Molecular Characterization of Type 2 Diabetes Mellitus by Single Nucleotide Polymorphism of Transcription Factor 7 Like 2 Gene

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Abstract

Type 2 diabetes mellitus (T2DM) is a multifactorial disease with a strong genetic component interacting with environmental factors. Many genes have been significantly associated with developing type 2 diabetes mellitus. Most of these genes have been linked to beta-cell dysfunction, impaired glucose homeostasis and insulin secretion. Transcription factor-7–like 2 (TCF7L2) gene has been found as an unexpected suspect for type 2 diabetes. The strongest and most commonly associated alleles of the TCF7L2 gene in T2DM in many countries are rs7903146 and rs12255372.

Aim: *The aim of this study was to evaluate the association between TCF7L2 gene in T2DM among Palestinian people. Two SNP's rs7903146C/T, rs12255372G/T alleles in the TCF7L2 gene were investigated in diabetic patients and control groups.*

Methods: *This is a case control study. A total of 326 participants were included in this study; 249 participants with T2DM and 77 normal glycemic controls. RFLP PCR was performed using two restriction enzymes RsaI and BseGI to identify the presence of the two specific mutations in the alleles of the TCF7L2 gene among the study population. Allele specific PCR was also performed to substitute for DNA sequencing on one hand and to genotype the TCF7L2 gene as homo or heterozygous. We used SPSS v.21 to compare the results obtained of the case and control groups.*

Results: *There was a strong association between the two SNP's rs7903146C/T, rs12255372G/T alleles and T2DM. Both alleles have statistically significant association with the disease. Each of the two alleles had stronger association with T2DM when tested alone than when both alleles were combined. We observed that several normal participants carried the SNP in one or both alleles. This indicates the possibility of future development of diabetes.*

Conclusion: *TCF7L2 gene is strongly associated with T2DM. This important finding can be utilized in screening the population at risk of developing diabetes. Furthermore, genetic testing can also be applied to the normal population to determine who can be prone to develop diabetes in the future.*

I. INTRODUCTION

Type 2 diabetes (T2D) is the most common form of diabetes mellitus (DM), it accounts for 90–95% of diabetic cases [1], it is characterized by hyperglycemia, insulin resistance, and relative insulin deficiency rather than absolute insulin deficiency. In Type 2 diabetes, the pancreatic β cells become progressively less able to secrete sufficient insulin to maintain normal carbohydrate and lipid homeostasis [2] T2D has become an epidemic in the 21st century. Unfortunately, the number of people with T2D diabetes is increasing in every country and the prevalence will continue to increase globally. It is projected that by 2030 the number would increase to 552 million. The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing obesity and physical inactivity[3]In Palestine in 2004, the reported prevalence in Palestinian Family Health Survey was 10.6% (8.7–12.5) versus an estimated 11.4% (9.7–13.4); in 2006, these values were 11.8% (9.8–13.8) and 12.3% (10.6–14.6), respectively and by 2010, the prevalence of type 2 diabetes had increased to 14.5% (12.2–16.7), in this period, prevalence in men rose from 11.7% (9.7–13.6) to 15.9% (13.4–18.1) and in women from 11.4% (9.3–13.3) to 13.2% (11.1–15.2). The forecasts for prevalence of diabetes are 20.8% (18.0–23.2) for 2020 and 23.4% (20.7–25.8) for 2030 [4] Diabetes or hyperglycemia over many years leads to damage to several tissues in the body, producing so-called diabetic complications and they are separated into macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, retinopathy and dermopathy)[5].

There are many different causes of this form of diabetes. Although the specific etiologies are unknown, type 2 diabetes can be caused by environmental (include behavioral factors) and genetic factors, insulin sensitivity and insulin secretion [6, 7].Although genes that predispose an individual to diabetes are considered to be an essential factor in the development of the disease, activation of a genetic predisposition is usually triggered by environmental factors, particularly those who are overweight with low physical inactivity and gestational diabetes [8].

