Association of *PPARG* and *FTO* polymorphisms with type 2 diabetes in Kurdistan Region

A Thesis

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DECLARATION

I declare that the Master of Science (M.Sc.) entitled: "**Association of PPARG and FTO polymorphisms with type 2 diabetes in Kurdistan Region**" is my own original work, and hereby certify that unless stated, all work contained within this thesis is my own independent research and has not been submitted for the award of any other degree at any institution, except where due acknowledgment is made in the text.

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We certify that we have read this thesis: **Association of PPARG and FTO polymorphisms with type 2 diabetes in Kurdistan Region** and as an examining committee examined the student (Sally Khalil Baqer) in its content and what related to it. We approve that it meets the standards of a thesis for the degree of MSc. in Master of Science in Medical Laboratory Technology.

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Dedication

This hard work is dedicated to:

My mother, father, husband and son …

My always encouraging, faithful brothers and sisters…

Sally *Khalil* *Baqer*

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SUMMARY

Type 2 Diabetes (T2D) is the most common form of diabetes; it develops when pancreatic cells produce an excess amount of insulin, resulting in insulin resistance by peripheral cells. T2D is an illness that caused by interaction between genetics and environmental factors. Genetic factors are involved in the development of T2D and several genetic variants increase the risk to have T2D. The single nucleotide polymorphisms (SNPs) rs1801282 in the Peroxisome Proliferator-Activated Receptors gammau8h (PPARG) gene and rs9939609 in the Fat mass and obesity associated protein (*FTO*) gene were discovered to be related to raising the risk of T2D in different populations. This research aimed to discover if rs1801282 in the *PPARG* gene and rs9939609 in the *FTO* gene are responsible for increasing the risk of T2D in Kurdistan region. In the current study DNA from 200 unrelated individuals (100 T2D, and 100 non-diabetic control) were genotyped using Allele-specific PCR for both SNPs. The accuracy of the PCR methods used were validated by the Sanger sequencing method.

The association analysis for the rs1801282 (adjusted by sex, age, and BMI) showed significant differences between the cases and the control groups for rs1801282, individuals with genotypes (GG, and GC) had a higher risk of the disease (p-value = 0.0045, OR = 3.96, 95%CI: 1.31-11.94) than genotype (CC). the contrary, there was no significant differences (p value= 0.39) between the case and control groups for the rs9939609 variant in the *FTO* gene.

The findings in this research suggested that the variant rs1801282 in the PPARG gene was a genetic suspectable SNP in T2D in the Kurdish population, while SNP rs9939609 in the *FTO* gene was not associated with T2D. Further investigations with larger number samples are required to validate these findings.

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List of Abbreviations	
WHO	World Health Organization
T2D	Type 2 Diabetes
GWAS	Genome Wide Association Study
Pro	Proline
Ala	Alanine
FTO	Fat Mass and Obesity
HDL	Hight Density Lipo Protein
T1D	Type 1 Diabetes
SNP	Singla Nucleotide Polymorphism
LD	Linkage Disequilibrium
NR	Nuclear Receptor
RXRA	Retinoid X Receptor Alpha
FFA	Free Fatty Acid
BMI	Body Mass Index
SS-DNA	Singla Strand- DNA

SS-RNA	Singla Strand- RNA
FBS	Fasting Blood Sugar
PCR	Polymerase Chain Reaction
HWE	Hardy- Weinberg equilibrium

CHAPTER ONE

INTRODUCTION

INTRODUCTION

The World Health Organization (WHO) estimated that more than 405 million people around the world are affected by diabetes (Basu et al., 2019). The most prevalent form of diabetes is type 2 diabetes (T2D), it affects between 80% and 90% of all cases that are recorded. (Prasad and Groop, 2015, Basu et al., 2019). T2D indicates defect in insulin usage by the body, characterized by elevated blood sugar and insulin resistance (Alkhalidy et al., 2018). T2D is an illness caused by interaction between genetic, epigenetic and environmental factors. In addition, the family history is considered as a risk factor. Environmental factors include obesity, lifestyle, weight at birth, stress, smoking, and age (Adeghate et al., 2006, Singh, 2011). The strongest risk factor for T2D is increased fat cells (Eckel et al., 2011, Roglic, 2016). Obesity tends to be related to T2D, it is responsible of increasing death rates around the world, losing weight in individuals that have prediabetes (early stages of T2D) can conserve the disease (Sarma et al., 2021). A balanced diet, regular exercise, maintaining a normal body weight, and quitting smoking can aid in preventing diabetes or delaying it (Siddiqui et al., 2014).

Diabetes is a fast-growing epidemic disease. The prevalence rates of diabetes around the world indicate that it is higher in the Middle East, which includes the Kurdistan Region (latif Amin and Al-Zandi, 2019). In 2018 the worldwide estimation of affected individuals with diabetes was more than 400 million, and this number is expected to be increased to more than 510 million in 2030 (Cho et al., 2018). Diabetes cause more than 1 million deaths per year, which makes diabetes the ninth reason of death (Khan et al., 2020a).

Genetic factors play a significant role in the development of T2D, and raise the risk of developing the disease. Having one parent with T2D increases the risk of

having the disease by 40%, and this will increase to 70% if both parents have the disease (Ali, 2013). Genome Wide Association Studies (GWAS) have confirmed that T2D is a polygenic disease that is susceptible to variations in many genes such as those that are responsible for insulin secretion (*TCF7L2, CDKAL1, JAZF1, KCNQ1, HHEX/IDE*) (Dowaidar, 2021), and other genes that are responsible for insulin sensitivity (*PPARG* and *FTO*) GWAS suggested that 63 % of T2D patients could be under the control of genetic variations in the form of SNPs (Kato, 2013).

The Peroxisome Proliferator-Activated Receptors gamma (PPARs), also called (nuclear receptor subfamily 1, group C, member 3) NR1C3 is found in the adipose tissue, colon, and immune cells (macrophages) (Escher and Wahli, 2000). It regulates the transcription of various genes (Astarci and Banerjee, 2009). The storage of fatty acids and glucose metabolism are both regulated by the *PPARG* gene and increases sensitivity to insulin by promoting storage of fatty acid in adipose tissue and by releasing adiponectin from adipose tissue (Ahmadian et al., 2013, Sarhangi et al., 2020). The *PPARG* gamma located on chromosome 3 is an important gene due to its effect on the evolution of diseases like T2D, obesity, cardiovascular diseases and cancer (Heinäniemi et al., 2007). The SNP in the *PPARG* gene results in an exchange of proline to alanine at codon 12 (Wang et al., 2013). This exchange is called (Pro12Ala) rs1801282, this mutation was reported to be associated with insulin resistance and higher risk of T2D (Deeb et al., 1998).

Fat mass and obesity associated protein *(FTO)* is an enzyme whose activity is regulated by the *FTO* gene and located on chromosome 16. (Jia et al., 2011a). Food intake and the obesity have been linked to the expression of the FTO gene. (Fredriksson et al., 2008, Olszewski et al., 2009). GWAS studies have confirmed that the *FTO* gene variants are related to obesity and T2D (Frayling et al., 2007,

Thorleifsson et al., 2009, 2009). The intronic region in the *FTO* gene has the SNP rs9939609, which causes obesity and raises the risk of T2D. (Kim et al., 2016).

Aims of the study:

1- To study the genetic background of patients with T2D and understand the risk factors for this disease.
2- To identify the frequencies of rs1801282 SNP in *PPARG* gene and rs9939609 SNP in *FTO* gene in the Kurdistan population.
3- To discover if rs1801282 in *PPARG* gene and rs9939609 in *FTO* gene are associated with the risk of T2D in the Kurdistan population.

CHAPTER TWO

LITRETURE REVIEW

2. LITERATURE REVIEW AND THEORETICAL BACKGROUND

2.1 Diabetes

In Greek diabetes means fainting and mellitus is a Latin term that means sugar or honey (Alam et al., 2017). People with diabetes typically live 7 years less than the normal average person. The disease is an epidemic illness that rises with obesity rates (Seidell, 2000). Recent research summarized an epidemiological progression in diabetes around the world. The rise in diabetes worldwide is due to the lack of awareness in choosing a healthy lifestyle, and poor health care (Kaul et al., 2013).

Diabetes is a chronic disease marked by elevated blood glucose levels and irregularities in the metabolism of fat. Which means that either the pancreas is not secreting enough insulin or the cells are unable to utilize the produced insulin as it should. In turn, this lead to an increase of blood sugar levels (Roglic, 2016). Diabetes is composed of three main types, first; type 1 diabetes (T1D) also called insulin-dependent diabetes in which the pancreas does not yield insulin (Von Herrath et al., 2007). Hyperglycemia in T1D only develops after destroying 90% of beta cells. The second type is T2D, which is the most frequent type of diabetes. It is characterized by resistance of body cells to pancreatic insulin or an insufficiency of insulin (Thévenod, 2008). Finally, type 3 diabetes which influence pregnant women and it is called gestational diabetes and can lead to complications during pregnancy and at birth as well as an increased future risk of T2D in the mother and obesity in the infant (Chew et al., 2015). The kidneys, heart, nerves, eyes, and blood arteries are organs and tissues affected by diabetes. Furthermore, it can cause heart attacks

and strokes (Cade, 2008). In 2012, 1.5 million people lost their lives due to diabetes(Roglic, 2016).

2.2 Prevalence of Diabetes

The prevalence of diabetes is growing, which is also causing an increase in obesity rates. In 2000, an estimated 171 million cases of diabetes were reported worldwide (Ogurtsova et al., 2017). In Asia, Africa, Latin America, and Europe the prevalence of diabetes is rising by 111%, 93%, 82%, and 51% respectively. Millions of people worldwide have diabetes, and Asia is likely the region with the highest prevalence (Leasher et al., 2016). In 2013 ten nations with the largest populations of diabetes are forecasted the International Diabetes Federation Atlas (Zimmet et al., 2014). Four of these nations are in Asia (China, India, Indonesia, and Japan). Asia is at the epicenter of the world's diabetes epidemic as a result of its fast urbanization, and economic growth (Bishwajit et al., 2014).

2.3 Type 1 Diabetes

Type 1 diabetes is an autoimmune illness that occurs when the immune system harms the cells that create insulin (beta cells). Insulin is a hormone that helps to regulate sugar levels in the bloodstream and it is essential for cells to utilize blood sugar for energy. (Al-Ishaq et al., 2019). Symptoms of T1D includes high rates of urination, thirst, hunger, weight loss, and other significant issues are typical signs of excessive blood sugar (Kumar et al., 2020).

Type 1 diabetes has unknown causes, but it is thought to be related to genetics and environmental factors (Wang et al., 2017). Although T1D can be detected at any age, it is one of the most common chronic illnesses among kids. The disease occurs between the ages of 5 to 7 years (Eizirik et al., 2009). There are two subtypes of this disease; First type is less common and has unknown etiology and influences predominantly people of Asian or African nations. Patients with the second type have varying degrees of insulin deficiency (Atkinson, 2012).

2.4 Type 2 Diabetes

Type 2 diabetes (T2D) is considered a global disease and a health problem related to obesity, and it is also known as adult diabetes. T2D increases the risk of evolving other diseases such as (cardiovascular illness, kidney illness, and neuropathy) (Halpern et al., 2010). The correlation between age and resistance to insulin in T2D remains unclear (Reed et al., 2021).

This type of diabetes is characterized by high glucose levels in the bloodstream, resistance to insulin, and relative defects in insulin secretion(Mukhtar et al., 2020). Insulin resistance, known as cells inability to react to regular insulin

levels, occurs in the muscles, liver, and fat tissue (Muoio and Newgard, 2008). Insulin controls glucose release in the liver. In the case of resistance to insulin, the liver incorrectly release glucose into the bloodstream. Individuals differ in the proportion of insulin resistance (Ziolkowska et al., 2021). Other critical mechanisms related to T2D and insulin resistance include a high level of lipid breakdown inside adipocyte tissue, preservation of salt and water in the body, and incorrect regulation of body metabolism by the nervous system (Petersen and Shulman, 2018). New evidence also suggests that a region in the brain called the hypothalamus plays an important role in the development of insulin resistance (Schriever et al., 2020). Normally the autonomic nervous system receives inputs from hypothalamic cells that control blood sugar levels. In diabetic people, the autonomic nervous system regulates glucose abnormally (Lundqvist et al., 2021).

T2D is related to obesity, it can be avoided by maintaining a healthy weight, exercising regularly, and choosing the right nutritious diet (Schulze and Hu, 2005). The right lifestyle reduces the risk by 28%, according to a 2017 assessment (Haw et al., 2017). Furthermore, T2D is characterized by high rates of urination, and thirst (polydipsia), with the felling of hunger (polyphagia) (Kumar et al., 2014). In addition, other symptoms may have an impact on vision, peripheral neuropathy, vaginal infections, and tiredness. The loss of taste is one of the other symptoms (Ship, 2003). Diagnosis of patients with T2D is accomplished by routine testing even when they have no symptoms for the first few years (Ripsin et al., 2009).

Factors that influence the prevalence of T2D are environment, genetics, and epigenetics factors that cause a defect in insulin, and insulin resistance by peripheral cells (DeFronzo et al., 2015). Organs such as the kidneys, pancreas, brain, small intestine, liver, muscles, and fat cells are linked to the predominance of

T2D. Individuals with the disease have 15% more complications than healthy persons and also increasing the risk of death (Gæde et al., 2003, DeFronzo, 2004). According to the International Diabetes Federation, 537 million individuals around the world had diabetes in 2021, with 90-95% of them being affected with T2D (Sharchil et al., 2022).

2.4.1 Environmental Factors That Effects T2D

Environmental factors include the type of food intake, almost 10% of obese adults progress T2D in 3 years (Oram et al., 2016). A study in the USA concluded that unhealthy food like fast-food is related to overweight and obesity increases insulin resistance and triglyceride concentration, which leads to the risk of T2D (Petersen and Shulman, 2018). In addition, built environment, which means a human-made environment that includes where people live and work and if they need physical activity to do their daily work such as going to work walking is better than going by car so the built environment is related to T2D through daily physical activities (Gorman et al., 2021). Other important factors are the chemical and physical environment associated with the air pollution, which increase the risk of having T2D by triggering inflammation and insulin resistance (Li et al., 2019). Furthermore, long-term exposure to chemicals (like pesticides) affects the metabolism of glucose and leads to resistance of insulin (Lee et al., 2011). Food contaminated by additional chemicals and exposure to various types of toxins have been associated with increased incidence of T2D and diabetic mortality (Murea et al., 2012). The exposure to noise is also considered as an environmental stressor that increases the prevalence of T2D (Dzhambov, 2015). High body temperature, heavy metals, organic pollutants, birth weight, aging, smoking, pesticides, and

stress can induce unhealthy diet and affect sleeping also considered as environmental factors that increase the risk of T2D (Beulens et al., 2022)

2.4.2 Genetic Background of T2D

It has been estimated that more than 70% of diabetes cases are hereditary and the risk of type 2 diabetes is influenced by more than 80 SNPs in more than 36 genes (Herder and Roden, 2011). T2D is a polygenic illness controlled by many genes including (*PPARG,CDKAL1,FTO,TCF2L7,IRS1,IRS2 KCNJ11, WFS1, HNF1A, HNF1B and HNF4A*) (Ali, 2013) (table 2.2) (Murea et al., 2012).

