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Transcription factor 7-like 2 (TCF7L2) Gene Polymorphism rs7903146 is Associated with Lipid Profile and Risk of Cardiovascular Disease in Metabolic Syndrome Subjects

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ABSTRACT

Transcription factor 7-like 2 (TCF7L2) polymorphisms especially rs7903146(C/T) are related to type 2 diabetes (T2D) and other metabolic diseases; Metabolic Syndrome(MetS), atherosclerosis and cardiovascular disease (CVD) risk, this study is performed in order to evaluate the relationship between TCF7L2 polymorphism rs7903146 to Metabolic Syndrome (MetS) and CVD risk. Blood samples were collected from the MetS (n=92) and control (n=80) subjects. The subjects were characterized according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP-III). Blood DNA was extracted and genotyped by mismatch PCR-RFLP. A logistic regression model was performed to analyze the data. T-TEST and ANOVA (Analysis Of Variance) were used to compare differences between CC and CT+TT rs7903146 genotypes. The biochemical factors showed that BMI, SBP, DBP, FBS, TG levels in the MetS subjects were meaningfully higher than Normal subjects (P=0.001, P=0.000, P=0.000, P=0.031, P=0.000) and HDL level in the MetS subjects was Lower than Normal subjects (P=0.003). The biochemical factors and genotype analysis indicated that FBS and TG levels in the CT+ TT genotypes were significantly higher than that of CC genotype in MetS subjects (*P* =0.000); The HDL level and BMI in CC genotype were higher (P=0.003, P=0.002). Furthermore, logistic regression analysis of the genotype frequencies for people with a history of cardiovascular disease and people without cardiovascular disease in metabolic syndrome subjects, showed that the odds ratios (OR) of CT+ TT genotypes carriers for CVD risk were higher than those of CC genotypes carriers (OR: 2.8889; 95 % CI : 1.0344 - 8.0680; P = 0.0429). There was relative risk for CT+ TT genotypes versus CC genotype (RR: 1.3542; 95 % CI: 1.0386 - 1.7656; P = 0.0251). Results of this study revealed that the CT+ TT genotypes carriers rs7903146 variation could be predisposed to CVD risk.

Key words: Transcription factor, Cardiovascular disease, Metabolic Syndrome, Gene polymorphism.

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1. INTRODUCTION

A set of factors that lead to the danger of type 2 diabetes and cardiovascular disease (CVD) were defined as the metabolic syndrome (1). According to the NCEPATP III definition people who have three or more of the following conditions are suffering from metabolic syndrome (2, 3):

- Central obesity (waist circumference >102 cm in men and >88 cm in women).
- Triglycerides (TGs) ≥1.7 mmol/l.
- HDL Cholesterol <1.03 mmol/l in men and <1.29 mmol/l in women.
- Systolic BP ≥130 mmHg and/or Diastolic BP ≥85 mmHg.
- Fasting Plasma Glucose ≥6.1 mmol/l.

To produce the metabolic syndrome environmental factors for example physical activity and regime, interact with still mostly unknown genetic factors, the pathogenesis of metabolic syndrome is uncertain (4-6). The presence of metabolic syndrome varies from one ethnic group to another (7). According to previous studies, people with metabolic syndrome between three and five times suffere from heart attacks more than those who have not the syndrome (8). Laaksonen et al argued that men with

metabolic syndrome have approximately ninefold greater possibility of developing diabetes than men without the metabolic syndrome (9). Several studies have shown that the appearance of the MetS or diabetes is related with increased CVD risk. In the light of European DECODE (Diabetes Epidemiology: Collaborative Analysis Of Diagnostic Criteria in Europe) study, MetS- nondiabetics subjects had an increased risk of all-reason mortality as well as from CVD (10). Surveys such as that conducted by, Lakka *et al.* have shown that men with the metabolic syndrome have more possibility to developing cardiovascular disease and all-cause mortality, even if baseline CVD and diabetes do not exist (11). The TCF7L2 gene is one of the most important transcription factors of the WNT ligand family, this gene has significant role in regulate of cell proliferation and differentiation, also TCF7L2 affecting preadipocyte differentiation, inflammatory status, pancreatic beta cell function and cortisol/ aldosterone secretion (12). The relationship of TCF7L2 gene variants, particularly rs7903146 and rs12255372, and Type 2 diabetes has been highlighted by several studies (13-17). There is an integration between TCF7L2 polymorphisms and MetS components such as dyslipidemia and waist circumference (18, 19). It has been demonstrated that the TCF7L2 rs7903146 polymorphism might develop the risk factor components of the metabolic syndrome (20, 21) but these findings have been inconsistent. Cawthorn et al and Gustafson mentioned that in vitro, TNF-α has been shown to increase the transcriptional activity of TCF7L2 leading to reduced adipogenesis (22, 23). The existence of the risk allele (T) of rs7903146 gene variation has been connected to other diseases except for diabetes, for instance the occurrence of cancer (24), MetS (21), or the harshness of coronary disease (25). In spite of the fact that lipid metabolism may be a connection between TCF7L2, adipocyte metabolism and coronary disease, this study was undertaken to evaluate the association between TCF7L2 polymorphism rs7903146 and Metabolism Syndrome and CVD risk in MetS subjects.