Many genes have been significantly associated with developing type 2 diabetes, until 2011 more than 36 genes have been found that contribute to the risk of type 2 diabetes, most of the discovered gene variants have been linked to beta-cell dysfunction, impaired glucose homeostasis and insulin secretion rather than insulin resistance [9].Prior to 2006, only two genes were implicated in type 2 diabetes (PPARG 1 and KCNJ11) [10]. After 2006, transcription factor-7–like 2 gene (TCF7L2) has been found as an unexpected suspect for type 2 diabetes by the DECODE group in Iceland [11]. They reported that a common microsatellite in TCF7L2 gene region DG10S478 within intron 3 was associated with type 2 diabetes in Icelandic case control sample. Two noncoding SNPs rs7903146 (C/T), and rs12255372 (G/T) were in strong linkage disequilibrium with DG10S478 (r^2=o.95 and 0.78 respectively) [12]. They then genotyped three extra single nucleotide polymorphisms (SNPs) with the strong correlation to DG10S478 (rs7901695, rs11196205, and rs7895340) and showed association between all three SNPs and type 2 diabetes but the rs7903146 and rs12255372 were the strongest[13,14] .Subsequently, this association was confirmed in other populations such as Indians [15], French[16], Tunisians [17], Japanese [18], Mexican Americans [19], and West Africans [20]Amish [21], Polish [22] Chinese[23], Swedish[24] , Persian [25],and Palestine [26],all of

these studies showed a strong association between TCF7L2 polymorphism and type 2 diabetes.

while the specific mechanism driving the development of type 2 diabetes remains unclear, there is sufficient evidence to demonstrate that TCF7L2 variants strongly predict the development of type 2 diabetes and/or the progression to diabetes from impaired glucose tolerance [27,28] . TCF7L2 is a transcription factor and key component of the Wingless-type integration site family (Wnt) signaling pathway, which exerts many important physiological and pathophysiological functions in different cell lineages and organs [29] .

Potential mechanisms by which TCF7L2 variants influence type 2 diabetes include its role in adipogenesis, myogenesis, and pancreatic islet development, as well as transcription of the genes for proglucagon and the glucagon-like-peptides GLP-1 and GLP-2, which play a role in post-prandial insulin secretion [30].

In this study we will focus on the Transcription Factor7Like2 gene (TCF7L2). It is a candidate gene which strongly related type 2 diabetes (T2D) locus identified to date [31]. To determine the rate of SNP rs7903146, and rs12255372 in patients with T2DM and compare that with normal non-diabetic controls. To determine the significance of the two SNPs individually and combined in T2DM patients as compared to controls. And to determine the possibility of conducting genetic testing on TCF7L2 gene in predicting possible development of diabetes in healthy people.

II. MATERIALS AND METHODS

A case-control study was used , a total of 326 blood samples included 249 type 2 diabetic patients and 77 healthy controls without diabetes were collected from the West Bank and East Jerusalem during 2011 to 2012 ,the distribution of the samples is summarized in Table **(1),** all samples were stored at -80 ^{o} C until further DNA purification and subsequent molecular applications. Informed consent was obtained from each participant, data collected for each participant included: age, gender, BMI, FBS, total cholesterol, blood pressure and family history of 1st degree relatives with T2D. The DNA was extracted from whole blood by using the salting out method.

III. GENOTYPING

Polymerase chain reaction (PCR) was used for amplification of the two SNPs rs7903146 and rs12255372 under the same conditions by using different annealing temperature, the primers used for: **rs7903146** amplification 5’TTAGAGAGCTAAGCACTTTTTAGGTA-3’(forward) and 5’ACTAAGTTACTTGCCTTCCCTG-3’ (reverse)

for **rs12255372** 5’-CCCAGGAATATCCAGGCAAGGAT-3’(forward)

5’-CAAATGGAGGCTGAATCTGGCA-3’ (reverse) [32]

Approximately120 base pairs the PCR products for both SNPs, PCR products were then digested with the restriction enzyme ***RsaI*** for rs7903146 and ***BseGI*** for rs12255372 , The C allele creates a restriction site and gives two fragments less than 100 base pairs in non-mutated genes after digestion with the restriction enzymes,3% Agarose gel was used to separate the restriction fragments. . Finally Allele-specific polymerase chain reaction (ASPCR) was used instead DNA sequencing, this allowed us to confirm the presence of the mutations detected previously by the RFLP PCR as follow: **For RS rs7903146**

Forward Normal:
AGAGAGCTAAGCACTTTTTAGATA**C**
Forward Mutated:
AGAGAGCTAAGCACTTTTTAGATA**T**

FOR rs12255372

Forward Normal: ccaggaatatccaggcaagaat**g**

Forward Mutated: ccaggaatatccaggcaagaat**t**

IV. STATISTICAL ANALYSIS

The data were analyzed using the SPSS v.21 program (Chicago, IL), the independent-samples t-test was used to compare the differences between means, the logistic regression was used to predict disease status (diabetes) and to adjust for different variables (covariates). *P* -value less than 0.05 was considered significant.