GWAS found many variants in these genes increasing the risk of T2D (Mahajan et al., 2014). GWAS also discovered 10 variants (SNPs) in the *FTO* gene and 23 variants in the *PPARG* gene that is responsible for T2D (Mahajan et al., 2014). Few successful case-control studies and family-based association researches found SNPs in *PPARG*, *TCF7L2*, and *WFS1* genes that were related with raising the risk of T2D (Mahajan et al., 2014). Another research found that *FTO* gene variants are also related to T2D through body mass index (BMI) (Fall et al., 2013). In addition another study predicted, three new loci found in (*FTO, PREX1,* and *MTNR1B*) genes to be associated in both obesity and T2D (Lewis et al., 2010). Furthermore, family history and twin studies also increases the risk of T2D (Larsen et al., 2007, Dayeh et al., 2016). Family history showed that 66% of individuals with T2D had at least one individual from relative affected by T2D. Mothers were double more frequent than fathers, offspring were more likely to inherit T2D from mothers than the fathers ($P < 0.01$) (Thomas et al., 1994). However a parent with T2D has a 40% lifetime risk of passing it on to their children's, this risk rises to 70% if both parents have the disease (Altshuler et al., 2000). Some ethnic groups

are at a higher risk of T2D than others; for example, Indians have a 10-fold higher prevalence of T2D than the general population in the United States. Nevertheless, ethnic differences in T2D susceptibility may be caused by shared or particular environmental and genetic influences (Rowley and Bezold, 2012).

Gene-environment interactions are described as hereditary influences on diseases features that vary in strength, depending on the environment influence. The majority of GWAS research make the assumption that genetic effects are equal over the same ranges of environmental exposure through a population (Franks, 2011).

Table 2. 1 Lists of the main genes according to gene impact, that are related to T2D (Zimmet et al., 2014).

Genes impact		
Genes affect insulin secretion	Genes affect resistance of insulin	Unknown affect
KCNJ11, HNF1A/1B, WFS1, GCK, TCF2, TCF7L2, HNF4A, CAPN10, PROX1, DGKB, FOXO1, HHEX, IDE, TSPAN8, JAZF1,	*PPARG, ENPP1, ADIPOQ, ADAMTS9, IRS1, FTO, GCKR, SREBF1, KLF14*	*NOTCH2, LGR5, RBMS1, TP53INP1, PTPRD, ZBED3, ZFAND6, PRC1, CRY2, FADS1, GLIS3, SLC2A2, HMGA2, C2CD4A/4B,*

2.5 Single nucleotide polymorphisms (SNPs)

Single nucleotide polymorphism is a genetic change at a single position (Robert and Pelletier, 2018). SNPs are the most prevalent kind of sequence variations in the human genome, if it happens in an unfunctional region (intron) of a gene it will not have a significant effect, but if it happens in a vital location of a gene it may cause disease due to disrupting the function of that gene and cause implications to the phenotype (Conrad et al., 2010). For example, it may occur in genes related to an immune response and it cause an illness to the immunity, and if this occurs in the glucose metabolism controlling gene it may cause diabetes (Vallejos-Vidal et al., 2020). However, SNPs can be used as markers to identify diseases (Jorde, 2000).

Nowadays SNPs are identified in genes that are responsible to cause disease. It has been predicted that SNPs happen at a frequency of 1 in each 1000 bp (Ching et al., 2002). When SNPs occur in the non-coding region of a gene, they cannot change the amino acid function, this type of SNP is called synonymous SNP. Furthermore, nonsynonymous SNPs change the amino acid function and can alter the protein binding to its substrate or the inhibitors leading to change in the mRNA stability, that is why SNPs may become a reason for a disease (Kimchi-Sarfaty et al., 2007).

Singla nucleotide polymorphism in association studies are applied in two forms, direct testing of the SNP associated with the disease, or by using SNP as markers for linkage disequilibrium (LD). LD is known as finding the degree of association of 2 genetic markers to be used to detect these sequences of the gene related with disease in a population, this used in the identification of disease associated with illness in families. In another word LD is a non-random connection of alleles at distinct loci in a particular population (Gray et al., 2000). Identifying SNPs have a huge benefit in the medical field by finding the relation between the genetic background of an individual and the drug response and using SNPs as markers for disease by association studies (Vallejos-Vidal et al., 2020, Emilien et al., 2000).

2.6 *PPARG* Gene

The human Peroxisome Proliferator-Activated Receptors (*PPARs*) are found on chromosome 3 (3p25) and cover a genomic region of around 150 kb. It has nine exons (A1, A2, B, and 1-6) (Youssef and Badr, 2013). The *PPARG* gene binds to chemicals that induce the generation of peroxisomes to oxidize fatty acid, also they

are part of the nuclear receptor (NR) superfamily (Khan and Heuvel, 2003, Vanden Heuvel et al., 2006). The *PPARG* gene is found in the liver and fat cells and have a great function in the metabolism of energy (Evans et al., 2004). Furthermore, the *PPARG* has a significant role in regulating the expression of different genes, also this gene is implicated in the metabolism of lipids, inflammation caused by obesity, metabolic syndromes, and atherosclerosis (figure 2.2) (Darwish et al., 2022). The *PPARG* with RXRA both control glucose metabolism (Gampe Jr et al., 2000).

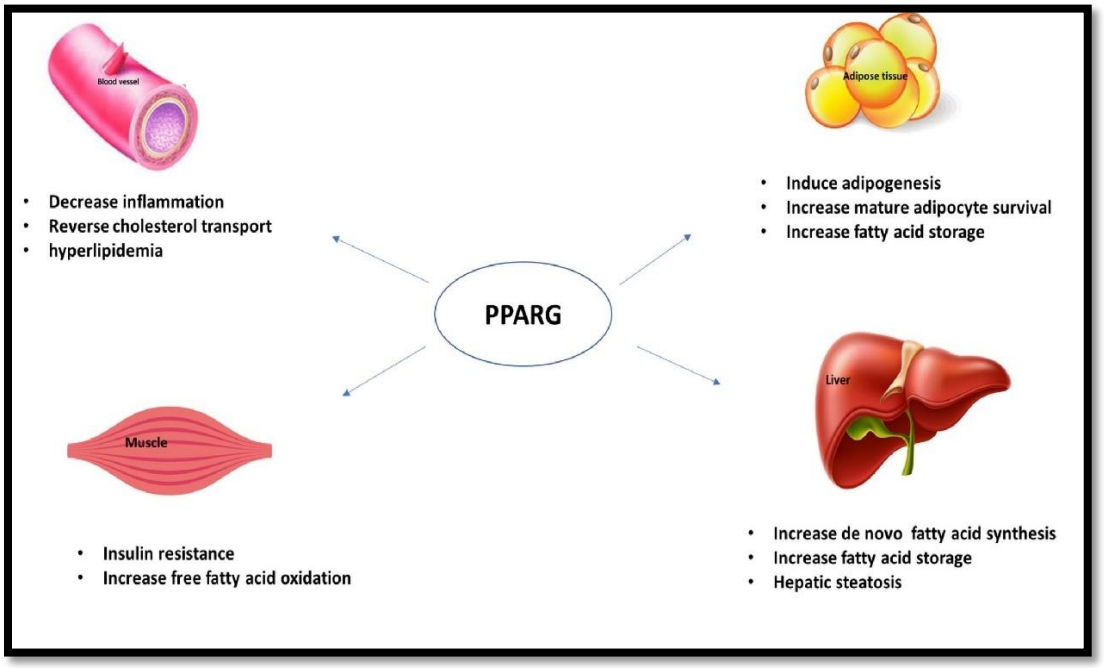

Figure 2. 1 Effect of the *PPARG* gene on liver, adipocytes, blood vessels and muscles (Zimmet et al., 2014).

The *PPARG* gene receptors when they are activated, they form a complex with RXR and they activate genes that store fatty acids in fat cells to decreases fatty acid circulating in the blood so the body become more specific to oxidase carbohydrates (Kersten, 2014). Moreover, *PPARG* stimulates the intake of free

fatty acids (FFA) and encourages their release from circulating lipoproteins. Inactivated *PPARG* gene induces hyperlipidemia and insulin resistance (Vergotine et al., 2014, Veelen et al., 2021). The *PPARG* gene was found to be associated with increased insulin sensitivity in the Finnish population and this conclusion is supported by other investigations in various different studies that evaluated insulin plasma levels and insulin resistance using a homeostasis model (HOMA-IR) (Veelen et al., 2021). *PPARG* has been associated genetically to T2D (Lefterova et al., 2014). Variations in the *PPARG* gene show a strong insulin resistance and high blood pressure (Tsai et al., 2009). Furthermore, Polymorphism known as (the Pro12Ala allele) was found to be protective against T2D in Caucasians population (Altshuler et al., 2000). In addition, it was considered to increase the risk of having T2D in South Asian populations (Vionnet et al., 2000). This polymorphism is also called rs1801282 (Andrulionyte et al., 2007).

2.6.1 rs1801282 in *PPARG* Gene

Many studies have shown that a SNP in the *PPARG* gene plays a significant role in regulating the metabolism of lipid and glucose (Povel et al., 2011). The SNP rs1801282 is a C→G transition SNP in the *PPARG* gene resulting in alanine in the place of proline at codon 12 reducing the transcriptional proteins (Matsuo et al., 2009). Worldwide there were inconsistency in the association between rs1801282 and T2D (Deeb et al., 1998). Several case-control studies have shown that the Pro12Ala (Ala12) variation is linked with reducing the risk of T2D, such as a study in Japan in 2001 on 2201 diabetic cases and 1212 individuals as control, which concluded that the rs1801282 variant is linked with protection against the development of T2D (Mori et al., 2001). A study in 2000 which also was in Japan assumed the same conclusion and predicted that the rs1801282 variant is related to

protection against the risk of T2D (Hara et al., 2000). Furthermore, in the Czech population, a study in 2004 concluded that the rs1801282 variant is also linked with a reduction of T2D risk (ROMéOV et al., 2004). In addition, another study in Scotland on the association between rs1801282 and T2D had the same conclusion that the SNP is related to the protection from T2D not increasing the risk (Doney et al., 2004).

On the other side, there are several additional studies concluded that rs1801282 is responsible for increasing the risk of T2D. In Russia they studies the association between the SNP and T2D, and they concluded that the *PPARG* gene SNP increases the risk of T2D and insulin resistance (Chistiakov et al., 2010). It was the same conclusion in South Asia, South Africa, Finnish and Chinese that rs1801282 increases the risk of T2D (Chang et al., 2012, Majid et al., 2017, Sarhangi et al., 2020).

A different conclusion was discovered in other studies, case and control studies in the South Indian population concluded no association between rs1801282 and T2D (Pattanayak et al., 2014). As well as there was no association between the SNP and the disease in the Bangladeshi population (Saleh et al., 2016). There was another study in the Iraqi Arab population in 2018 that included 97 individuals with T2D and 95 individuals as control, they concluded that rs1801282 is not associated with the risk of T2D (Al-Naemi and Ahmad, 2018).

2.7 *FTO* gene

Fat mass and obesity-associated protein also known as alpha-ketoglutarate-dependent dioxygenase, the *FTO* gene encodes for the *FTO* enzyme, found on chromosome number 16. The *FTO* gene consists of 505 amino acid polypeptide with 9 exons (de Oliveira et al., 2019). Some alleles located in the *FTO* gene appear to be related to obesity and lead to increasing the risk of T2D (Jia et al., 2011b, Loos and Yeo, 2014). Although the *FTO* gene is expressed in different organs, the hypothalamus and brain have the highest levels of expression. It is connected to controlling food intake, inflammation, and metabolic rate of the body (Branavan et al., 2020). A GWAS study in 2007 on T2D in the European population comparing 1,924 cases and 2,938 controls led to the initial discovery of the *FTO* gene (Frayling et al., 2007). Another research on a group of SNPs in the first intronic region of the *FTO* gene showed a strong relation with increasing the risk of T2D, this research suggests that the *FTO* gene related with T2D and mediated by the impact of *FTO* on BMI (Frayling et al., 2007). After the first suggestion on the *FTO* gene, a BMI GWAS study on 4,741 individuals found extremely significant relationships for SNPs from the same intronic region in the *FTO* gene. This finding was then confirmed in 2,335 European and Americans study (Scuteri et al., 2007). There were GWAS studies reported the relation between the *FTO SNPs* and obesity, identified different SNPs in the first intron of the gene and showed strong relation to the BMI, one of them is rs9939609 (Frayling et al., 2007, Hinney et al., 2007, Tung and Yeo, 2011). It is found that SNPs in the *FTO* gene do not affect the weight at birth but it can affect the weight at early age of childhood (Sovio et al., 2011).

FTO proteins function as DNA demethylates (Kang et al., 2018). *FTO* is a part of the alpha-ketoglutarate-dependent hydroxylase superfamily, they are non-heme iron proteins which catalyze huge number of reactions, these proteins function as catalyzing demethylation of 3-methylthymine in SS-DNA and 3-methyluracil SS-RNA (Gerken et al., 2007). A study on the *FTO* gene found that the gene is a 2-oxyglutarate and Fe dependent demethylase. In addition, inside the body recombinant *FTO* can catalyze the Fe- and 2OG- demethylation of 3 methyl thymine in SS-DNA, as well as it works on SS-RNA on 3 methyl uracil, and 6 methyl adenosines. This shows that *FTO* may have a function in nucleic acid modification and repair (Loos and Yeo, 2014).

In middle-aged and older people, obesity is a significant causative risk factor for morbidity and mortality. As well as T2D, cardiovascular illness, coronary heart disease, cancer, dementia, and stroke are diseases that linked to obesity (figure 2.3) (Ganeff et al., 2019). *FTO* proteins are enzymes encoded by the *FTO* gene which increase the *FTO* expression and related with controlling of energy (Olszewski et al., 2009). The *FTO* gene is not related directly with diabetes but increasing the body fat in which lead to increase the risk of future T2D (Drong et al., 2012). The *FTO* gene can cause obesity, and resistance to insulin which lead to the development of T2D (Zhang et al., 2020). Yet, GWAS studies found the relation between SNPs in the *FTO* gene and risk of T2D (Scott et al., 2007). The SNP rs9939609 is found in the *FTO* gene is related to increasing the body fat that lead to future risk of T2D (Sandholt et al., 2012).

