1±7.1	**0.057**	28.75±5.8	29.77±6.1	28.89±6.96	0.688
SBP (mmHg)	135.4±20.4	138.2±16.4	133.9±16.9	0.166	121.4±9.5	124.2±10.7	119.3±16.3	0.222
DBP (mmHg)	81.3±10.5	80.9±10.1	78.6±11.9	0.206	78.5±8.1	76.7±10.2	72.8±9.2	0.087
FBS (mg/dl)	169.9±65.6	163.9±58.9	159.1±54.3	0.553	86.9±8.8	87.7±7.4	85.9±6.9	0.693
TC (mg/dl)	183.7±39.8	188.5±49.2	189.1±49.3	0.783	180.4±28.2	188.5±48.8	168.1±40.1	0.165

P<0.05 is considered to be significant.

3.6.8. Association of *FTO* (rs9939609) gene polymorphism with macrovascular and microvascular complications in T2DM patients

The association of the *FTO* (rs9939609) genotype and T2DM complications (Retinopathy, Nephropathy, Diabetic Foot and CVD) of study participants were studied. Our results demonstrated insignificant association (*P*>0.05) of various complications with *FTO* (rs9939609) genotype as shown in table (3.15).

Table 3.15: Type 2 diabetes complications according to *FTO* (rs9939609) genotype.

Complications	**No. of T2DM complications across FTO genotypes**			***P*-value**
	TT n (%)	**TA n (%)**	**AA n (%)**	
Microvascular complications				
Retinopathy	5 (9.8%)	6 (4.6%)	6 (5.9%)	0.425
Nephropathy	6 (11.7%)	17 (13.2%)	11(10.9%)	0.867
Diabetic Foot	3 (5.8%)	14 (10.8%)	5 (4.9%)	0.216
Macrovascular complication				
CVD	5 (9.8%)	18 (13.9%)	18 (17.8%)	0.401
Total	19 (37.1%)	55 (42.5)	40 (39.5%)	

P<0.05 is considered to be significant

Chapter Four

4.1. Discussion

Type 2 diabetes mellitus is a common metabolic disorder characterized by hyperglycemia with disturbances in fat, carbohydrate and protein metabolism. The two major pathophysiological defects in T2DM are impaired insulin secretion, insulin action, or both. These metabolic abnormalities lead to long term damage of different organs, causing their dysfunction and failure. Type 2 diabetes related microvascular complications are responsible for the majority of new cases of kidney failure, blindness and diabetic foot amputation. Furthermore, macrovascular complications such as CVD and stroke related deaths are 2-4 times more frequent in adults with diabetes than in the general population.

The number of confirmed loci involved in the development of T2DM has increased from 3 in 2006 to over 70 to date. Taking into consideration the impact of genetics and environmental factors such as diet and physical activity on developing this disease (Maggie et al., 2014). The differences in susceptibility of different ethnic groups may be explained. Most of these variants have modest effects on disease risk (with an odds ratio ~ 1.2) and their role in the development of T2DM is still poorly understood. However, the association of *FTO* variants with T2DM in the MENA (Middle East and North Africa) region is not well studied. In the present case-control study, we investigated the potential association of the minor A allele at rs9939609 of *FTO* gene with T2DM and its related complications among Palestinian population.

Microvascular (retinopathy, nephropathy and diabetic foot), and macrovascular (CVD) complications are common among people with T2DM and have profound effects on patient's morbidity and mortality. In this study, we noted that 74.7% of T2DM patients had first relative family history to diabetes and 41% developed T2DM complications. The prevalence of retinopathy was significantly higher among patients without family history to diabetes this could be attributed to several factors -other than family history- that widely recognized as major risk factors for the development of diabetic retinopathy including severity of hyperglycemia, present of hypertension and diabetes duration (Antonetti et al., 2012). CVD was the most prevalent (14.6%) compared to other complications which is in line of several studies reported that diabetes is recognized to be an independent risk factor for CVD (Palem,

2017; M. K. Poulsen et al., 2010) As expected, the levels of biochemical parameters that refer to T2DM risk factors were significantly higher in participants with T2DM compared to the healthy control group confirming that T2DM is associated with hypertension, hyperglycemia and other abnormal metabolism dysfunctions.