V. RESULTS

By using Restriction Fragment Length Polymorphism – PCR (RFLP-PCR) for TCF7L2 gene to detect the presence of SNPs for both sites rs7903146 and rs12255372, there were 47.4% (118/249) of the cases tested were positive for the mutation **rs7903146** and 52.6 % (131/249) were negative. In the **controls,** there were 15.6% (12/77) positive and 84.4% (65/77) negative.

For **rs12255372**: in **cases** 37.8% (94/249) were positive, 62.2% (155/249) were negative and in **controls** 9.1% (7/77) were positive, 90.9% (70/77) were negative. By using the t-test ,mean age for cases and controls was significantly different ,mean age for the cases was 58.66 ±9.59 years and the controls was 42.99 ±8.12 years. In this study, there was no significant difference between cases and controls regarding the gender of the participants .Mean BMI, systolic blood pressure, family history of type 2 diabetes, fasting blood sugar FBS and total cholesterol all of them showed significant difference between cases and controls at 0.05 level of significance (P <0.005) except for the diastolic blood pressure was not statistically significant. All of these data are summarized in Table **(2)**. By using RFLP-PCR, after adjusting for all risk factors and exclusion family history and FBS the logistic regression yielded significant odds ratio suggesting that the risk alleles confers significant risk for developing T2DM with reference to both SNPs: rs7903146 and rs12255372. For rs7903146 odd ratio OR=3.0 (95%CI 1.24-7.24), rs12255372 OR= 5.5 (95%CI 1.97-15.19), There was significant difference at 0.05 level of significance (P <0.005) in the obese people only [OR= 3.5(1.3-9.5)]. Systolic blood pressure was significant OR =4.11(1.2-14.2) but diastolic was not significant OR=o.78 (0.6-9.8), and the age was significant for 51-70 years, OR =12.7(5.55-29.03), for 71-85years, OR = 28(3.3-233.3).Here the gender was not significant no difference between males and females to have the disease, the results of this analysis in table **(3).**

When studying the effect of each mutation alone after adjusting for other risk factors and exclusion of family history and FBS , for **rs7903146** it showed significant association with diabetes [OR=3.34(1.46-7.65)] , Comparing the following parameter for all participants

(cases and controls), BMI for the obese people, total cholesterol, systolic blood pressure ,and age all showed statistical significant results as shown in Table**(4)**.Statistical significant results were also observed indicating positive association with T2DM when testing the previously mentioned parameters for the second mutation r**s12255372 [**OR= 5.83(2.18-15.56)], results are shown in Table **(5).**

We analyzed both mutations simultaneously (**rs7903146** and **rs12255372)** in the total population tested for all the parameters with possible development of T2DM.Although we observed significant results the OR was 3.6(1.08-11.98), much smaller than when each mutation tested alone, OR was 15.52(5.42-44.5) as shown in Table **(6)**.

Allele specific PCR was used to detect the presence of SNPs on both alleles (homozygous genotype), or present in one allele only (heterozygous genotype), as shown in Table **(7)** and **(8).** Allele frequencies were also calculated, the allele specific frequencies were 0.1039 for rs7903146 T allele in the controls and three folds higher 0.297in the cases. For the second mutation rs12255372 T allele, the allele specific frequency was 0.065 in controls and about four folds 0.2168 in cases .OR was calculated for both SNPs to assess its association with type 2diabetes as shown in the same Tables (7) and (8) before and after adjustment for sex, age, BMI, systolic, diastolic blood pressure and total cholesterol as covariates, the results showed evidence of association of the rs7903146 T allele with T2D in agreement with previous studies. The OR for **heterozygous** CT before adjustment was 5.77 (2.6-12.6),*P* value 0.000, and after adjustment OR was 3.5(1.37-8.8), *P* value 0.009. The OR For **homozygous** before adjustment was 3.1(1.0-9.3), *P* value 0.043 and after adjustment was 3.7(1-13.5) *P* value 0.044.