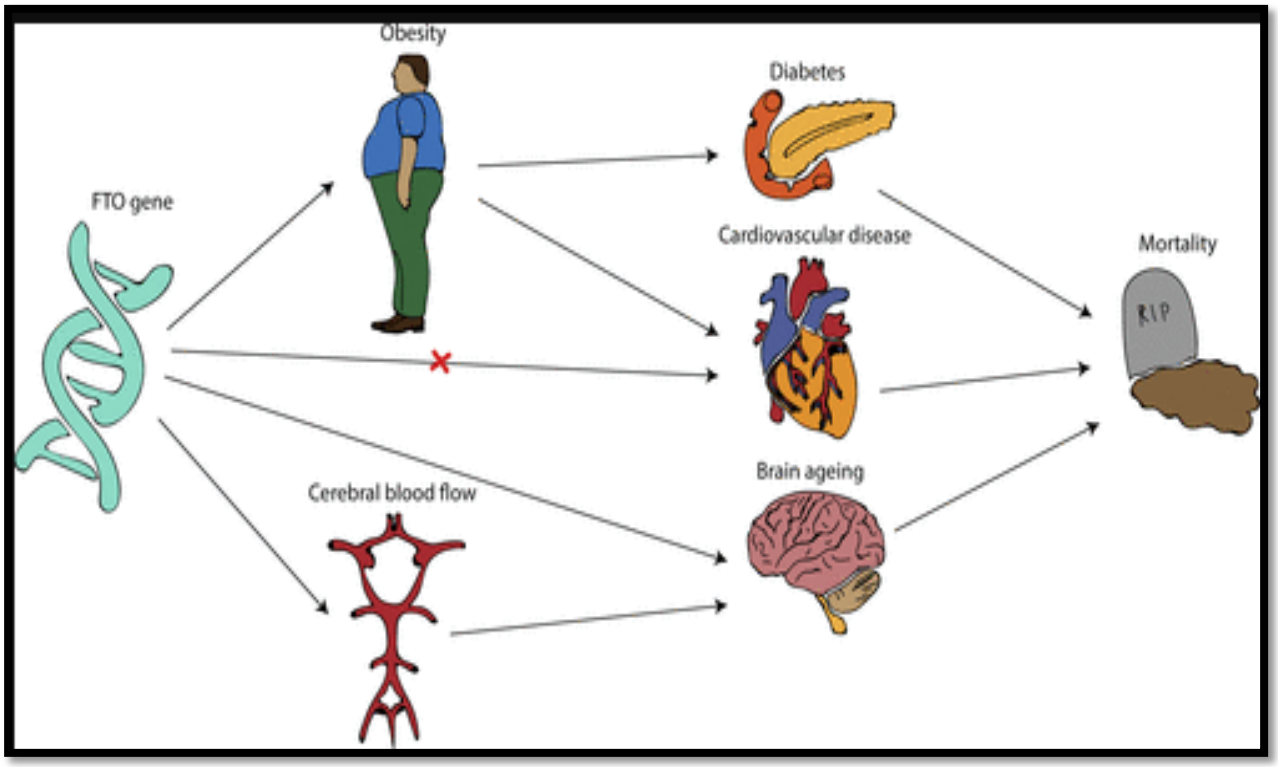

Figure 2. 2 *FTO* gene related disease(Ganeff et al., 2019)

2.7.1 rs9939609 in the *FTO* gene

Studies on *FTO* have identified several distinct single-nucleotide polymorphisms (SNPs), rs9939609 being the most often researched in relation to obesity and BMI. The risk allele rs9939609 is widely known for it is relation with obesity or features related to obesity, and insulin resistance related to T2D. Moreover, it might be crucial in the control of gluconeogenesis, and rs9939609 play a strong role in beta cell functions (De Soysa et al., 2021). The SNP rs9939609 is T→A transversion in the *FTO* gene that result in A allele in the place of T allele and located within 47 kb in the first intronic region (Al-Serri et al., 2019). This gene produces the protein alpha-ketoglutarate, which is a Fe^{+2}-dependent

dioxygenase enzyme that restores alkylated DNA and RNA through oxidative demethylation (Hess et al., 2013). A case-control study included 7052 individuals (3,430 were T2D cases and 3,622 were control) suggested that the association between rs9939609 and T2D depends on the type of diet (Ortega-Azorín et al., 2012). However, research on obese females in Pakistan showed no association between rs9939609 and increasing levels of BMI, FBS and plasma insulin which leaded to the risk of T2D (Shahid et al., 2013). In Palestine, the SNP were assessed to prove the association again between the rs9939609 and T2D (Sabarneh et al., 2018). Furthermore another research made the same suggestion that rs9939609 is responsible for future T2D in Vietnamese individuals (Binh et al., 2022). In numerous European cohorts, the fat mass and obesity-associated *FTO* gene rs9939609 polymorphism has been strongly linked to childhood obesity (Moleres et al., 2012). In 2012 in a Polish children study aimed to determine how genetic background influences the onset of overweight, obesity, insulin resistance and metabolic syndromes. Characteristics of diabetic and healthy Polish children suggested that rs9939609 was associated to insulin resistance (Luczynski et al., 2012). Another study in Spain on 345 child and adults ranged from 6-18 years aimed to determine whether dietary fatty acid intake distribution could interact with the *FTO* genetic variant and obesity. The study demonstrates for the first time the relationship between the distribution of dietary fatty acids and the effect of the rs9939609 polymorphism of the *FTO* gene on the risk of childhood and adolescent obesity (Moleres et al., 2012). Moreover, another study in Iran in 2019 on 168 case and control individuals suggested that genetic variants at the *FTO* rs9939609 may alter insulin metabolism and play significant roles in the emergence of T2D via insulin resistance. A French Study also verified the same impact of the *FTO* rs9939609 polymorphism's A allele on the risk of obesity and T2D (Legry et al.,

2009). Same conclusion in Tunisian population (Elouej et al., 2016). A systematic review composed of 42 study on 11 different obesity loci including rs9939609 loci concluded that some loci are strongly associated with T2D risk, and some others increase the T2D risk through obesity (Xi et al., 2014). Hence, analyzing these polymorphic areas may be useful for making predictions about T2D (Khoshi et al., 2019)

2.8 T2D Diagnosis and Prevention.

T2D is diagnosed using blood tests such fasting and random blood glucose, oral glucose tolerance test, or glycated hemoglobin (A1C) (table 2.8) (Chai et al., 2017). Another blood test is used which is C-peptide, in T2D C-peptide levels are normal to high vice versa with T1D which indicates low levels of C-peptide.

Table 2. 8 blood tests used in diagnosis of diabetes (Fox et al., 2015).

ADA diabetes diagnostic criteria in 2015		
	Diabetes mellitus	**Prediabetes**
Level of HbA_{1c}	≥6.5%	5.7-6.4%
Fasting glucose	≥126 mg/dL	100-125 mg/dL
Random blood glucose (2h glucose)	≥200 mg/dL	140-199 mg/dL
Random glucose with classic symptoms	≥200 mg/dL	Not available

Prevention of T2D is a major global concern. T2D can be controlled or delayed with appropriate nutrition and frequent exercise which mean choosing the right life style and diet can prevent T2D and insulin resistance (Muscogiuri et al., 2022).

2.9 Genotyping Methods

There are different types of genotyping methods that used to identify SNPs. Below are the most common methods.

2.9.1 Restriction Fragment Length Polymorphism (RFLP)

This quick and accurate enzymatic method for amplifying DNA fragments has been used for different purposes, mostly used to find nucleic acid polymorphisms and determine the biological significance of genetic variations. This methods is identified by restriction enzyme (Ota et al., 2007). Choosing the best primer combination and identifying the restriction enzymes that will recognize the SNPs in the PCR-amplified products are the first steps in every PCR-RFLP procedure (Ota et al., 2007). This technique is used in many cases-control studies, such method was used to identify rs9939609 SNP association with T2D in Iranian study in 2019 on 168 individuals by using specific restriction enzyme (Khoshi et al., 2019). Another study in India used the same technique on 400 subjects, 300 were T2D and 100 were control by using ScaI restriction enzyme (Khan et al., 2020b). Same method also was used in Egypt (RAHMAN et al., 2018).

2.9.2 Polymerase Chain Reaction (PCR)

The genotyping of hundreds of variants was necessary. As a result, there is a need for a quick, affordable genotyping technique. Polymerase chain reaction (PCR) is used for genotyping of SNPs (Erlich, 1989). Such methods that use PCR for genotyping are PCR-RFLP (Urakawa et al., 1997), DNA sequencing (Kan et al., 2004), ARMS PCR (Medrano and De Oliveira, 2014) and Allele Specific PCR (Gaudet et al., 2009), PCR is used in most of the genotyping methods (Wang et al., 2005, Falk et al., 2006, Long et al., 2017).

2.9.3 DNA Sequencing (Sanger method)

This method considered as direct method used for genotyping of SNPs, mostly used for conformation of PCR product because it considered expensive. Sanger sequencing method has been the industry standard for DNA sequencing. It is considered as the gold standard in, small number of samples could be examined simultaneously by Sangar sequencing, and its considered as expensive method (Fakruddin and Chowdhury, 2012). In 2021 a study in Pakistan used DNA sequencing for conformation of the PCR product (Shaikh et al., 2021). Another study in India on 518 T2D individuals and 518 controls to identify three SNP rs9939609 in the *FTO* gene, rs1801282 in the *PPARG* gene and rs16861194 in association with susceptibility of T2D, TETRA-ARMS PCR were used for genotyping and 10% of the samples were sequenced by Sangar sequencing to measure the validity of TETRA-ARMS PCR (Phani et al., 2016).

2.9.4 Allele Specific PCR (ASP-CR)

In the context of AS-PCR, PCR amplification is used to discriminate between alleles that differ by one or more nucleotides. AS-PCR makes it possible to be detected by testing the PCR results immediately on an ethidium bromide-stained agarose or polyacrylamide gel for any mutation in human DNA (Gaudet et al., 2009). This approach depends on employing allele-specific primers with 3´-end bases that are complementary to the SNP site to produce the PCR product that is unique to the SNP polymorphism (Ahmadian et al., 2001). The low cost and quick identification of the amplified products are two benefits of the AS-PCR (Latorra et al., 2003). This detection of SNPs in a single-tube experiment using at least two unique primers specific for the SNP (Germer and Higuchi, 1999).

The Amplification Refractory Mutation System (ARMS) is a part of AS-PCR and it is a fast and reliable technique for analysis of SNPs. ARMS PCR was developed from allele specific amplification avoiding using restriction enzymes. ARMS-PCR eliminates the need for expensive and frequently challenging post-PCR processing by allowing the identification of specific genotypes in a single PCR step(Collins and Ke, 2012). Tetra-primer ARMS-PCR, often known as T-ARMS, is a method that combines tetra-primer PCR with ARMS. T-ARMS combines two inner SNP-specific primers and two outer primers in a single reaction, with intentional mismatches at position -2 from the 3' end of the inner primers (Hamajima et al., 2000). This method is widely used as low-cost and quick method to identify SNPs (Lajin et al., 2012). Many studied used this method to identify SNPs (Phani et al., 2016, Saravani et al., 2017), (Kar et al., 2023, Amin, 2023).

Genome-wide association studies indicate that the pathogenesis of T2D is related with variations in the *PPARG* and the *FTO* genes, the SNP rs1801282 in the *PPARG* gene and the rs9939609 in the *FTO* gene are associated with increasing risk factor for T2D in different populations (Frayling et al., 2007, Mtiraoui et al., 2012). On the other side it is found that rs1801282 is also related to protection against T2D (Totomoch-Serra et al., 2018), that why it is important to study the genetic background of patients with T2D in Kurdistan region and identify if the rs1801282 in the *PPARG* gene and rs9939609 in the *FTO* are the responsible variants to develop T2D in Kurdistan region.

CHAPTER THREE

MATERIALS AND METHODS

3. MATERIAL AND METHODS

3.1 Sample collection

In this study, 100 blood samples of unrelated patients with T2D, and 100 non-diabetic controls were collected from two laboratories in Erbil city (Smart Lab, and Pharma Lab) and stored at -80ºC deep freeze for DNA extraction. The duration of the sample collection took more than 3 months. The characteristics and biochemical tests of the collected samples were also collected including (Age, BMI) and (insulin, RBS, and HbA1c) tests were ran on E411 and C311(Roche, Germany).

3.1.1 inclusion and exclusion criteria

The diabetic samples in this study were collected depending on the HbA1c, FBS, insulin and the history of the patients. Related patient (same family) was excluded from this study.

3.2 Equipments

Table 3.2 Shows the instruments that were used for the practical part in this study.

Table 3. 2. Equipment used in this project, production company and country of origin.

No.	Equipment	Company	Origin
1	Autoclave	Wisd	Germany
2	Centrifuge	Hettich	Germany
3	Gel electrophoresis tank	Biobase	China
4	Micro-centrifuge	Hettich	Germany
5	Micropipettes	PhysioCare	USA
6	Power supply	Consort	Belgium
7	Vortex	Isolab	Germany
8	Water bath	GFL	Germany
9	Sensitive Balance	KERN	……..
10	NANODROP LITE	Thermos scientific	UK
11	XP cycle	BIOER	……
13	Deep freeze		
14	Cylinder	LMS	Germany
15	Microwave	LG	Korea

16	Dark hood DH-40 Gel documentation system	Bio-tech fischer	Germany
17	EDTA tubes	ISMS	China
18	Serum tubes	ISMS	China
19	Cobas E411	Roche	Germany
20	Cobas 311	Roche	Germany

3.3 Chemical materials

Table 3.3 shows the chemical materials and the production companies with their country of origin used in this research.

Table 3. 3. List of chemical material used in this project, production company and country of origin.

No.	Materials	company	Origin
1	Ethanol 99.9%	ISMS	China
2	Agarose (Analytical Grade)	ISMS	China
3	Safe gel stain	Add bio	Korea
4	Loading dye 6X concentration	Geneaid	Korea
5	Tris-Boric acid EDTA buffer (TBE) buffer 5X	Inno-train	Korea
6	Add Taq Master	Add Bio	Korea
7	Sterilized water	----------	----------

3.4 Sterilization Methods

Sterilization was done by autoclaving for 15 minutes at 121°C.

3.5 DNA extraction from whole blood

DNA extraction from whole blood was done by following the manufacturer's protocol using (WizPrep™ gDNA Mini Kit (Blood); WizBio Solutions, Korea) kit. The following steps clarify the steps of the genomic DNA extraction:

Step one (lysis step)

1- Two hundred µl of whole blood was added to 1.5 microcentrifuge tube.

2- Two hundred µl of GB buffer and 20 µl of proteinase k was added and mix by vertexing.

1- The samples were incubated at 56ºC for 10 minutes, during incubation invert the tube each 5 minutes.

Step two (binding step)

1- Two hundred µl of 100% ethanol was added to the sample lysate and mixed by vertexing.
2- Spin column was connected to 2ml collection tube.
3- Applied the mixture to the spin column and centrifuge for 1 minutes at 13000rpm.
4- The spin column was transferred to a new collection tube

Step three (wash step)

1- Five hundred µl of W1 buffer were add to the spine column and centrifuge for 1 minute at 13000 rpm.
2- The flow-through was discard and reconnect the spin column.
3- Five hundred µl of W2 buffer was added and centrifuge for 1 minute at 13000 rpm.
4- The flow-through was discarded and reconnect the spin column and centrifuge for 2 minutes at 13000 rpm.

Step four (elusion step)

1- The spin column was connected to new 1.5 ml tube.
2- 50µl of elusion buffer was added and incubate it at the room temperature for 1 minutes.
3- Centrifuge for 1 minutes at 13000 rpm
4- The spin column was discarded and the purified genomic DNA was obtained.

3.6 DNA concentration and purity

The concentration and purity of the extracted DNA was evaluated by Nano Drop device.

1- 1µl of the DNA sample was added to the loading position on the Nano Drop device
2- The samples were diluted using sterilized D.W by using C1V1=C2V2 equation.

3.7 Tris-Boric acid EDTA buffer (TBE) buffer 5X preparation

20 ml of 5X TBE was added to 80 ml of sterilized water (D.W).

3.8 Agarose preparation

The agarose gel was prepared in different concentration in each level for testing the genomic DNA 1% agarose was used, for SNP rs1801282, 3% agarose was used and for rs9939609 2.5 % of agarose was used. The following steps were done for agarose preparation.