The association between obesity and T2DM has long been established and recognized across all ethnic groups. In this investigation, we found that 86.4 % of all participants were overweight and obese compared. These results reflect the increase of obesity in the Palestinian population as a result of lifestyle turns toward physical inactivity, urbanization and increased fast food consumption. Thus, there is a need to educate people with increased risk of metabolic diseases that associated with obesity and its related traits. In morbid obesity, the expression rate of various insulin signaling molecules is reduced and several mechanisms such as docking translocation and fusion of Glucose transporter-4 (GLUT-4) containing vesicles with the plasma membrane are impaired leading to insulin resistance, a major risk factor for T2DM (Goodyear et al., 1995; Kahn et al., 1992). Insulin resistance is a risk factor of T2DM and many other metabolic traits. However, it has been shown that not all individuals with insulin resistance should develop T2DM, only about 20% of obese individuals develop the disease while most are able to maintain blood sugar level (Lan et al., 2003). Aging has also been identified as an important risk factor for many metabolic disorders including T2DM (Kautzky-Willer et al., 2016). In the current study, we observed a significant difference in age between T2DM patients (58.15±12.09) and control groups (49.62±8.71), P <0.0001, indicating that age was one of the factors contributing to the high prevalence of T2DM noted in our population.

In this study, we evaluated the effect of *FTO* (rs9939609) variant on predisposition of T2DM in the Palestinian population. Statistically significant association was found between the minor allele A of the *FTO* gene and increased risk of T2DM with an allelic OR of 1.84 after adjustment for age, gender and BMI. The association with *FTO* rs9939609 variants with T2DM was first described by Frayling et al, 2007. Afterwards, similar findings were reported in different populations including Europeans (Freathy et al., 2008) and South Asian Indians (Yajnik et al., 2009).

In this study, the minor allele frequency (MAF) was 58.8% in diabetic group which is higher than A allele frequency in South Asian population (35%) (Rees et al., 2011), Chinese (12%)(Li et al., 2008), Japanese (18%) (Omori et al., 2007) , Europeans (45%) (Freathy et al., 2008), South Asian Indians (30%) (Yajnik et al., 2009) and Egyptian (39%) (Khella, Hamdy, Amin, & El-Mesallamy, 2017) indicating that genetic makeup may play a critical role in leading of T2DM in Palestinian population.

On the other hand, several studies have demonstrated that the association of *FTO* polymorphisms with T2DM is mediated by obesity (Cauchi et al., 2008; Thorleifsson et al., 2009). However, our results showed no association with *FTO* (rs9939609) gene and BMI in diabetic patients and control group (*P*= 0.057 and P=0.688, respectively) indicating that *FTO*-T2DM association was not mediated by BMI and the high prevalence of obesity in our studied population may be attributed to other *FTO* variants / other genes and environmental factors. In addition, our results were inconsistent with a study by Chang et al. showed that the minor A allele at rs9939609 of *FTO* gene was strongly associated with obesity and BMI in the Chinese population. Also, a study conducted by (Dina et al., 2007) identified SNPs in *FTO* gene that which were strongly associated with early-onset obesity among both children and adults in European population. In contrast, another study found a borderline evidence that *FTO* genotypes are associated with BMI in Asians population (Ng et al., 2008).). Furthermore, several studies showed no association of *FTO* variants and BMI (Jacobsson et al., 2009). The inconsistency of the reported studies revealed the differences in the genetic background, diets and lifestyle. Several studies reported about the effect of physical activity and diet on *FTO* variants, some of these studies have been clarified that its obesity-increasing effect may be suppressed in individuals who are physically active (Ahmad et al., 2011). While, other studies have not replicated this interaction, leaving it unresolved whether physical activity can reduce *FTO* impact on the risk of obesity (Jonsson et al., 2009).

Moreover, we investigated the association of *FTO* variant with T2DM related complications, no significant difference between T2DM complications across *FTO* genotypes, *P*>0.05 was observed. These results are in contrast to several studies which reported an association of *FTO* variants with T2DM complications. A study conducted by Hubacek et al., 2012 described the association of variants in this gene with end-stage renal disease (Hubacek et al., 2012). Another study conducted by (Doney et al., 2009) described that AA genotype of the *FTO* rs9939609 variant was associated with increased CVD risk in men with either impaired

glucose tolerance or type 2 diabetes and the association was not explained by the conventional CVD risk factors.

4.2. Conclusions

This study is the first in Palestine that investigated the effect of *FTO* (rs9939609) variant on T2DM and BMI. Our results showed strong association between *FTO* (rs9939609) variant and T2DM but not BMI. Moreover, no significant association was observed between this variant and age, gender, T2DM complications or other biochemical parameters.