For rs12255372 OR for **heterozygous** GT before adjustment was 5.12(2.3-11.8*) P* value 0.000, and after adjustment OR was 3.77(1.4-9.8) *P* value 0.007 giving significant results for both. For rs12255372 **homozygous** TT, it was significant before adjustment OR was 4.5(1-19) *P* value 0.047 but not significant after adjustment, OR was 3.6 (1-21) *P* value 0.15 thus it was not significant.

As a result, for all diabetic participants who carry a single mutation or both mutations in this research, Bethlehem took first place ,Ramallah Jerusalem then the North (Qalqelya, Tulkarem, and Nablus)

VI. DISCUSSION

This study aimed to assess the effect of TCF7L2 gene in T2DM in Palestinians, the main two SNP's rs7903146C/T, rs12255372G/T in the TCF7L2 gene were investigated in diabetic patients and compared to a control group. The results of the RFLP showed the presence of significant association at0.05 level of significance between the two investigated single nucleotide polymorphisms and T2DM. This is the first study that evaluated the association of two mutations with T2DM in this country, a study conducted by Abdeen et al. in 2010 in Al-Quds University [26] rs7903146C/T reported similar results as compared to ours. We used allele specific PCR to genotype the gene investigated instead of the sequencing analysis they used. Previous studies evaluated the association of both mutations as used by us conducted by researchers in the US, Poland, Amish population in the US ,Finnish, and French have similar results for rs7903146C/T, rs12255372G/T both of these SNPs are associated with T2DM[33,34,35,36,37,38]. The study performed on the association of TCF7L2 gene and T2DM on the Palestinian population by Abdeen et al., reported an association between the **rs7903146** T allele and T2DM as reported in this study. The population selected for Abdeen study was restricted to one geographic area, Ramallah, Palestine. Their samples were collected from the UNRWA clinics in the Ramallah area. Our study is more comprehensive and more representative of the Palestinian population and the different geographical regions of Palestine. Furthermore, our study addressed the two strongest and most commonly involved alleles in T2DM, while Abdeens study addressed only one allele.

A study conducted on Israeli Ashkenazi Jewish population on 2010[39] included **rs7903172** T allele ,indicating that this allele is strongly associated with T2DM in Palestinian population as compared to weak association among the Ashkenazi Jews. In a Mexican study conducted by Parra EJ, in 2007[32] reported significant association between rs12255372 while SNP rs7903146 showed similar trends, but its association with T2D is not as strong as rs12255372. This may reflect geographic distribution regarding the polymorphism of TCF7L2 gene.

The nature of TCF7L2 gene polymorphism differs from one geographic area and another. In China for example, they did not detect association of the risk allele (rs7904146 T and rs12255372 T) with type 2 diabetes as detected in our study. These risk alleles were found to be rare with low frequencies in the Chinese population [40]. A possible explanation for this result could be attributed to differences in the ethnic background among the Chinese population or the effects of environmental factors, such as life-style as well as geographical distribution of these alleles.

By using RFLP –PCR with exclusion family history and FBS because both are very significant and strongly related to type 2 diabetes so if both included in analysis the association between two mutations and the disease will be less significant thus both were excluded. The same for **rs7903146** after adjusting for other risk factors ,age, sex , BMI, total cholesterol, and blood pressure it showed significant association with diabetes OR=3.34(1.46-7.65, as shown in table (4).

The allele specific PCR for **rs12255372 T** allele has a higher frequency in the patients with T2D than the controls as shown in Table (8). It was noteworthy that the heterzygote genotype for this allele was statistically significant before and after adjustment for the confounding factors (risk factors, age, sex, BMI, total cholesterol, and blood pressure). Contrary to that, it was found that for the homozygous genotype of this allele was statistically significant before adjustment but not statistically significant after adjustment for the confounding factors. This can be due to scarcity of this mutation among the control group. Similar results have been reported by a Mexican study but on the **rs12255346 T** allele [32].