1- (1gm, 2.5gm and 3gm) of agarose added to 100ml of 1x TBE buffer in a beaker than heat it in microwave till boiling, then the agarose was left to cool till 60ºC to 70ºC.
2- When agarose reaches 60ºC to 70ºC safe stain added and let it cool to reach 55ºC.
3- The liquid of agarose was poured in to gel tray and left to solidify at room temperature for 20-30 minutes.
4- 1X TBE buffer was poured in to a gel tank, and the gel tray was placed inside the electrophoresis tank.
5- 5 µl of 50 bp DNA ladder was loaded inside the first well of the gel

6- 1 µl of 6X loading dye was added 5 µl of extracted DNA to be loaded inside the gel wells.
7- Power supply was turned on 90 volts for an hour for genomic DNA, 50 and on 100 volts for 1:45hr for the *PPARG* gene rs1801282 and 120 volts for 1:30hr for the *FTO* gene rs9939609.
8- The bands of allele specific PCR product visualized using UV light.

3.9 Primers list and their sequences

primers were designed by using Tetra ARMS - PCR Primer Design Tool (Ye et al., 2001). After designing they were bought from Integrated DNA Technologies (IDT) U.S.A in a lyophilized form, each primer concentrated 100 mM and dissolved in sterilized D.W. eight primers were used in this research and listed in (table 3.3).

Table 3. 1. Primer sequences used in this research.

No.	Primers	Sequence of primers 5’ → 3’
1	S5	5’-TCCTTGCGACTGCTGTGAATCTT-3’
2	S6	5’-AACAGAGACTATCCAAGTGCATCGCT-3’
3	S7	5’-TTCCAGTCATTTTTGACAGCATGG-3’
4	S8	5’-AGCCCAAGGATGGTGTTTCTAAGG-3’
5	S9	5’-GAAACTCTGGGAGATTCTCCTATTGTCC-3’
6	S10	5’-ATCAGTGAAGGAATCGCTTTCAGC-3’
7	S11	5’-CTCCTAATAGGACAGTGCCAGCCA-3’
8	S12	5’-TTTAAATGAACGCGATAGCAACGAG-3’

3.10 Taq Master Mix

Master mix (Add Taq Master; Add bio, Korea), contain loading dye.

3.11 Allele specific PCR (ARMS-PCR)

All the samples were genotyped using allele-specific PCR for two SNPs, rs1801282 in the *PPARG* gene, and rs9939609 in the *FTO* gene. For the rs1801282 SNP four primers were designed using Tetra ARMS - PCR Primer Design Tool (Medrano and De Oliveira, 2014). Two outer and two inner primers, the forward

outer primer S11-5'-CTCCTAATAGGACAGTGCCAGCCA-3' and reverse outer primer S12-5'-TTTAAATGAACGCGATAGCAACGAG-3', were used as a control with a product size of 453bp. The forward inner primer S09- 5'-GAAACTCTGGGAGATTCTCCTATTGTCC-3' worked with the reverse outer primer (S12) and gave a product size of 238 bp for the (C) allele, and the reverse inner primer S10- 5'-ATCAGTGAAGGAATCGCTTTCAGC-3' worked with the forward outer primer (S11) and gave a product size of 267 bp for the (G) allele.

SNP rs9939609 in *FTO* gene were genotyped similar to rs1801282 by using allele-specific PCR. Four primers were designed, two outer and two inner primers. The forward outer primer S07-5'-TTCCAGTCATTTTTGACAGCATGG-3', and reverse outer primer S08-5'- AGCCCAAGGATGGTGTTTCTAAGG-3' were used as control with a product size of 473bp. The forward inner primer S05-5'-TCCTTGCGACTGCTGTGAATCTT-3' worked with the reverse outer primer (S08) and gave a product size of 242bp for the T allele, and reverse inner primer S06-5'-AACAGAGACTATCCAAGTGCATCGCT3' worked with forward outer primer (S07) and gave a product size of 280bp for the A allele.

PCR mixture of both SNPs contained, 10 µl of the master mix, primers, sterilized DW needed for the allele specific PCR reaction were mixed in a sterilized 1.5ml tubes. Second, we added 9µl of the mixture into labeled 0.5 ml sterilized Eppendorf tube. Finally, we added the sample DNA into each tube and loaded in to the conventional PCR device (table 3.4).

Table 3. 2. Shows the components that were used for the PCR reaction.

Order	Components	Volume	Concentration
1	Master Mix	10µl	2X
2	Forward inner primer	1µl	10µm
3	Reverse inner primer	1µl	10µm
4	Forward outer primer	1µl	10µm
5	Reverse outer primer	1µl	10µm
6	D.W	5µl	------
7	DNA sample	1µl	10 ng/µl
Total volume		20µl	

The allele specific PCR program for *PPARG* gene rs1801282 was run as follows:

Initial denaturation	95°C for 5 minutes	
Denaturation	95°C for 30 sec	35 cycle
Annealing	61°C for 1 minute	
Extension	72°C for 2 minutes	
Final extension	72°C for 5 minutes	

Then the amplified DNA was analyzed in 3% agarose gel electrophoresis.

And the allele specific PCR program for *FTO* rs9939609 was run as follows:

Initial denaturation	95°C for 5 minutes	
Denaturation	95°C for 30 sec	35 cycle
Annealing	59°C for 1 minute	
Extension	72°C for 45 sec	
Final extension	72°C for 5 minutes	

Then the amplified DNA was analyzed in 2.5% agarose gel electrophoresis.

3.12 DNA sequencing

Samples from both SNPs were selected for sequencing by Sanger method (Applied Biosystems 3730xl). For rs1801282 in the *PPARG* gene four samples with different genotypes were sequenced, two heterozygotes (CG), one homozygote (CC), and one homozygote (GG). Similarly, for SNP rs9939609 in the *FTO* gene, two heterozygous (TA), one homozygous (AA), and one homozygous (TT) were sequenced.

3.13 Statistical analysis

The genotype and allele frequencies were calculated by SNPStats web tool (Solé et al., 2006). Hardy-Weinberg equilibrium (HWE) were assessed for T2D individuals and control groups by using Chi square test (Solé et al., 2006, Teo et al., 2007). Logistic regression model was used for the association study between each SNP and T2D in the case and control groups. To analyze allele specific risk the Odd Ratios (OR) with associated 95% confidence intervals (CIs) were assessed. To choose the best mode of inheritance for the SNPs, Akaike information criterion (AIC) and Bayesian information criterion (BIC) were assessed (Teo et al., 2007).

CHAPTER FOUR

RESULTS

4.RESULT

4.1 Samples characteristics

In the current study a total of 200 samples were enrolled, which were 100 T2D (49% males and 51% females) and 100 non-diabetic controls (52% male and 48% females). All samples were subjected to biochemical tests (HbA1c, insulin and RBS), mean of (Age ±51.05, BMI ±30.01, insulin ±22.55, RBS ±145.2, HbA1c ±6.81) in all status. The mean of case group had higher levels of BMI, random blood sugar, insulin and HbA1C than control group (table 4.1).

Table 4. 1. Means of parameters (Age, BMI, Insulin, HbA1c, RBS). In all the 200 samples, cases and controls.

Parameters	All status	Case	Control
Age	51.05	54.58	47.51
BMI	30.01	30.47	29.72
Insulin	22.55	30	15.1
HbA1c	6.81	8.32	5.3
RBS	145.2	191.08	99.51

4.2 Genomic DNA

Genomic DNA was successfully extracted from 200 whole blood samples (stored in EDTA tubes) using DNA extraction kit (WizPrep™ gDNA Mini Kit (Blood); WizBio Solutions, Korea). The extracted DNA concentration ranged from 8.5-346 ng/µl and the purity ranged from (1.79-2.1). The concentrations were unified to 10ng/µl.

4.3 Allele Specific PCR and DNA sequencing

All the samples were subjected to allele-specific PCR (ARMS-PCR) for both variants (rs1801282 in the *PPARG* gene and rs9939609 in the *FTO* gene) using 4 primers in each assay to identify each variant.

4.4 Allele-Specific PCR products of rs1801282 in *PPARG* gene.

All the samples were genotyped successfully for SNP rs1801282. The PCR products of rs1801282 were resolved on 3% agarose gel electrophoresis to identify the heterozygous (CG) and homozygous (GG/CC) genotypes. Samples from 1-20 were all homozygous for C allele, except sample number 14 was heterozygous for both alleles GC (figure 4.1).

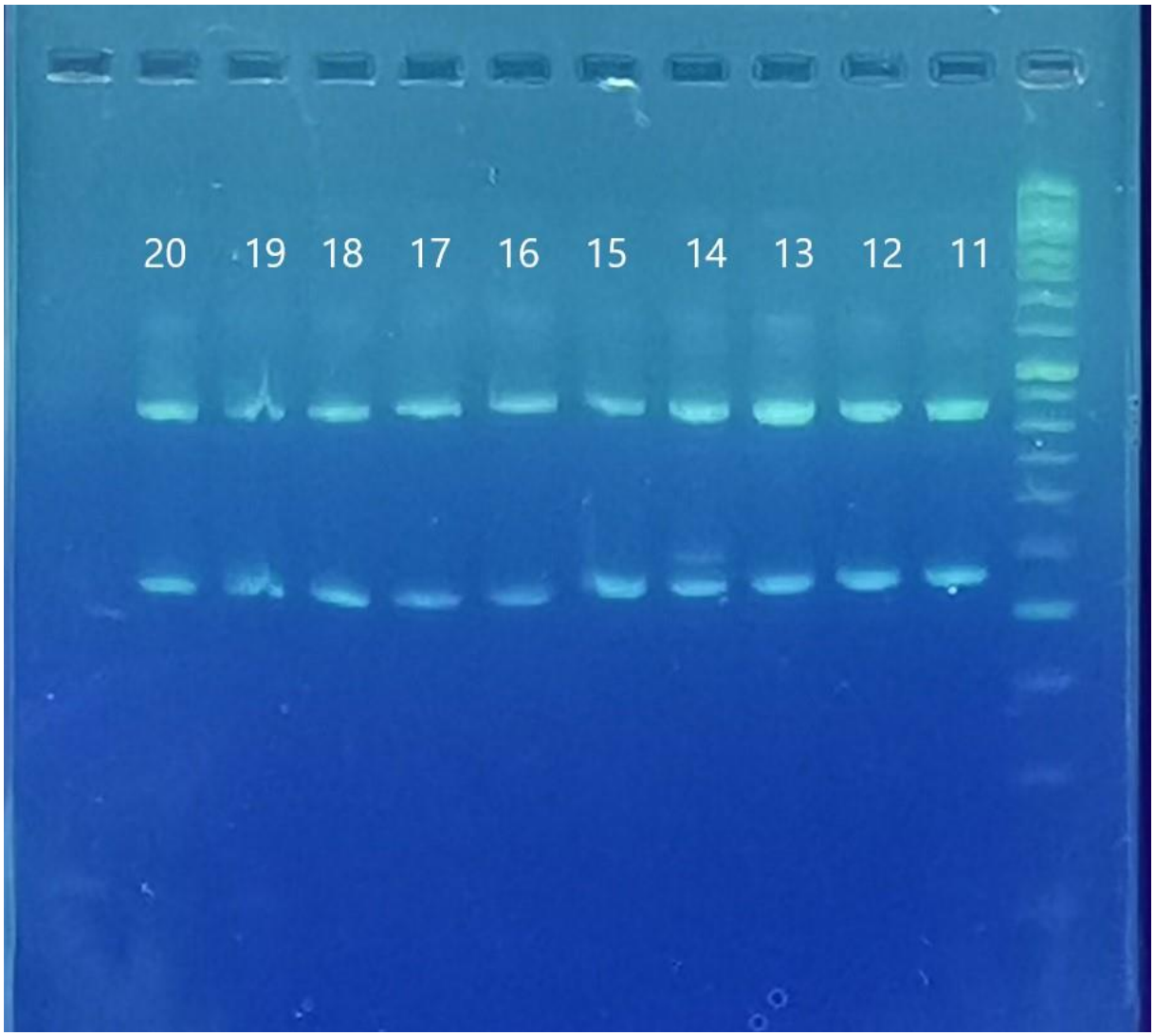

Figure 4. 1 PCR Products of rs1801282, shows three genotypes GG, CC and CG. The product size for the C allele is 238bp and for the G allele is 267bp, the outer primers product size is 453 bp and it was used as a control. The first lane is a 50 bp ladder. Samples from 11-20 were all homozygous for CC allele, except sample 14 was heterozygous for CG alleles. The PCR products were resolved on 3% agarose stained with safe gel stain, and ran for 1 hour at 95 volts.

PCR products for samples number 79, 80 and 86 were heterozygous CG alleles, samples number 71-78 and 81-85 were homozygous CC (figure 4.2).

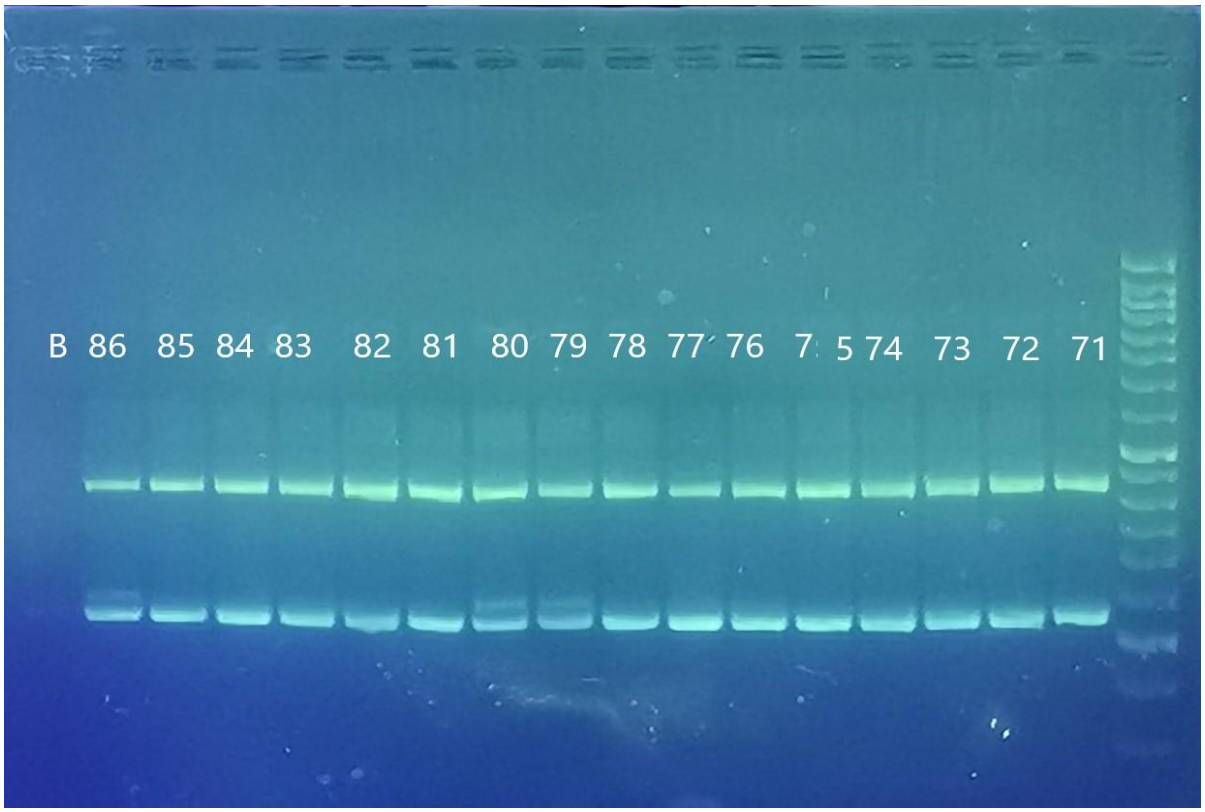

Figure 4. 2 PCR Products of rs1801282, shows three genotypes GG, CC and CG. The product size for the C allele is 238bp and for the G allele is 267bp, the outer primers product size is 453 bp was used as a control. The first lane is a 50 bp ladder. Samples number 79, 80 and 86 were heterozygous CG, samples number 71 ,72, 73 ,74 ,75 ,76 ,77 ,78 ,81 ,82 ,83 ,84 and 85 were homozygous CC. (B) is blank. The PCR products were resolved on 3% agarose stained by safe gel stain, and ran for 1 hour at 95 volts.