4.3. Recommendations

1. In diabetic clinics in UNRWA, BMI was used only for reference obesity. Other important measures such as WC, WHR should be added which can be of great importance for future studies.
2. Analysis of more SNPs of *FTO* genes to determine which one is more common in our population.
3. Evaluation of gene expression to determine the effect of SNPs on the various phenotypic parameters.
4. Further large-scale study is required to elucidate the role of other *FTO* variants/other gene and environmental factors on the high prevalence of obesity in Palestinian population.

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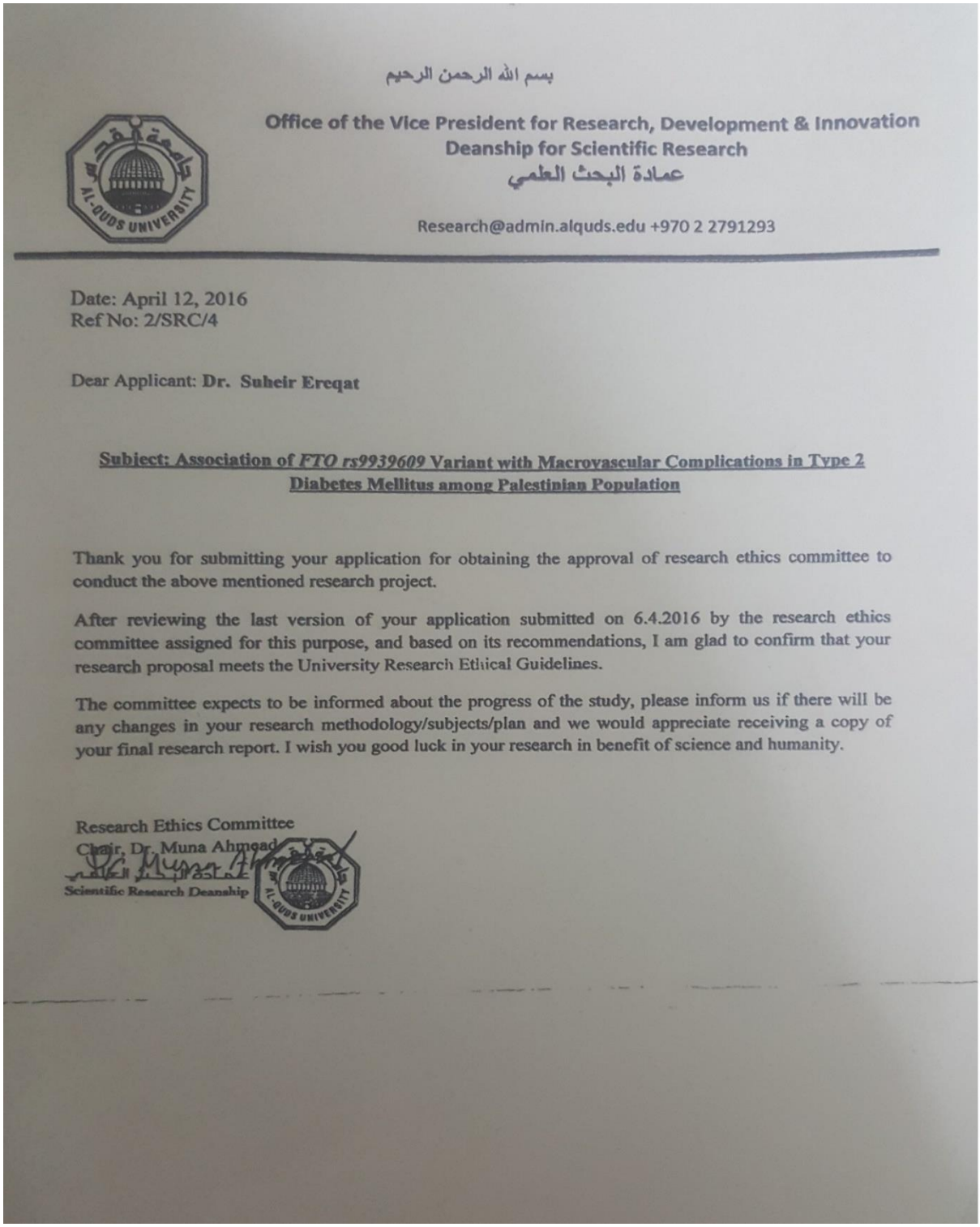
Appendix 1

Research Ethics Committee



بسم الله الرحمن الرحيم

Office of the Vice President for Research, Development & Innovation
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Date: April 12, 2016
Ref No: 2/SRC/4

Dear Applicant: **Dr. Suheir Ereqat**

Subject: Association of *FTO rs9939609* Variant with Macrovascular Complications in Type 2 Diabetes Mellitus among Palestinian Population

Thank you for submitting your application for obtaining the approval of research ethics committee to conduct the above mentioned research project.