The interaction between mutations rs7903146 and rs12255372 on the *TCF7L2* gene and T2DM combined had an OR= 3.6(1.08-11.98) but the OR was 15.52(5.42-44.5) when these mutations were considered singly as shown in Table (6). The differences in the OR in these situations may be explained by the considering that every mutation works independently with distinct mechanism and it is not common to find both mutations together in the same individual. The exact mechanisms, by which genetic variation within the intron of the

TCF7L2 gene that confer susceptibility to Type 2 diabetes remain to be elucidated. Genetic variations near the 3′ end of the *TCF7L2* gene may affect the action of *TCF7L2*, through the regulation of alternative splicing [38].

There was difference in the mutation distribution between south to north Palestine, mainly Bethlehem (south) occupied the first place in diabetic patients who carry the mutations in TCF7L2 gene, many reasons may interact like high trend of positive family history for diabetes among the study population in the south may be due to consanguineous marriages contributing to the perpetuation of genetic disorders or adopting of adverse health behaviors like eating habits, smoking and other environmental factors that differ from one geographic region to another in Palestine, which contribute to development of diabetes disease when interacting with a susceptible gene.

A limitation of this study could be that the number of the control participants was relatively small and may have caused some bias when comparing age and gender, blood pressure and cholesterol, these confounding factors have been adjusted in the statistical analysis to obtain a more real picture of the association between T2DM and TCF7L2 gene.

As a conclusion, a significant association of the *TCF7L2* variant with type 2 diabetes risk was observed in Palestinians for both polymorphisms rs7903146 and rs12255372, but rs7903146 was more significant than rs12255372 between Palestinians. As known, the strongest association with T2DM risk in most reported studies is with rs7903146 that shows a stronger association with type 2 diabetes than rs12255372.

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Table (1) Distribution of samples on the different regions of the West Bank and Jerusalem

City	***Clinic***	***Specimens***
Jerusalem	*UNRWA Clinic – Indian Hospice*	*60*
Bethlehem – South West Bank	*UNRWA Clinic- Khamashta*	*58*
Ramallah – Central West Bank	*Ministry of Health Clinics*	*46*
	Diabetes Society – Al Bireh Center	*18*
North West Bank	*Nablus – Medicare Labs*	*15*
	Tulkarem – Ministry of Health Clinics	*11*
	Qalqelia – Ministry of Health Clinics	*41*
Controls	*Clinics throughout West Bank and Jerusalem*	*77*
TOTAL		***326***

Table (2) General characteristics for the study population (mean ± SD)

	total	Controls (n=77)	Cases (n=239)	*P*
Age	54.96 ± 11.4	42.99 ±8.12	58.66 ±9.59	0.000*
Gender				0.830
Males	111 (34%)	27(35.1)	84(33.7)	
Females	215 (66%)	50(64.9)	165(66.3)	
BMI	30.2 ± 5.68	26.49 ±3.54	31.34±5.73	0.000
FBS	152.05 ±70.16	86.79 ±9.85	172.22±68.48	0.000
T. Cholesterol	181.19 ± 38.9	168 ±19.96	185.26±41.45	0.000
Family history				0.000
Yes	220 (67.5%)	22(28.6)	198(79.5)	
No	106 (32.5%)	55(71.4)	51(20.5)	
BP (Sys)	127.34 ±17.77	118±11.25	130.22±18.44	0.000
BP (Dias)	78.64 ±10.41	76.49±7.93	79.07±11.01	0.350
Mutation 1 (rs7903146)				0.000
Yes	126 (38.7%)	12(15.6 %)	118(47.4%)	
No	200 (61.3%)	65(84.4%)	131(52.6%)	
Mutation 2 (rs12255372)				0.000
Yes	101 (31%)	7(9.1 %)	94(37.8%)	
No	225 (69%)	70(90.9%)	155(62.2%)	
Area				0.000
1(Beit-lahem)	58 (17.8%)	0	58(23.3%)	
2(Ramallah)	64 (19.6%)	0	64(25.7%)	

3(Jerusalem) 4(controls=Different areas) 5(North west bank)	60(18.4%) 77(23.4%) 67(20.4%)	0 77(100%) 0	60(24.1) 0 76(26.6%)	

Table (3):*Adjusted for all risk factors in table, for rs7903146 and rs12255372# P < 0.05 include and without family history and Fasting blood sugar (FBS)