Samples from 92-99, 101-106, and 108 were homozygous for C allele, sample number 100 were heterozygous CG alleles, sample number 107 was homozygous G allele (risk allele) (figure 4.4). A set of samples from (92-102) were Duplicated and all the samples showed the same genotype (figure 4.3).

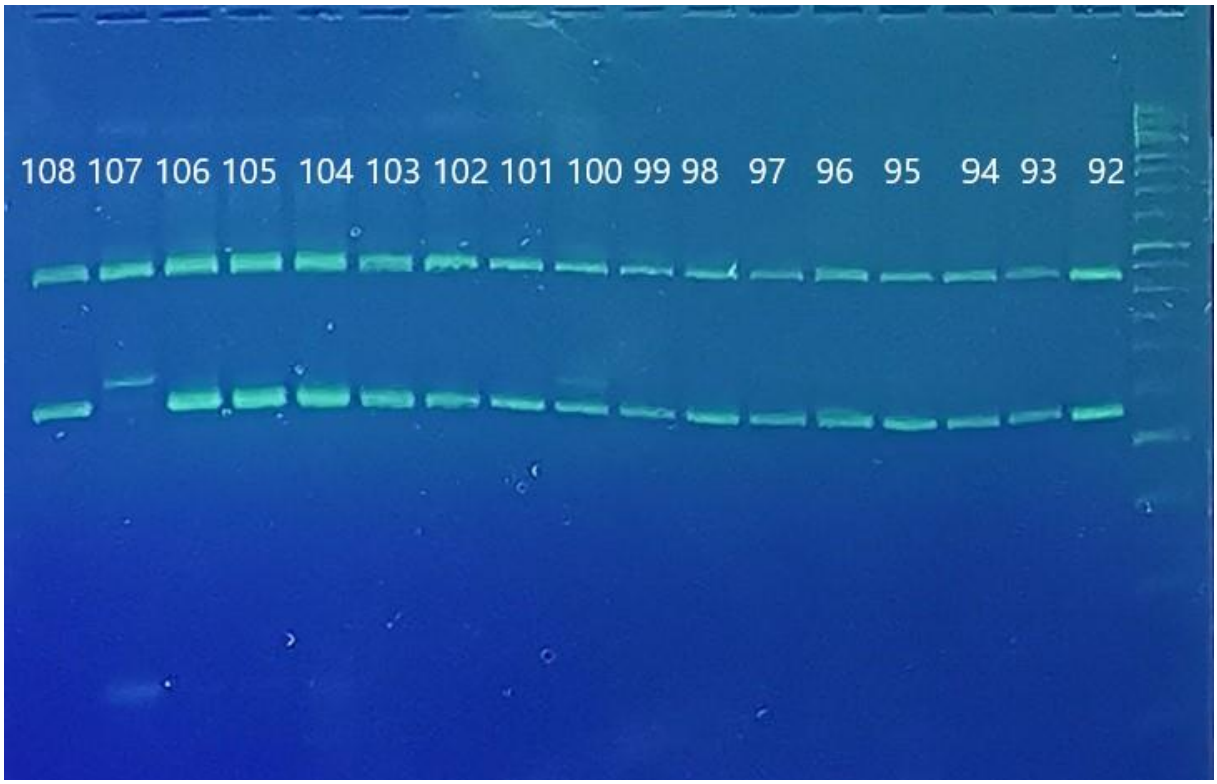

Figure 4. 3 PCR Products of rs1801282, shows three genotypes GG, CC and CG. The product size for the C allele is 238bp and for the G allele is 267bp, the outer primers product size is 453 bp was used as a control. The first lane is 50 bp ladder. Samples number 92, 93, 94, 95, 96, 97, 98, 99, 101, 102, 103, 104, 105, 106 and 108 were homozygous CC, samples number 100, were heterozygous CG, sample number 107 was homozygous GG (risk allele). samples from 92-102 were duplicated. The PCR products were resolved on 3% agarose stained with safe gel stain, and ran for 1 hour at 95 volts.

Samples from 124-139, Samples number 124-129, 131, 132, and 134-138 were homozygous C allele, samples number 133, 139 were heterozygous for CG alleles, sample number 130 was homozygous G allele (risk allele) (figure 4.4).

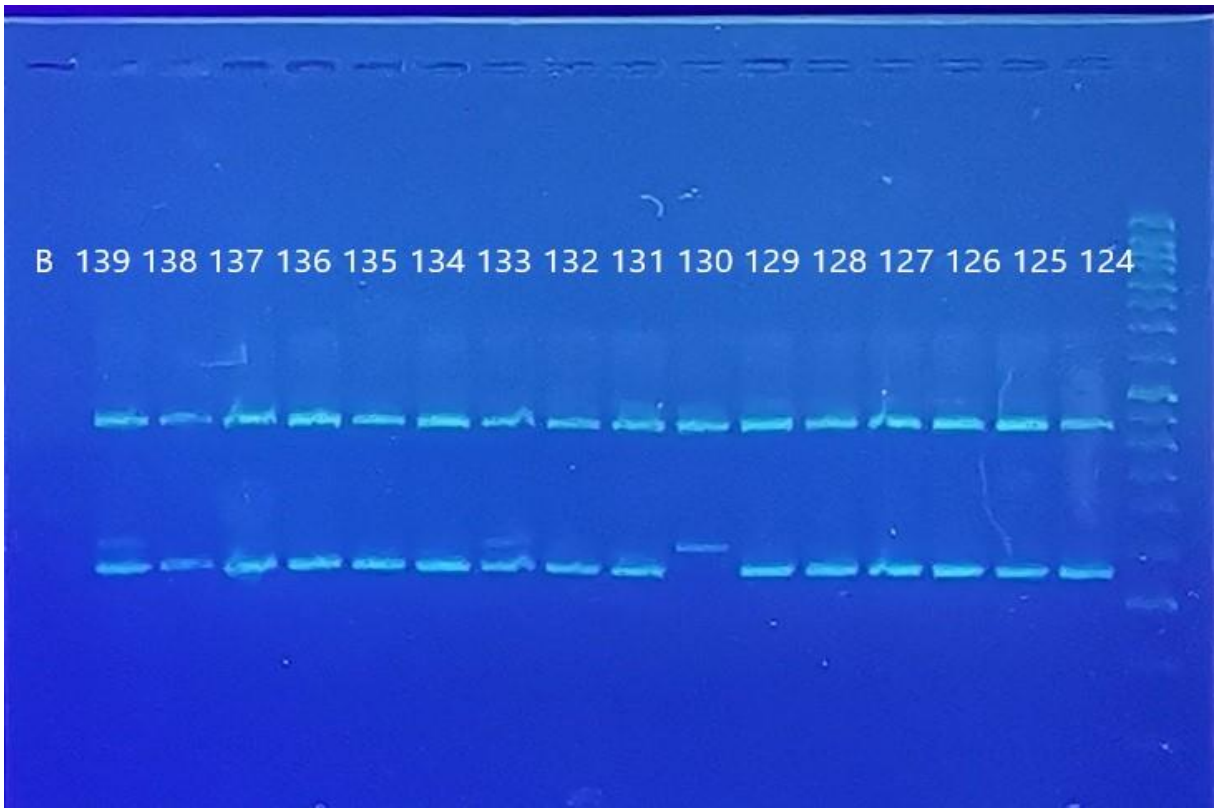

Figure 4. 4 PCR Products of rs1801282, shows three genotypes GG, CC and CG. The product size for the C allele is 238bp and for the G allele is 267bp, the outer primers product size is 453 bp was used as a control. The first lane is a 50 bp ladder. Samples number 124, 125, 126, 127, 128, 129, 131, 132, 134, 135, 136, 137, and 138 were homozygous CC, samples number 133, 139 were heterozygous for CG, sample number 130 was homozygous GG (risk allele. The PCR products were resolved on 3% agarose stained with safe gel stain, and run for 1 hour at 95 volts.

Samples from 140-156 samples number 141, 143, 146, 148, 149, 151, 152, 155 were homozygous for C allele, samples number 140, 142, 144, 145, 147, 153, 154, 156 were heterozygous for CG alleles, sample number 150 were homozygous for G allele (risk allele) (figure 4.5).

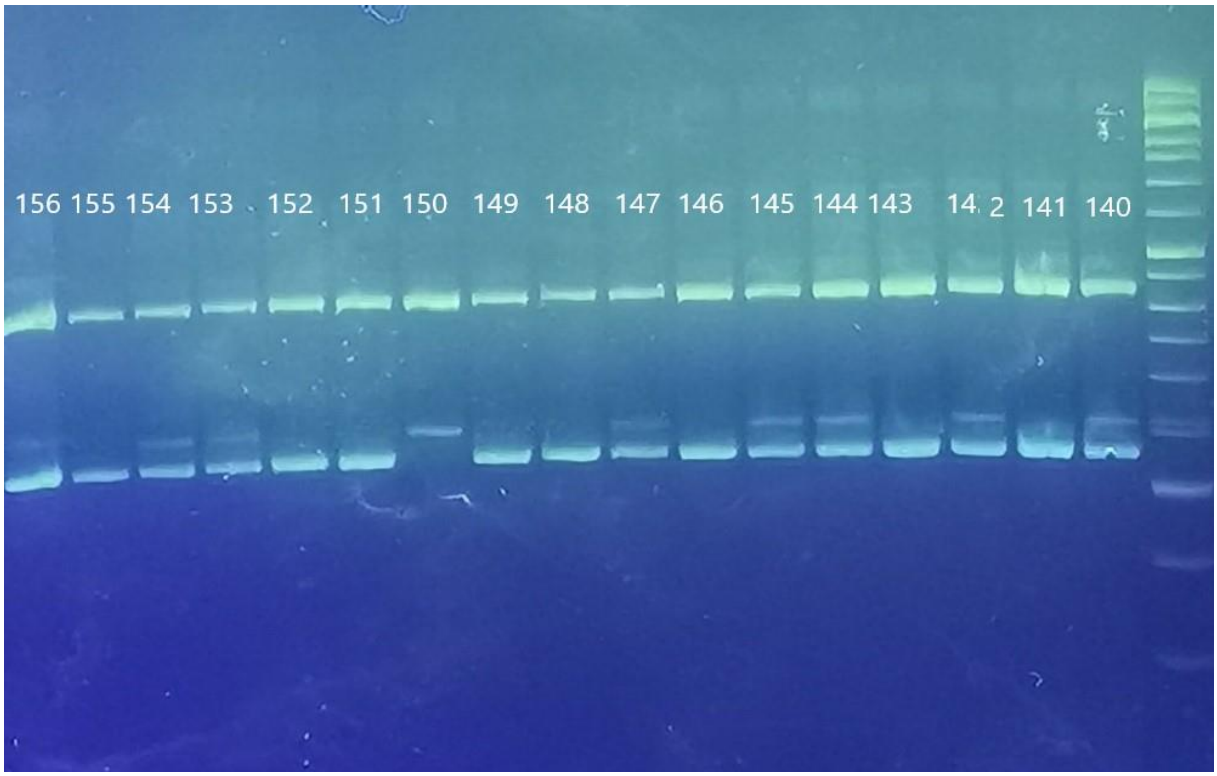

Figure 4. 5 PCR Products of rs1801282, shows three genotypes GG, CC and CG. The product size for the C allele is 238bp and for the G allele is 267bp, the outer primers product size is 453 bp was used as a control. The first lane is a 50 bp ladder. Samples number 141, 143, 146, 148, 149, 151, 152, 155, were homozygous for CC, samples number 140, 142, 144, 145, 147, 153, 154, 156 were heterozygous for CG, sample number 150 were homozygous for GG (risk allele). The PCR products were resolved on 3% agarose stained with safe gel stain, and ran for 1 hour at 95 volts.

4.5 DNA sequencing of rs1801282.

To validate the accuracy of the PCR method used for genotyping rs1801282 in the *PPARG* gene, PCR products of 4 samples (79, 145, 150, and 188) were sequenced by Sanger sequencing method (Applied Biosystems 3730xl). The sequencing results of samples number 79 and 145 was heterozygote CG, sample 150 was homozygous GG and 188 was homozygous CC (figure 4.6). All the sequencing results were similar to the genotyping method (see figures 4.2 and 4.5).

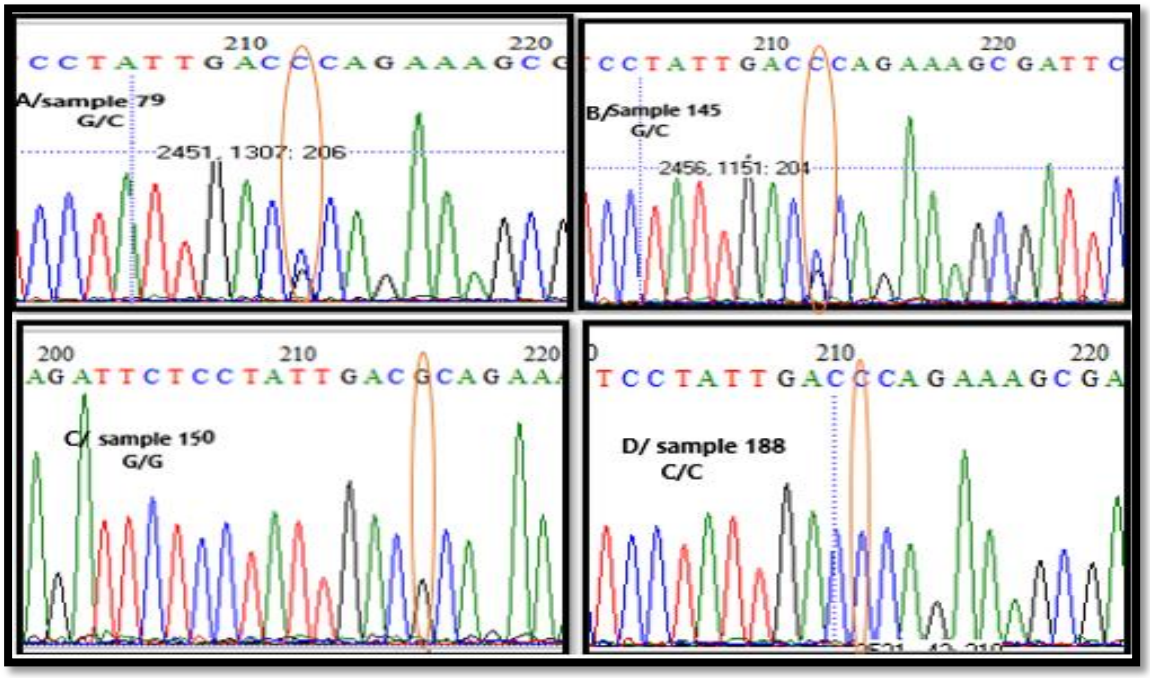

Figure 4. 6 Sanger sequencing traces of four genotypes that matched the PCR method. (A) shows sample 79 in which the genotype is CG, (B) shows sample 145 in which the genotype is CG, and (C) shows sample 150 in which the genotype is GG risk allele and (D) shows sample 188 in which the genotype is C/C. All the four genotypes are indicated by red circles.