After reviewing the last version of your application submitted on 6.4.2016 by the research ethics committee assigned for this purpose, and based on its recommendations, I am glad to confirm that your research proposal meets the University Research Ethical Guidelines.

The committee expects to be informed about the progress of the study, please inform us if there will be any changes in your research methodology/subjects/plan and we would appreciate receiving a copy of your final research report. I wish you good luck in your research in benefit of science and humanity.

Research Ethics Committee
Chair, Dr. Muna Ahmead
Scientific Research Deanship

Appendix 2

موافقة للإشتراك في البحث العلمي

اسم الباحث: أنس رزق صبارنه.

عنوان البحث:

"Association of *FTO rs9939609* Variant with Macrovascular Complications in Type 2 Diabetes Mellitus among Palestinian Population"

مكان إجراء البحث: جامعة القدس- ابو ديس

أنت مدعو(ة) للمشاركة ببحث علمي سريري سيجرى جامعة القدس. الرجاء أن تأخذ(ي) الوقت الكافي لقراءة المعلومات التالية بتأن قبل أن تقرر(ي) إذا كنت تريد(ين) المشاركة أم لا. بإمكانك طلب إيضاحات أو معلومات إضافية عن أي شيء مذكور في هذه الاستمارة أو عن هذه الدراسة.

أ. سيتناول هذا البحث الخصائص الجينية والوراثية لمرض السكري من النوع الثاني، والمخاطر الناجمة عن هذا المرض كأمراض القلب والاوعية الدموية بحيث سيتم البحث بالمسببات الوراثية والتشوهات الجينية التي يمكن ان تكون عاملا رئيسيا في التسبب بمثل هذه المخاطر والامراض.

ب. مع استمارة الموافقة هذه، يطلب منك تقديم عينة دم دون أي من المخاطر أو الآثار الجانبية التي يمكن حدوثها من جراء مشاركتك/ي في هذه الدراسة. كما تتضمن الدراسة الاطلاع على سجلاتك الطبية تاريخك الطبي.

ت. اخي المواطن اشتراكك في هذا البحث قد يسهم في عملية التشخيص المبكر لأمراض القلب والاوعية الدموية وبالتالي سيساعد في الوقاية والعلاج وكذلك ليقي ابناءكم واقربائكم بالمستقبل من هذا المرض.

ان مشاركتك في هذه الدراسة مسألة تطوعية تماما، وفي حال وافقت على المشاركة في هذه الدراسة، سيبقى اسمك طي الكتمان وبسرية تامة. لن يكون لأي شخص، ما لم ينص القانون على ذلك، حق الاطلاع على ملفك الطبي باستثناء الطبيب المسؤول عن الدراسة ومعاونيه، ولجان الأخلاق المهنية المستقلة، ومفتشين من الإدارات الحكومية المنظمة.

<u>موافقة المشترك</u>:

لقد قرأت استمارة القبول هذه وفهمت مضمونها. تمت الإجابة على أسئلتي جميعها. وبناء عليه فأنني، حرا مختارا، أجيز إجراء هذا البحث وأوافق على الإشتراك فيه، وإني أعلم ان الباحث أنس رزق صبارنه وزملاءه سيكونون مستعدين للإجابة على أسئلتي.

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اسم المشترك توقيع المشترك

التاريخ----------

Appendix 3

Questionnaire

Al-Quds University		**جامعة القدس**
Faculty of Medicine		**كلية الطب**
Abu-Dies, Jerusalem		أبوديس – القدس

Case ☐ **Control** ☐ **Q. No.:** ☐☐☐☐

Name----------

Age---------- Gender----------

Religion ---------- Geographic area ----------

Weight (kg)---------- BMI---------- WHR----------

Length (cm)----------

Physician's name: ---------- Address---------- Tel. ----------

Have you ever suffered from?

Hypertension ☐ Diabetes Mellitus (T2D) ☐ Cardiovascular disease ☐

Sys/Dias blood pressure (SBP/DBP) ----------

Family history for D.M/ 1st relative degree:

☐ ☐

Yes No

For diabetic patients only:

Age of onset/at diagnosis ---------- (yrs.)