	Number	**Crude OR (95% CI)**	**Adjusted OR (95%CI)* with FBS**	**Adjusted OR for all factors with family history except FBS**	**Adjusted OR for all factors without family history and FBS**
Mutation rs7903146 No (ref) Yes	 196 130	 1 4.88 (2.51-9.48)#	 1 4.31 (1.02-18.18)#	 1 2.1(0.81-5.2)	 1 3.0(1.24-7.24)
Mutationrs12255372 No (ref) Yes	 225 101	 1 6.065(2.68-13.74)	 1 0.41(0.08-2.1)	 1 3.3(1.12-9.1)	 1 5.5(1.97-15.19)
BMI (kg/m2) 18-24.9 25-29.9 30-55	 52 111 163	 1 1.78 (0.910-3.48) 9.86 (4.55-21.32)#	 1 2.26 (0.41-12.5) 7.83 (1.34-45.8)	 1 1.12(.4-3.2) 2.5(0.8-7.3)	 1 1.24(0.49-3.2) 3.5(1.3-9.5)
Total cholesterol(mg/dL) 40-239 240-400	 275 51	 1 19.01(2.6-140.7)	 1 27.2(1.7-434.7)	 1 12(1.2-122)	 1 13.9(1.6-122.67)
Diastolic(mmHg) 60-95 96-110	 310 16	 1 4.87(0.63-37.49)	 1 0.66(0.04-11.8)	 1 0.8(.06-9.3)	 1 0.78(0.6-9.8)
Systolic(mmHg) 90-139 140-210	 242 84	 1 15.8 (6.40-39.01)	 1 1.64 (0.4-7.2)	 1 2.5(0.7-8.7)	 1 4.11(1.2-14.2)
Gender Male Female	 111 215	 1 1.06(0.6-1.8)	 1 0.5(.15-1.7)	 1 1.05(0.45-2.5)	 1 0.92(0.416-2.04)
Age(years) 30-50 (1) 51-70 (2) 71-85(3)	 117 180 29	 1 22.0(10.5-45.9) 36.2(4.8-275.3)	 1 16.97(5.2-56) 44.4(3.8-521.6)	 1 14.9(6-36.6) 29(3.4-250.6)	 1 12.7(5.55-29.03) 28(3.3-233.3)
Family history No Yes	 106 220	 1 9.7(5.4-17.4)	 1 4.5(1.4-14.5)	 1 6.1(2.6-14.2)	 ----------

Table (4):*Adjusted for all risk factors in table, for rs7903146 # P < 0.05.

	Number	**Crude OR (95% CI)**	**Adjusted OR (95% CI)* with Family history**	**Adjusted OR (95% CI)*without family history**
Mutation(1)rs7903146 No (ref) Yes	 196 130	 1 4.88 (2.51-9.48)#	 1 2.28(0.93-5.58)	 1 3.34(1.46-7.65)

BMI (kg/m2) 18-24.9 25-29.9 30-55	 52 111 163	 1 1.78 (0.910-3.48) 9.86 (4.55-21.32)#	 1 0.99(0.36-2.72) 2.41(0.82-7.1)	 1 1.032(0.42-2.51) 3.81(1.45-10.03)
Total Cholesterol(mg/dL) 40-239 240-400	 275 51	 1 19.01(2.6-140.7)	 1 11.24(1.15-109.6)	 1 12.12(1.42-103.2)
Diastolic(mmHg) Normal Abnormal	 310 16	 1 4.87(0.63-37.49)	 1 0.83(0.07-9.66)	 1 0.85(0.73-9.82)
Systolic (mmHg) Normal Abnormal	 242 84	 1 15.8 (6.40-39.01)	 1 2.23(0.66-7.51)	 1 3.57(1.095-11.62)
Gender Male Female	 111 215	 1 1.06(0.6-1.8)	 1 1.12(0.49-2.55)	 1 1.01(0.47-2.15)
Age 30-50 (1) 51-70 (2) 71-85 (3)	 117 180 29	 1 22.0(10.5-45.9) 36.2(4.8-275.3)	 1 15.25(6.21-37.4) 28.26(3.27-244.23)	 1 12.35(5.544-27.53) 23.97(2.93-196.34)
Family history No Yes	 106 220	 1 9.7(5.4-17.4)	 1 7.41(3.26-16.82)	----------

Table (5):*Adjusted for all risk factors in table, for rs 12255372 # P < 0.05.