4.6 Allele-specific PCR products of rs9939609 in *FTO* gene

All the samples were genotyped successfully for SNP rs9939609. The PCR products of rs9939609 in *FTO* gene were resolved by agarose gel electrophoresis, to identify the heterozygotes (AT) and homozygotes (AA/TT) genotypes. Samples number 11-43, Samples number 11, 12, 14-17, 28-35, 42 were heterozygous TA, samples number 13, 20, 22, 25, 26, 27, 36-41, 43, were homozygous TT, samples number 18, 19, 21, 23, 24 were homozygous AA (risk allele) (figure 4.7).

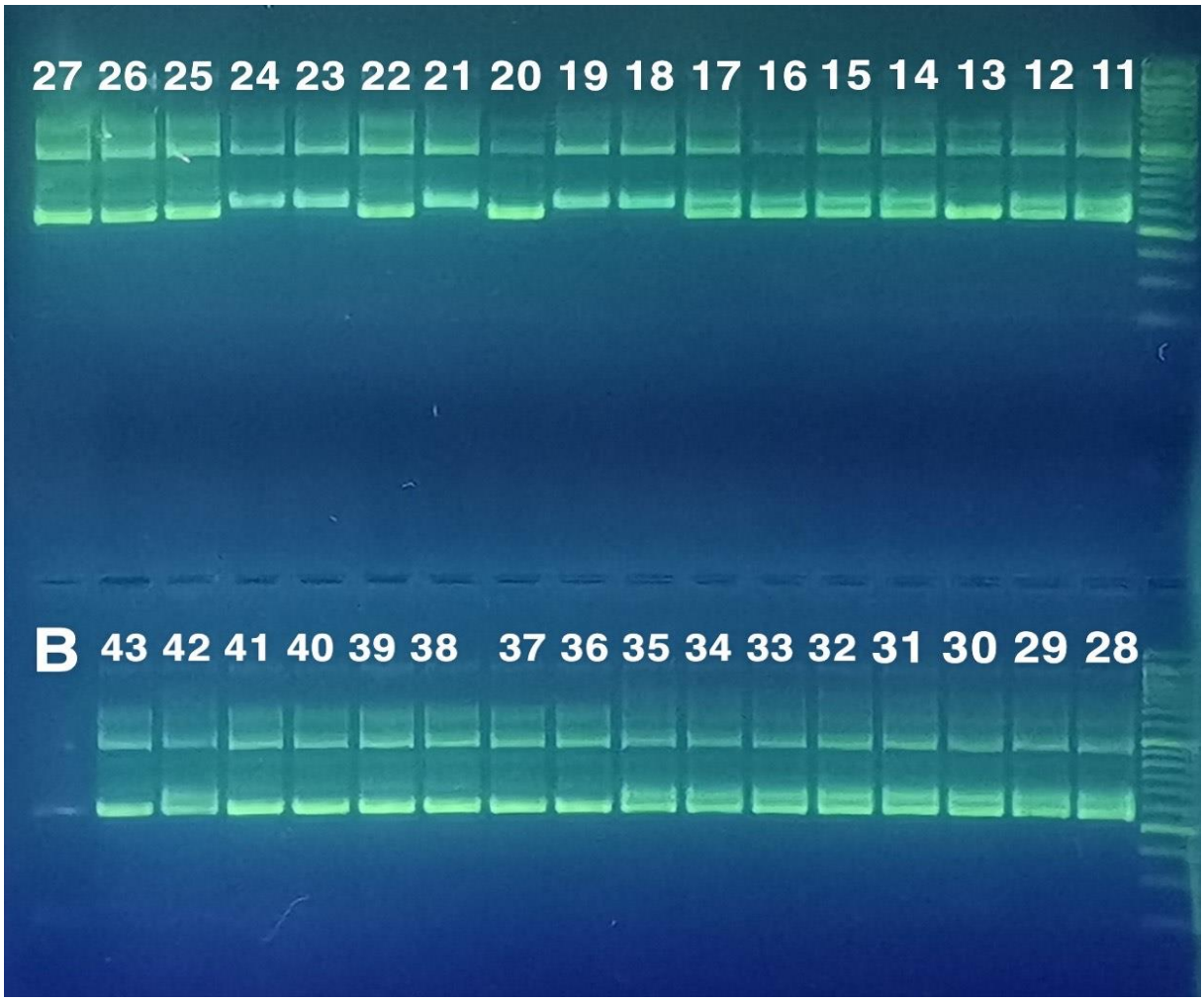

Figure 4. 7 PCR products of rs9939609 (FTO gene) shows three genotypes formation TT, TA and AA. The product size for the T allele is 242 bp, and for the A allele 280 bp, the outer primer product size 473 bp. The first lane is a 50 bp ladder. Samples number 11, 12, 14, 15, 16, 17, 28, 29, 30, 31, 32, 33, 34, 35, 42 heterozygous TA, samples number 13, 20, 22, 25, 26, 27, 36, 37, 38, 39, 40, 41, 43 were homozygous TT, samples number 18, 19, 21, 23, 24 were homozygous AA (risk allele). The PCR products were resolved on 2.5, and ran for at 110 volts for 1 hour.

Samples from 71-100, h Samples number 78, 83, 84, 85, 87, 90, and 96 were homozygous AA (risk allele), samples number 71, 72, 77, 86, and 88 were homozygous for TT, samples number 73, 74, 75, 76, 79, 82, 89, 91, 92, 93, 94, 95, 79, 98, 99 and 100 were heterozygous TA (figure 4.8).

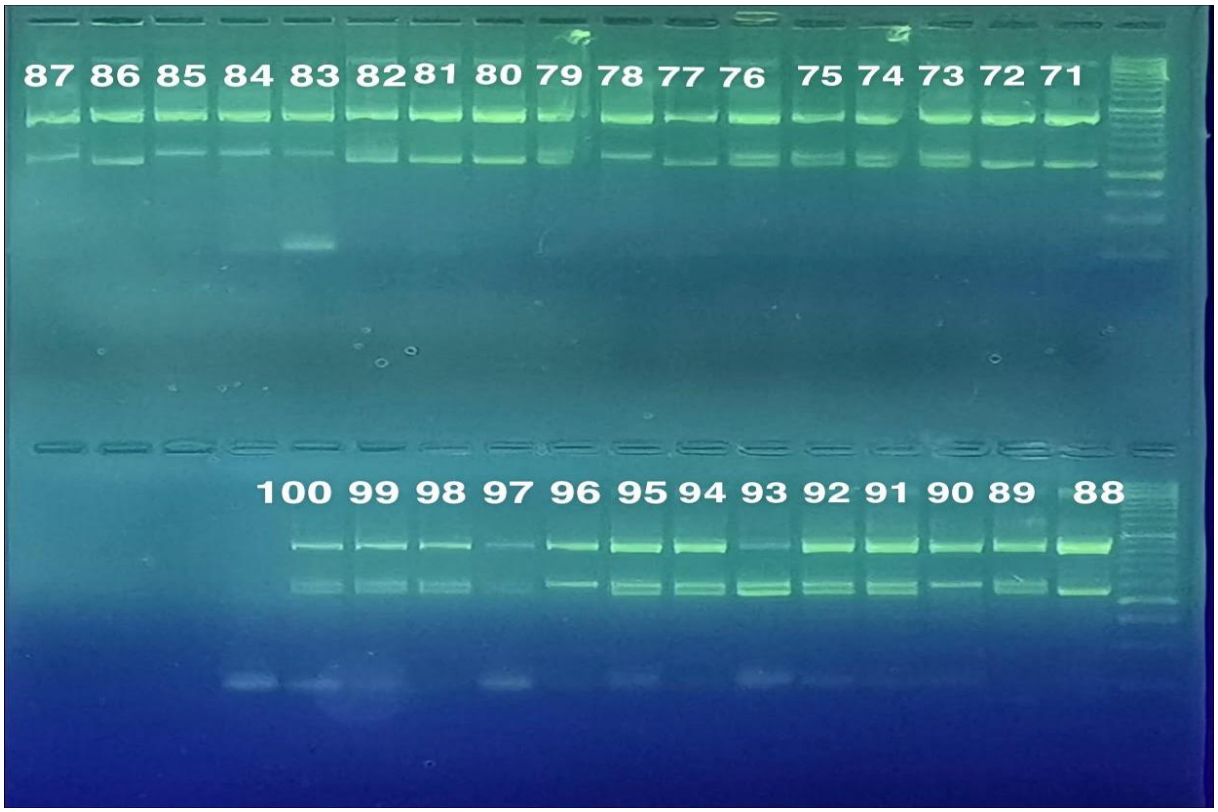

Figure 4. 8 PCR products rs9939609 (FTO gene) shows three genotypes formation TT, TA and AA, product size for T allele is 242 bp and for A allele 280 bp, the outer primer size 473 bp. The first lane is 50 bp ladder. Samples number 78, 83, 84, 85, 87, 90, and 96 were homozygous AA (risk allele), samples number 71, 72, 77, 86, and 88 were homozygous for TT, samples number 73, 74, 75, 76, 79, 82, 89, 91, 92, 93, 94, 95, 79, 98, 99 and 100 were heterozygous TA. The PCR products were resolved on 2.5% agarose stained by safe gel stain, ran at 110 volts for 1 hour.

Samples from 112-128, Samples number 113, and 127 were AA (risk allele), samples number 116, 117, 120, 121, 122, 126, and 128 were heterozygous TA, samples number 112, 114, 115, 118, 119, 123, 124, and 125 were homozygous TT (figure 4.9).

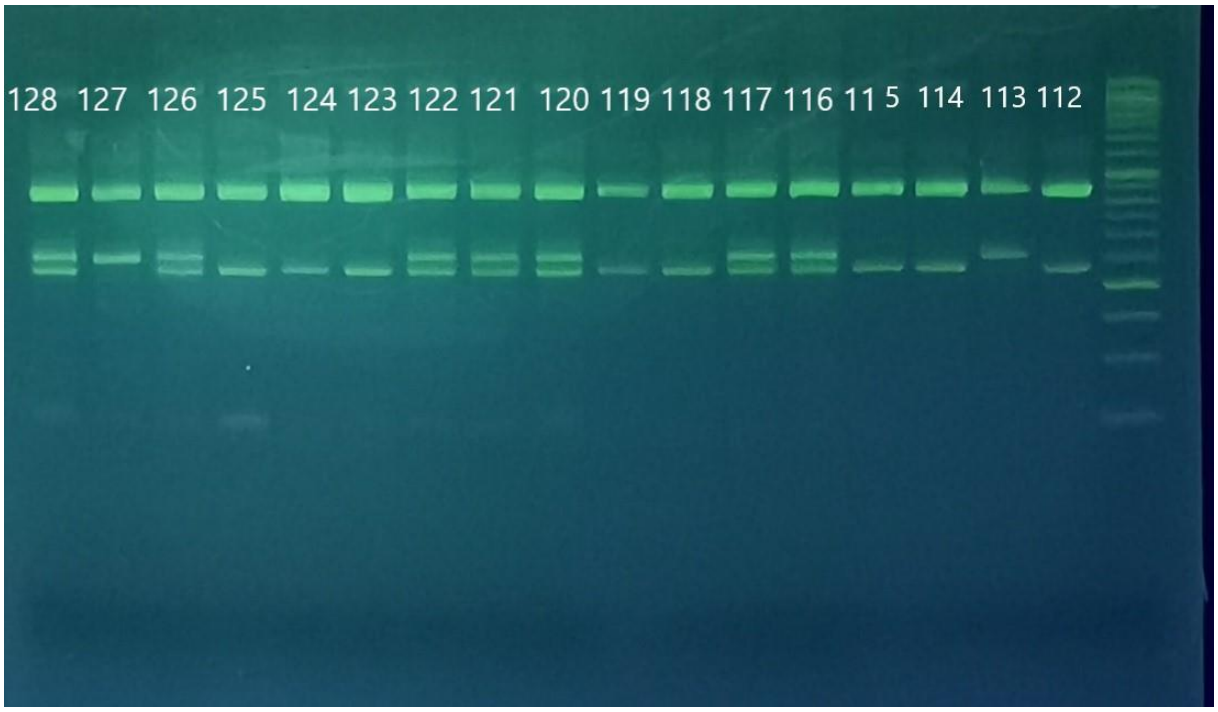

Figure 4. 9 PCR products of rs9939609 (FTO gene) shows three genotypes formation TT, TA and AA. The product size for the T allele is 242 bp and for the A allele 280 bp, the outer primer product size 473 bp. The first lane is a 50 bp ladder. Samples number 113, and 127 were AA (risk allele), samples number 116, 117, 120, 121, 122, 126, and 128 were heterozygous TA, samples number 112, 114, 115, 118, 119, 123, 124, and 125 were homozygous TT. The PCR products were resolved on 2.5% agarose, stained by safe gel satin, and ran at 110 volts for 1 hour.

Samples from 131-164 samples number 138, 148, 156, 159, 161, 162 and 164 were homozygous AA (risk allele), samples number 132, 135, 136, 137, 139, 141, 142, 144, 145, 146, 150, 152, 153, 155, and 158 were heterozygous TA, samples number 131, 133, 134, 140, 143, 147, 149, 151, 154, 157, 160, and 163 were homozygous TT (figure 4.10).