Weight at diagnosis ---------- (kg)

Complications:

Coronary heart disease ☐ Nephropathy ☐ Retinopathy ☐

Medication:

Anti-diabetic agents ☐ Anti-hypertensive ☐ Lipid lowering agents ☐

Biochemical Tests:

FBS	**mg/dl**
HbA1C	**%**
Total cholesterol	**mg/dl**
Triglycerides	**mg/dl**
HDL-c	**mg/dl**
LDL-c	**mg/dl**
VLDL-c	
LDL/HDL	

العلاقة بين المتغير الجيني (rs9939609) *FTO* مع مضاعفات الاوعية الدموية Macrovascular Complications في النوع الثاني من مرض السكري Type 2 diabetes mellitus بين السكان الفلسطينيين

إعداد: أنس رزق حسن صبارنه

إشراف: د. سهير عريقات

الملخص

مرض السكري من النوع الثاني (T2DM) هو مجموعة من الأمراض الايضية التي تتميز بارتفاع سكر الدم الناجم عن خلل في إفراز الأنسولين، أو عمل الأنسولين، أو كلاهما. أظهرت الأبحاث العلمية في السنوات الاخيرة أن هناك علاقة بين الوراثة وهذا النوع من السكري أيضا المضاعفات الناجمة عن المرض والتي تشمل كل من اعتلال الشبكية (Retinopathy)، اعتلال الكلى (Nephropathy)، أضرار القدم (Diabetic foot) وايضا امراض القلب والأوعية الدموية. تم تحليل العديد من المتغيرات (Variants) في جين ال (*FTO*) حيث وجد انها مرتبطة ارتباطا قوية مع هذا النوع من السكري والسمنة (Obesity) التي تعتبر عاملا من العوامل التي فرص الاصابة بهذا المرض في مختلف السكان. لذلك صممت هذه الدراسة لتشخيص العلاقة بين التباين الجيني (rs9939609) *FTO* مع مرض السكري من النوع الثاني (T2DM)، والمضاعفات الناجمة عن هذا المرض.

اجريت هذه الدراسة خلال الفترة الزمنية الممتدة من 2016-2017 م على مجموعتين الأولى تشمل مرض السكري من النوع الثاني (T2DM) وعددهم 281 مريضا (181 مريضا كانوا يعانون من السمنة المفرطة و15 لا يعانون من السمنة المفرطة)، أما المجموعة الثانية شملت على اشخاص سليمين ولا يعانوا من أي مشاكل صحية وعددهم 118 (52 شخصا كانوا يعانون من السمنة المفرطة و39 شخصا لا يعانون من السمنة). جميع المشاركين في هذه الدراسة من كلا المجموعتين لا تجمعهم صلة القرابة (unrelated)، وتتراوح أعمارهم فوق سن الأربعين عاما. تم جمع البيانات المتعلقة بالمشاركين في هذه الدراسة من خلال استبيان منظم اشتمل على كافة البيانات السريرة والكيميائية الحيوية. تم تحديد المتغير الجيني ل (*FTO*) جين من خلال PCR متبوعا بتقنية (PCR-RFLP). تم استخدام التحاليل الإحصائية الوصفية لتقيم المعطيات والتي شملت كل من (Pearson's Chi-square test) للمقارنة بين مجموعتي الدراسة، ايضا استخدام (R statistics (v2.8.0). software).

أظهرت نتائجنا التي توصلنا اليها الى وجود ارتباط قوي للأيل (A) للمتغير الجيني (rs9939609) *FTO* مع زيادة خطر الاصابة بمرض السكري من النوع الثاني (T2DM) (95%CI [1.04-3.05], OR=1.84، *P*=0.034) بعد التعديل بالنسبة للعمر والجنس ومؤشر كتلة الجسم (BMI) . أيضا لوحظ ان هناك ارتباطا ضعيفا للأيل (A) للمتغير الجيني (rs9939609) *FTO* مع مؤشر كتلة الجسم (BMI) لدى مجموعة المصابين بمرض السكر من النوع الثاني (*P* = 0.057)، في حين لم يتم العثور على هذا الارتباط في المجموعة الثانية (*P* = 0.688). أيضا لم يلاحظ وجود أي

ارتباط بين المتغير الجيني (rs9939609) *FTO* وكل من العمر والجنس والمضاعفات الناجمة عن هذا المرض ($P>0.05$).

في الختام، أظهرت نتائجنا ان هناك ارتباطا قويا بين المتغير الجيني (rs9939609) *FTO* وزيادة خطر الاصابة بمرض السكري من النوع الثاني (T2DM). مع ذلك، هناك حاجة قوية الى مزيد من الدراسات واسعة النطاق لتوضيح دور هذا المتغير (rs9939609) *FTO* ايضا في زيادة مؤشر كتلة الجسم (BMI) لدى السكان الفلسطينيين.