	Number	**Crude OR (95% CI)**	**Adjusted OR (95% CI) *with Family history**	**Adjusted OR (95% CI)* without Family history**
Mutation(2)rs12255372 No (ref) Yes	 225 101	 1 6.065(2.68-13.74)	 1 3.5(1.28-9.5)	 1 5.83(2.18-15.56)
BMI (kg/m2) 18-24.9 25-29.9 30-55	 52 111 163	 1 1.78 (0.910-3.48) 9.86 (4.55-21.32)#	 1 1.1(0.38-2.99) 2.3(0.8-6.74)	 1 1.19(0.48-2.97) 3.26(1.22-8.74)
Total Cholesterol(mg/dL)				

40-239 240-400	275 51	1 19.01(2.6-140.7)	1 12.77(1.33-123.1)	1 15.57(1.83-32.8)
Diastolic(mmHg) Normal abnormal	 310 16	 1 4.87(0.63-37.49)	 1 0.66(0.06-7.9)	 1 0.65(0.05-8.1)
Systolic(mmHg) Normal abnormal	 242 84	 1 15.8 (6.40-39.01)	 1 2.53(0.73-8.8)	 1 4.4(1.29-14.99)
Gender Male female	 111 215	 1 1.06(0.6-1.8)	 1 1.12(0.49-2.58)	 1 1.07(0.5-2.3)
Age 30-50 (1) 51-70 (2) 71-85 (3)	 117 180 29	 1 22.0(10.5-45.9) 36.2(4.8-275.3)	 1 15.77(6.46-38.54) 31.71(3.74-268.52)	 1 13.8(6.13-30.96) 33.99(4.15-278.4)
Family history No Yes	 106 220	 1 9.7(5.4-17.4)	 1 6.90(3.02-15.81)	----------

Table (6) Adjusted for all risk factors in table. For interaction and combination between mutations rs7903146 and rs12255372 together, # P < 0.05.
**** (0): have not both mutations, (1): have single mutation either rs7903146 or rs 12255372 , (2): have both mutations rs7903146 and rs12255372 .**

	Adjusted OR (95% CI) * with Family history	**Adjusted OR (95% CI) * without Family history**
Mutation(1)rs7903146+mutation(2)rs12255372 No (ref)(0)** Yes(1) (2)	 1 10.143.34-30.8) 2.21(0.65-7.5)	 1 15.52(5.42-44.5) 3.6(1.08-11.98)
BMI (kg/m2) 18-24.9 25-29.9 30-55	 1 1.27(0.43-3.77) 3.4(1.08-10.9)	 1 1.29(0.48-3.46) 4.4(1.53-12.8)
Total Cholesterol (mg/dL) 40-239 240-400	 1 13.3(1.21-147.7)	 1 15.1(1.064-139.0)
Diastolic(mmHg) Normal abnormal	 1 0.56(0.042-7.5)	 1 0.57(0.042-7.85)
Systolic Normal abnormal	 1 2.88(0.78-10.66)	 1 4.93(1.37-17.8)
Gender Male Female	 1 0.76(0.3-1.89)	 1 0.65(0.27-1.54)
Age 30-50 (1) 51-70 (2) 71-85 (3)	 1 19.3(7.5-49.9) 34.7(3.82-315.02)	 1 17.14(7.1-41.45) 33.53(3.85-291.8)
Family history No Yes	 1 5.46(2.3-13.01)	----------

Table (7) the genotype frequency of the r7903146 polymorphism in the *TCF7L2* gene

Genotype	Control	T2DM	*P* value	Crude OR	Adjusted OR	*P*
CC	65	131	reference			
CT	8	88	0.000	5.77 (2.6-12.6)	3.5(1.37-8.8)	0.009
TT	4	30	0.043	3.1(1.0-9.3)	3.7(1-13.5)	0.044
Total	77	249				
Allele frequency						
Allele C	0.896	0.7028				

Table (8) the genotype frequency of the rs12255372 polymorphism in the *TCF7L2* gene

Genotype	Control	T2DM	*P* value	Crude OR	Adjusted OR	*P* value
GG	69	154	reference			
GT	6	76	0.000	5.12(2.3-11.8)	4.0(1.4-10)	0.009
TT	2	19	0.047	4.5(1-19)	3.6(1-21)	0.15
Total	77	249				
Allele frequency						
Allele G	0.935	0.783				
Allele T	0.065	0.2168				