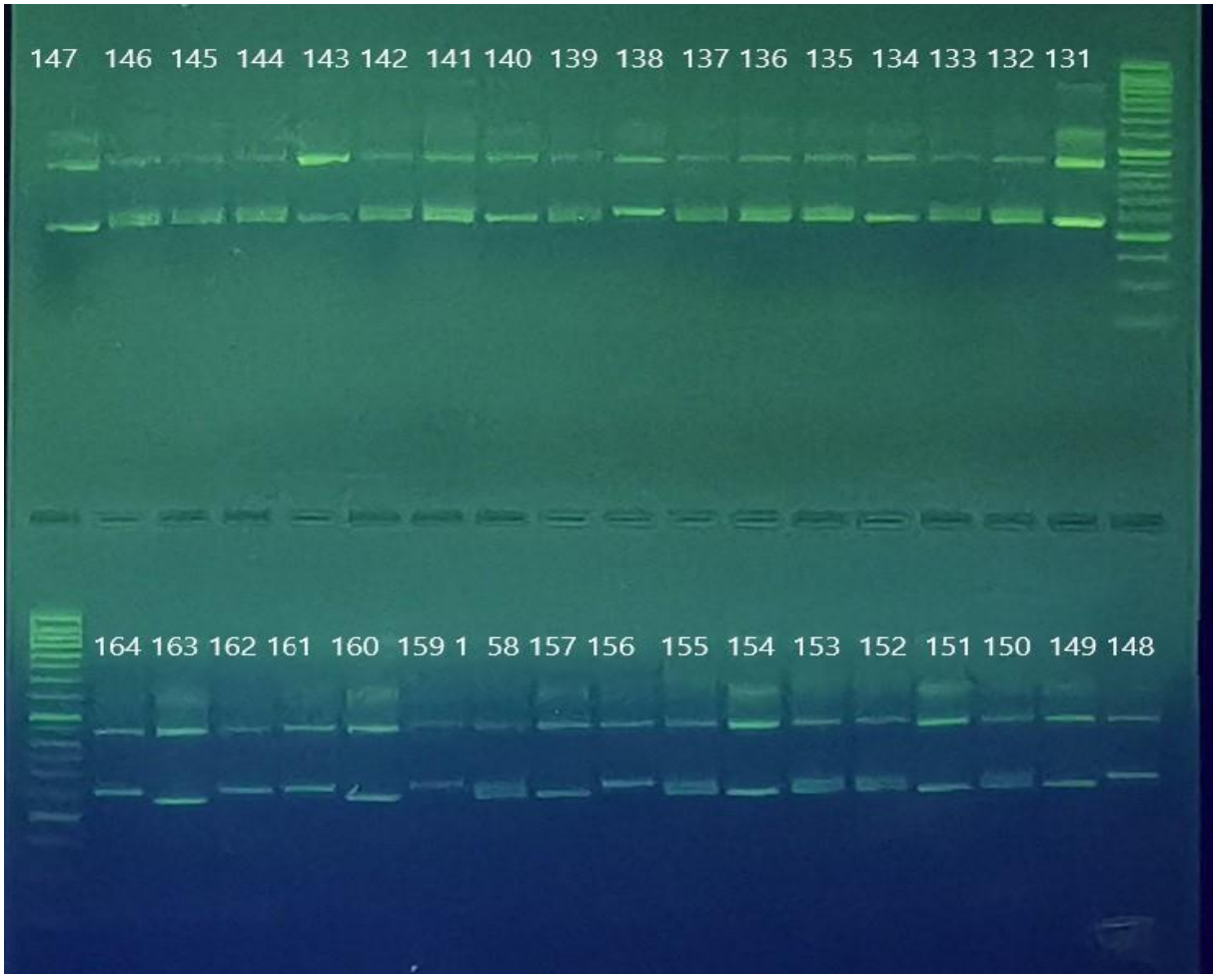

Figure 4. 10 PCR products of rs9939609 (FTO gene) shows three genotypes formation TT, TA and AA. The product size for the T allele is 242 bp and for the A allele 280 bp, the outer primer product size 473 bp. The first lane is a 50 bp ladder. Samples number 138, 148, 156, 159, 161, 162 and 164 were homozygous AA (risk allele), samples number 132, 135, 136, 137, 139, 141, 142, 144, 145, 146, 150, 152, 153, 155, and 158 were heterozygous TA, samples number 131, 133,134, 140, 143, 147, 149, 151, 154, 157, 160, and 163 were homozygous TT. The PCR products

were resolved on 2.5% agarose, stained with safe gel stain, and ran at 110 volts for 1 hour.

4.7 DNA sequencing of RS9939609

To validate the accuracy of the PCR method used for genotyping rs9939609, PCR products from 4 samples (112, 113, 116, and 117) were sequenced by Sanger sequencing method (Applied Biosystems 3730xl). The sequencing results of samples number 116 and 117 were heterozygote TA, sample number 113 was homozygous AA (risk allele), sample number 112 was homozygote for TT (figure 4.11). All the sequencing results were similar to the genotyping method (see figure 4.9).

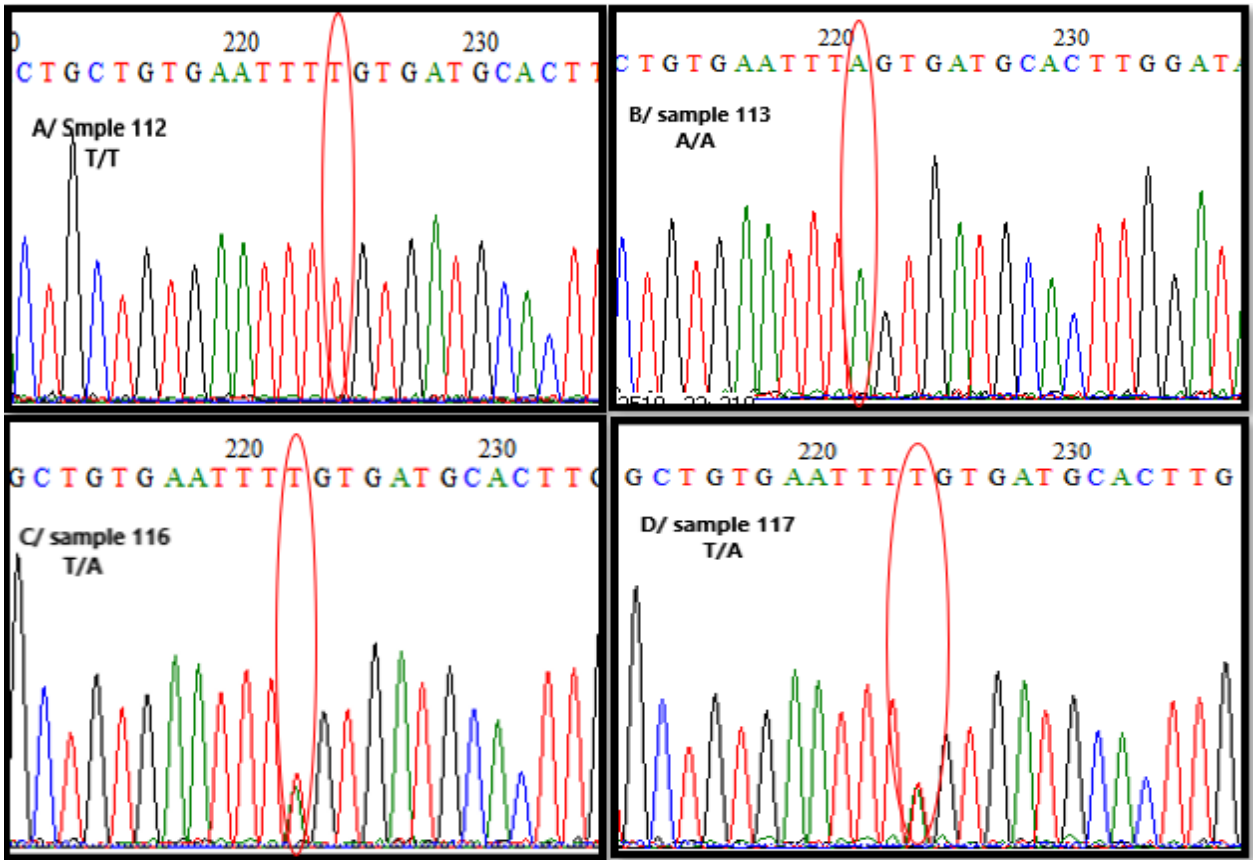

Figure 4. 11 FTO rs9939609, Sanger sequencing traces of four genotypes that matched the PCR method, (A) shows sample 112 in which the genotype is TT, (B) shows sample 113 in which the genotype is the risk alleles AA, (C) shows 116 in which the genotype is TA, and (D) shows sample number 117 in which the genotype is TA. All the four genotypes are indicated by red circles.

4.8 Genotype and allele frequencies

4.8.1 Allele frequency of rs1801282 in the *PPAR* gene and rs9939609 in the *FTO* gene

Allele frequency of rs1801282 in the *PPARG* gene and rs9939609 in the *FTO* gene are listed in (table 4.2). The frequencies of rs1801282 (C) allele were 98%, 90%, 94%, in cases, control groups, and all status respectively. the G allele (risk allele) was 2%, 10%, 6% in cases, controls, and all status respectively. Allele frequencies of rs9939609 in *FTO* (T) allele were 58%, 60% and 59% in case, control, and all status respectively. the A allele (risk allele) were 42%, 40% and 41% in cases, controls, and all status respectively.

Table 4. 2. Allele frequency of rs1801282 in *PPARG* gene and rs9939609 in *FTO* gene.

Allele frequency of rs1801282 in *PPARG* gene						
	All subjects		Case		Control	
Allele	Count	Proportion	Count	Proportion	Count	Proportion
C	377	0.94	196	0.98	181	0.9
G	23	0.06	4	0.02	19	0.1
Allele frequency of rs9939609 in *FTO* gene						
	All subjects		Case		Control	
Allele	Count	Proportion	Count	Proportion	Count	Proportion
T	237	0.59	117	0.58	120	0.6
A	163	0.41	83	0.42	80	0.4

4.5.2 Genotype frequency of rs1801282 and rs9939609

Genotype frequencies of rs1801282 in the *PPARG* gene and rs9939609 in the *FTO* gene are listed in (table 4.3). Genotype frequencies of rs1801282 C/C were 96%, 84% 90%, in case, control groups, and all status respectively, C/G were 4%, 13%, 8%, in case, control groups, and all status respectively, G/G were 0%, 3%, 2%, in case, control groups and all status respectively. While Genotype frequencies of rs9939609 A/A 20%, 19% 20% case, control groups, and all status respectively, T/A were 43%, 42%, 42%, case, control groups and all status respectively, T/T were 37%, 39%, 38% case, control groups and all status respectively.

Table 4. 3. Genotype frequency of rs1801282 in *PPARG* gene and rs9939609 in *FTO* gene

Genotype frequency of rs1801282 in *PPARG* gene						
	All subjects		Case		Control	
Genotype	Count	Proportion	Count	Proportion	Count	Proportion43%
C/C	180	0.9	96	0.96	84	0.84
C/G	17	0.08	4	0.04	13	0.13
G/G	3	0.02	0	0	3	0.03
Genotype frequency of rs9939609 in *FTO* gene						
	All subjects		Status=ca		Status=co	
Genotype	Count	Proportion	Count	Proportion	Count	Proportion
A/A	39	0.2	20	0.2	19	0.19
T/A	85	0.42	43	0.43	42	0.42
T/T	76	0.38	37	0.37	39	0.39

4.5.3 Hardy-Weinberg equilibrium (HWE) of rs1891282 and rs9939609

The genotype frequency for SNP rs9939609 was in HWE for case and control groups (P>0.05) (table 4.4). The genotype frequency of SNP rs1801282 was in HWE for the cases (P> 0.05), while there was a slight deviation from the HWE in the control group (p-value = 0.039) (table 4.5.3)

Table 4. 4. Hardy-Weinberg equilibrium for rs1801282 in *PPARG* gene and rs9939609 in *FTO*.

Hardy-Weinberg equilibrium for rs1801282 in *PPARG* gene(n=200)	
	P-value
All subjects	0.019
Case	1
Control	0.039
Hardy-Weinberg equilibrium for rs9939609 in *FTO* gene(n=200)	
	P-value
All subjects	0.11
Case	0.3
Control	0.22

4.9 Association analysis for SNPs rs1801282 and rs9939609 with T2D

In the current study, association of both SNPs with T2D was conducted using logistic regression models. Different mode of inheritance (codominant, dominant, recessive, over-dominant, and log-additive) were compared together. To choose the best mode of inheritance that fit the data compared to the most common one (co-dominant), the lowest AIC (Akaike information criterion) and BIC (Bayesian information criterion) were used (Franckowiak et al., 2017)

4.9.1 Association analysis of rs1801282 with T2D before adjustment of age, sex, BMI

Before adjustment by sex, age and BMI the best mode of inheritance for SNP rs1801282 was the log-additive model. The logistic regression analysis showed significant association between the SNP and T2D (p-value=0.002), (OR=4.22, 95%CI:1.44-12.35) (table 4.5).

Table 4. 5. Rs1801282 association with T2D before adjustment by sex, age and BMI.

Model	Genotype	Status=ca	Status=co	OR (95% CI)	P-value	AIC	BIC
Codominant	C/C	96 (96%)	84 (84%)	1.00	0.0068	273.3	283.2
	C/G	4 (4%)	13 (13%)	3.71 (1.17-11.83)			
	G/G	0 (0%)	3 (3%)	NA (0.00-NA)			
Dominant	C/C	96 (96%)	84 (84%)	1.00	0.0035	272.7	279.3
	C/G-G/G	4 (4%)	16 (16%)	4.57 (1.47-14.21)			
Recessive	C/C-C/G	100 (100%)	97 (97%)	1.00	0.04	277.1	283.7
	G/G	0 (0%)	3 (3%)	NA (0.00-NA)			
Overdominant	C/C-G/G	96 (96%)	87 (87%)	1.00	0.02	275.8	282.4
	C/G	4 (4%)	13 (13%)	3.59 (1.13-11.41)			
Log-additive	---	---	---	4.22 (1.44-12.35)	0.002	271.7	278.3

4.9.2 Association analysis of rs1801282 with T2D after adjustment of age, sex, BMI

After adjustment by sex, age and BMI the best mode of inheritance for SNP rs1801282 was the log-additive model. The logistic regression analysis showed significance association between the SNP and T2D (p-value=0.0045), (OR=3.96, 95%CI:1.31-11.94) (table 4.6).

Table 4. 6. Rs1801282 association with T2D after adjustment by (Sex +Age +BMI).

Rs1801282 association with T2D (n=200, adjusted by Sex +Age +BMI)							
Model	Genotype	Status=ca	Status=co	OR (95% CI)	P-value	AIC	BIC
Codominant	C/C	96 (96%)	84 (84%)	1.00	0.015	266.4	286.2
	C/G	4 (4%)	13 (13%)	3.52 (1.07-11.54)			
	G/G	0 (0%)	3 (3%)	NA (0.00-NA)			
Dominant	C/C	96 (96%)	84 (84%)	1.00	0.0072	265.7	282.2
	C/G-G/G	4 (4%)	16 (16%)	4.24 (1.33-13.52)			
Recessive	C/C-C/G	100 (100%)	97 (97%)	1.00	0.062	269.4	285.9
	G/G	0 (0%)	3 (3%)	NA (0.00-NA)			
Overdominant	C/C-G/G	96 (96%)	87 (87%)	1.00	0.03	268.2	284.7
	C/G	4 (4%)	13 (13%)	3.41 (1.04-11.18)			
Log-additive	---	---	---	3.96 (1.31-11.94)	0.0045	264.8	281.3

4.9.3 Association analysis of rs9939609 with T2D before adjustment of age, sex, BMI

The best mode of inheritance for SNP rs9939609 was Dominant, Recessive, Overdominant, and Log-additive models before adjustment by sex, age and BMI. However, there was no significance differences between the case and control groups, for the SNP rs9939609 (table 4.7)

Table 4. 7. Shows rs9939609 association with T2D before adjustment by age, sex, BMI.

Rs9939609 association with T2D (n=200)							
Model	Genotype	Status=ca	Status=co	OR (95% CI)	P-value	AIC	BIC
Codominant	T/T	37 (37%)	39 (39%)	1.00	0.96	283.2	293.1
	T/A	43 (43%)	42 (42%)	0.93 (0.50-1.72)			
	A/A	20 (20%)	19 (19%)	0.90 (0.42-1.95)			
Dominant	T/T	37 (37%)	39 (39%)	1.00	0.77	281.2	287.8
	T/A-A/A	63 (63%)	61 (61%)	0.92 (0.52-1.63)			
Recessive	T/T-T/A	80 (80%)	81 (81%)	1.00	0.86	281.2	287.8
	A/A	20 (20%)	19 (19%)	0.94 (0.47-1.89)			
Overdominant	T/T-A/A	57 (57%)	58 (58%)	1.00	0.89	281.2	287.8
	T/A	43 (43%)	42 (42%)	0.96 (0.55-1.68)			
Log-additive	---	---	---	0.95 (0.65-1.38)	0.77	281.2	287.8

4.9.4 Association analysis of rs9939609 with T2D after adjustment of age, sex, BMI

The best mode of inheritance after adjustment by same covariates (age, sex, BMI) was over-dominant. However, there was no significance differences between the case and control groups, for the SNP rs9939609 (p-value= 0.39, OR = 0.77, 95%CI: (0.42-1.40) (4.8).

Table 4. 8. rs9939609 association with T2D after adjustment by Sex + Age + BMI)

rs9939609 association with T2D (n=200, adjusted by Sex +Age +BMI)							
Model	Genotype	Status=ca	Status=co	OR (95% CI)	P-value	AIC	BIC
Codominant	T/T	37 (37%)	39 (39%)	1.00	0.66	274.1	293.9
	T/A	43 (43%)	42 (42%)	0.74 (0.38-1.43)			
	A/A	20 (20%)	19 (19%)	0.89 (0.39-2.00)			
Dominant	T/T	37 (37%)	39 (39%)	1.00	0.43	272.3	288.8
	T/A-A/A	63 (63%)	61 (61%)	0.78 (0.43-1.44)			
Recessive	T/T-T/A	80 (80%)	81 (81%)	1.00	0.91	272.9	289.4
	A/A	20 (20%)	19 (19%)	1.04 (0.50-2.16)			
Overdominant	T/T-A/A	57 (57%)	58 (58%)	1.00	0.39	272.2	288.6
	T/A	43 (43%)	42 (42%)	0.77 (0.42-1.40)			
Log-additive	---	---	---	0.91 (0.61-1.36)	0.64	272.7	289.2

CHAPTER FIVE

DISCUSSION

5. DISCUSSION

This study aimed to identify the association of variants rs1801282 and rs9939609 (located in the *PPARG* and *FTO* genes, respectively) with T2D in Kurdistan Region/Iraq. For this purpose, a total of 200 samples were enrolled, 100 were T2D and 100 were non-diabetic controls. These variations were chosen due to their significance as adiposity mediators. The analysis demonstrated that the variant rs1801282 was associated with the risk of T2D under control of log-additive model GG genotype have a double risk than heterozygous GC genotype, nevertheless rs9939609 was not associated with the risk of T2D in Kurdistan Region.

GWAS discovered thousands of SNPs over the past ten years. Linking between a particular gene and diseases, some of these studies failed to bring definitive results. But identification of the SNP in genes and understanding the pathway implicated in particular diseases gives a strong route to new treatment, better diagnosis and better protection from disease (20 and A. 2, 2007).

A robust and accurate genotyping method is an important step to proceed with case-control association studies (Chen et al., 2017). There is no 100 % correct genotyping method, genotyping error raise mistakes in disease association studies, one way to identify the genotyping error is to test for Hardy-Weinberg equilibrium (HWE) in the study samples, specifically the control groups (Gordon and Ott, 2000). HWE relay on the predictions in huge, random population, genotype frequencies must interact with HWE proportions. Any small deviation from these ratios is due to many reasons, one of these reasons is genotyping error. However, there are many other reasons, including copy number variation (CNV) (Lee et al., 2008, Wang and Shete, 2012).

The case and control groups for SNP rs9939609 were in HWE, indicating an accurate genotyping method. However, the Chi square test for SNP rs1801282 showed a slight deviation from the HWE in the control group (*p-value*= 0.039), which might indicate a loss of heterozygosity. Yet, this possibility was excluded in the current study for several reasons. First, the p-value threshold for a SNP not in HWE ranges between 0.001- 5.7 x 10^{-7} (Clarke et al., 2011, Anderson et al., 2010). The p-value for rs9939609 was 0.039, which is below the threshold. Second, duplicate samples were genotyped and all gave the same results which were clarified in the results section (see figure 4.6). Third, the PCR method for both variants were validated by using Sanger sequencing method (see figure13 and figure20), all the sequenced samples were consistent with the genotyping method by the allele specific PCR. Finally, the error rate in this study is very low, in addition to the fact that our sample size is small, hence it is not an indication for a genotyping error in our study(Leal, 2005).

In this study both SNPs were selected and been analyzed in the Kurdish population due to their importance by increasing the risk of T2D in different populations worldwide (Binh et al., 2013). The diabetic samples in this study were collected depending on the HbA1c, FBS, insulin and the history of the patients. The association analysis demonstrated that the SNP rs1801282 was associated with the risk of T2D before adjustment by sex, age, and BMI (*p-value = 0.002*, OR=4.22, 95%CI. 1.44-12.35), and after adjustment by age, sex, and BMI (*p-value* = 0.0045, OR= 3.96, 95%CI1.31-11.94). The analysis indicated that rs1801282 allele increase the risk of T2D in the Kurdistan region population similar to study in German on 433 T2D cases and 438 controls, the study predicted that rs1801282 was associated with increasing the risk of T2D (*p- value*<0.05) (Završnik, 2019). This prediction was suggested in American-Caucasian population (*p- value= 0.002)* which indicate

that rs1801282 increase the risk of T2D (Florez et al., 2007). This suggestion is Similar to other deferent population Hong Kong (ERIKSSON et al., 2007), Indian (Chauhan et al., 2010), Pakistani (Rees et al., 2011), Finnish (Bo and Jie, 2009), Arab (Wakil et al., 2006), and Tunis (Rees et al., 2011).

There is other predictions about rs1801282 in other studies, these studies suggesting that rs1801282 is linked to reduce the risk of T2D like Japanese population showed *p-value* less than 0.005 with OR= 0.413, 95% CI;0.220–0.735) (Hara et al., 2000). Similar to studies in Korea (Moon et al., 2005), Iran (Meshkani et al., 2007), Scotland, Danish (Doney et al., 2004, Frederiksen et al., 2002), and French populations (Jaziri et al., 2006). The reason of rs1801282 being protective against T2D is due to involving of the SNP in improving insulin resistance. Other side of rs1801282 is considered important due to other studies results on different population suggesting that the SNP is not associated with increasing risk of T2D or with the protection against the disease. This theory was found in South Indian and Bangladeshi populations (Pattanayak et al., 2014), (Saleh et al., 2016).

For the SNP rs9939609, the analysis showed no significant differences between the cases and control groups (*p-value*= 0.39). Similar a meta-analysis in east Asian populations suggested that rs9939609 is related to obesity not to predictions of future T2D, this is due to several reasons including environmental factors, dietary habits, different physical activity, these reasons may interact with the association between rs9939609 and T2D, in addition the small sample size may interfere the statical power (Bo and Jie, 2009). While several studies found that rs9939609 was associated with the risk of T2D in different population, such as a study in Pakistan on women (Shahid et al., 2013), in Vietnamese population (Binh et al., 2013), Iran (Khoshi et al., 2019), and Kuwait population(Al-Serri et al., 2018).

The lack of association between rs9939609 in *FTO* gene and T2D in our study might be due to the small sample size or this variant has no effect on the risk of T2D in the Kurdish population. These discrepancies in the relations between SNPs and disease among different populations might be due to the diversity in the genetic background of each population and exposure to different environmental factors (Ellegren and Galtier, 2016)

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6. CONCLUSION

In conclusion this study showed that the

1- Variant rs1801282 in *PPARG* gene increase the risk of T2D in the Kurdistan region.
2- Variant rs9939609 in *FTO* gene was not associated with T2D in the Kurdistan region.
3- Genotype and allele frequencies were identified which indicate a different frequency from other populations this may be due to diversity in life style, environment, genetics and epigenetics factors.
4- These finding leads us to understand that the genetic background of individuals in Kurdistan region differ from other population
5- Risk factors implicate genetic background of individuals in a different way

7. RECOENDATIONS

1- Further studies are needed to investigate other SNPs that influence future T2D in Kurdistan region.

2- Additional studies is required with a larger sample size and better technique.

3-Necessary to do more studies on risk factors that interfere the genetic background that effect T2D in Kurdistan region.

4- Better labs for genetic should be provided in Kurdistan region.

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Appendix

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Date: 4 Apr. 2023 **Manuscript #:** 1254

Authors: **Sally Khalil Baqer[1]; Nzar Ali Ameen Shwan[1]**

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Article Acceptance Letter

Article Title: **PPARG and FTO gene variants and their association with type 2 diabetes in the Kurdish population**

We are pleased to inform you that your article paper has been peer reviewed and **accepted** by our editorial board for publication in the *Passer* journal of basic and applied sciences on (http://passer.garmian.edu.krd/). The article will be published online after uploading the 'corrected proof' version of the article in issue 1, volume 5, which will be released in June 2023.

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ERBIL POLYTECHNIC UNIVERSITY

فرەجۆری FTO و PPARG پەیوەندی

لەگەڵ جۆری دووەمی شەکرە لە ناوچەی کوردستان

تێزێک

پێشکەش بە ئەنجوومەنی بەشی تەکنەلۆژیای تاقیگەی پزیشکی، کۆلێژی تەکنیکی تەندروستی و پزیشکی هەولێر، زانکۆی پۆلیتەکنیکی هەولێر بۆ وەرگرتنی بڕوانامەی ماستەر لە زانستی تەکنەلۆژیای تاقیگەی پزیشکی

لە لایەن

سالی خەلیل باقر

مایکرۆبایۆلۆجی پزیشکی- زانکۆی کۆیا – B.Sc. ٢٠١٦

سەرپەشتیکراوە لەلایەن

دکتۆر نزار علی امین شوان

هەولێر-کوردستان

٢٠٢٣

گوڵان٢٧٢٣ **نیسان ٢٠٢٣** **شە وال ١٤٤٤**

پوختە

جۆری شەکرەیە ، ئەم تاریکیە کاتێک دروست دەبێت کە لە ـــــ (T2D) جۆری دووەمی شەکرە بەنکریاس بریکی زۆر ئەنسۆلین بەرهەم دەهێنیت کە دەبێتە هۆی بەرگری ئەنسۆلین لە لایەن خانەکانی نەخۆشەکە ،ئەمەش بەهۆی کارلیکی نێوان هۆکارە بۆ ماوەیی یەکان و هۆکارە ژینگەی یەکان دروست دەبێت .

و چەندین هۆکاری گۆرانی T2D هۆکارە بۆماوەیی یەکان بەشدارن لە گەشەکردنی جۆری دووەمی شەکرە بۆ ماوەیی بەشدارن لەم ناریکیە ، وە ئەبنە هۆی زیاد بوونی ترسی ئەم نەخۆشی یە هەمە (چەشتی) لە دەرکەوتووە کە پەیوەندیان بە نەخۆشی (FTO) لەجینی rs9939609 و PPAR لەجینی rs1801282 NSNP-ـــــ شەکرە جۆری (٢) لەناودانیشتواندا .ئامانج لەم توێژینەیە دیاری کردنی پەیوەندی لە سەر نەخۆشی شەکرەی جۆری دوو لە کوردستانی FTO لەrs993960 و PPARG لە rs1801282 عیراقدا لەم توێژینە ـــــ- خوێن (٢٠٠) کەسی ناپەیواندار لە دانیشتوانی کوردستانی عیراق وەرگیرا بۆ ئەم نەخۆشی جینوتایپیان بۆکرا بەبەکارهێنانی (و ١٠٠ کەسی تەندروست T2D نەخۆشی 100) (AS PCR) بۆ پشت راستکردنەوەی بۆ پرۆسەی (SNP) بۆ هەموو دوو جینەکە Allele specific PCR ـــــ(sanger seg…) ، شێوازیەک خستنی

بۆ هەردوو rs1801282 لەکەسانی (و تەمەن ورمگەز BMI) جیاوازی بەرچاو هەبوو لەپەیوەندی نیوان) زۆرترە T2D دەرکەوت کە ئەم دوو جینوتایپیە مەترسی تووشی بوون بەنەخۆشی GCو GC جینوتایپی لەنێوان ـــــــ کۆنترۆل ($p<0.05$) کە جیاوازی بەرچاوی نەبوو (cc) لەجینوتایپی ($p<0.05$ هەشتاریکی PPARG لە جینی rs1801282 ونەخوشەکان دەرئەنجامەکان دەریان خست کەقرەچەشنی لە لە پیکهانەی کوردی لە (T2D) بۆ ماوەیی یان هەیە (یان ئامادەباشی بۆ ماوەیان) هەیە بۆ نەخۆشی پەیوەندی بەر چاوی تی بۆ ئەم نەخۆشی یە .بۆ زیاتر FTO کوردستانی عیراق بەلام قرەچەشتی لە جینی ـــــــ ئەم ـــــ- خانە زیاتر توێژینەوە و وەرگرتنی گونەی زیاتر پیویستە بکرێت

رابطة تعدد الأشكال FTO و PPARG

مع مرض السكري من النوع ٢ في إقليم كوردستان

أطروحة

مقدم الى مجلس الكلية الفنية الطبية والصحية في اربيل في جامعة التقنية اربيل في استسفاء جزئي لمتطلبات درجة الماجستير في تكنلوجيا المختبرات الطبية

بواسطة

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اربيل - كودستان

أبريل ٢٠٢٣ شوال ١٤٤٤ جولان ٢٧٢٣

الخلاصة

مرض السكري نوع الثاني هو الشكل الاكثر شيوعا لمرض السكري. يتطور عندما تنتج خلايا البنكرياس كمية زائدة من الأنسولين ، مما يؤدي إلى مقاومة الأنسولين بواسطة الخلايا المحيطية.

وهو مرض ناجم عن التفاعل بين العوامل الوراثية والبيئية. تشارك العوامل الوراثية في تطوير T2D والعديد من المتغيرات الجينية تزيد من خطر الإصابة بلمرض. تم اكتشاف أن تعدد أشكال النوكليوتيدات المفردة بزيادة نسبه خطوره الاصابه بلمرض rs1801282 (SNPs) في جين PPARG و rs9939609 في مجموعات سكانية مختلفة.

يهدف هذا البحث الى اكتشاف ما اذا كانت الطفرات الوراثية في هذين الجينين مسؤولان عن زيادة خطر الاصابه بلمرض في سكان اقليم كردستان

في الدراسه الحاليه تم استخلاص الحمض النووي من ٢٠٠ شخص ١٠٠ شخص مصاب بلسكري نوع الثانس و١٠٠ شخص غير مصاب بلمرض باستخدام تفاعل البوليمرايز المتسلسل بلاليلات الخاصة لكل طفرة وتم التحقق من دقة تفاعل ال بي سي ار عن طريق سنكر سكونسك.

اضهر تحليل الارتباط بحسب الجنس والعمر ومؤشر كتله الجسم فروقا ذات دلاله احصائية بين الحالات وطفره رس ١٨٠١٢٨٢ بلانماط الجنينيه الثالاثة قيمة بي ٠,٠٠٤٥ علر عكس الطفره الثاني رس ٩٩٣٩٦٠٩ قيمة ال بي ٠,٣٩

اشارت النتائج ان رس١٨٠١٢٨٢ يزيد من خطر الاصابة بمرض السكري نوع الثاني في منطقة كردستان بينما الطفرة الثانيه رس ٩٩٣٩٦٠٩ غير مسؤولة عن زيادة خطر الاصابه بلمرض في سكان الاقليم .مطلوب مزيد من التحقيقات مع عينات عدد أكبر للتحقق من صحة هذه النتائج.