2. MATERIALS AND METHODS

2.1. Study Subjects

This case-control study set out to assess the association between SNP rs7903146 with MetS and CVD risk. The patient population consist of 92 MetS subjects (29 men and 63 women) and the control group was 80 healthy subjects (38 men and 42 women) randomly selected from the general population. All blood samples were collected from patients from the day clinic of Isfahan University of Medical Sciences Hospital/ Iran. All participants were informed well the purposes of the study and hopefully all were happy to make positive contribution. Also written informed consent was obtained from patients and participants in the study to register for a confidential barcode. Metabolic syndrome diagnosed based on the definition proposed by the NCEPATP III. A Metabolic Syndrome subject should encounter three or more of the following criteria; Central obesity (waist circumference >102 cm in men and >88 cm in women), Triglycerides (TGs) ≥1.7 mmol/l, High Density Lipoprotein (HDL) Cholesterol <1.03 mmol/l in men and <1.29 mmol/l in women, Systolic Blood Pressure (SBP) ≥130 mmHg and/or Diastolic Blood Pressure (DBP) ≥85 mmHg, Fasting Blood Sugar (FBS) ≥6.1 mmol/l. The clinical data and biochemical factors were analyzed and the results were shown in Table 1.

Table 1. The Clinical and biochemical characteristics of the study participants

Characteristics	Normal subjects(80)	MetS subjects(92)	P value
N(Men/Women)	80 (38/42)	92(29/63)	-
Age(y)	53.5±7	59.80 ± 7.95	0.785
BMI(kg/m^2)	25.5±3.07	32.03 ± 4.5	0.001
SBP(mmHg)	113±16.72	131±21	0.000
DBP(mmHg)	73±8.31	78±9.67	0.000
FBS(mg/dl)	96.8±8.3	136.62 ± 58.84	0.031
CHO(mg/dl)	184±37.2	185.86 ± 45.26	0.608
TG(mg/dl)	161.7±88.2	206.84 ± 112.04	0.000
HDL(mg/dl)	43.86±13.2	39.02 ± 11.33	0.003
LDL(mg/dl)	105.08±27.03	102.44± 39.44	0.450
Waist(cm)	NA	102±8	NA

ıBody mass index (BMI), Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Fasting blood sugar (FBS), Triglyceride (TG), High density lipoprotein (HDL), Low density lipoprotein (LDL). Data analyzed by one way ANOVA/T-test. Means in same row with star are shown significantly ($P < 0.05$).

After being sure about biochemical factors and existence of metabolic syndrome conditions, medical history of each person in metabolic syndrome group was considered in medical records with confirming the therapeutic doctor. People who had any history of cardiovascular diseases (CVD) including diseases related to Coronary Heart diseases (CHD), Atherosclerosis, hypertension, Ischemic heart disease , peripheral vascular disease, heart failure, were coded in order to final study and study of relation to polymorphism.

2.2. Genotyping of SNP rs7903146 (C/T) Polymorphism

Peripheral blood samples of patients group were collected in EDTA-anticoagulated tubes. After wards DNA was extracted from whole-blood samples using the GENET BIO kit (Korea). DNA integrity was checked by UV spectrophotometer at 260/280 nm and by agarose electrophoresis to visualize the purity. Genotyping was performed by mismatched Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The modified primers were also used for PCR-RFLP:

Forward (26): 5′- TTA GAG AGC TAA GCA CTT TTT AGA TA- 3′

Forward - mismatch: 5′ - TTA GAG AGC TAA GCA CTT TTT AGG TA - 3′

Reverse: 5′-AGA GAT GAA ATG TAG CAG TGA AGT G-3′

The above specific PCR primers amplified a 201- bp fragment in which there is a specific restriction site to distinguish the allele differences of the rs7903146 variant. The PCR was conducted on an Eppendorf thermal cycler (AG2231, Eppendorf, Germany) under the following conditions: 94˚C for 4 min by 35 cycles, 94˚c for 30 s, 61.1˚c for 1 min, 72˚c for 20 s and a final extension of 72˚c for 5 min. Finally, the PCR products were then digested with 0.5 unit of RsaI restriction enzyme (Fermentas, Lithuania). After 4-h incubation at 37˚C, the digestive products were visualized by 3% agarose and /or Polyacrylamide gel electrophoresis.

2.3. Primer design

In this study Forward Primer is designed in a way that a cutting site is artificially created in SNP because SNP rs: 7903146 is located in a situation of gene in which there is no cutting site for any enzyme. This task is done by replacing a nucleotide at the end of 3' Forward Primer and nucleotide A has been replaced in sequence of gene by nucleotide G and thus cutting site (GT↓AC) was created for RsaI restriction enzyme. Beginning of sequence of gene in location of SNP is as follows, in this polymorphism C allele, normal allele, and T allele, mutant allele are considered.

5′- TTA GAG AGC TAA GCA CTT TTT AGA TA C/T- 3′

The sequence of primary Forward Primer:

5′ - TTA GAG AGC TAA GCA CTT TTT AGA TA - 3′

The sequence of Forward Primer with Mismatch

5'- TTA GAGA GC TAA GCA CTT TTT AGGTA -3'

2.4. Determination of SNP rs: 7903146 genotype using method of Mismatch PCR-RFLP

In the method of Mismatch PCR-RFLP particular restricting enzyme is used in order to distinct between normal and mutated alleles. The technique of Mismatch PCR-RFLP was created in order to distinct between T and C alleles by using RsaI restriction enzyme. In the case of SNP rs: 7903146 C/T polymorphism nucleotide C was replaced by T. In this study by performing method of Mismatch PCR-RFLP, using restricting enzyme and doing electrophoresis gel of productions of reaction, and regarding cutting site of enzyme which was created in primer, three following patterns are expected on electrophoresis gel:

People who are homozygote CC (normal): 2 bands with the lengths of 176 bp and 25 bp

People who are homozygote (variant risk) TT: 1 band with the length of 201bp

People who are heterozygote: 3 bands with lengths of 25, 176, 201 bp

Therefore, according to the number of observed bands on gel electrophoresis after enzyme reaction we can identify the type of SNP (Figure 1).

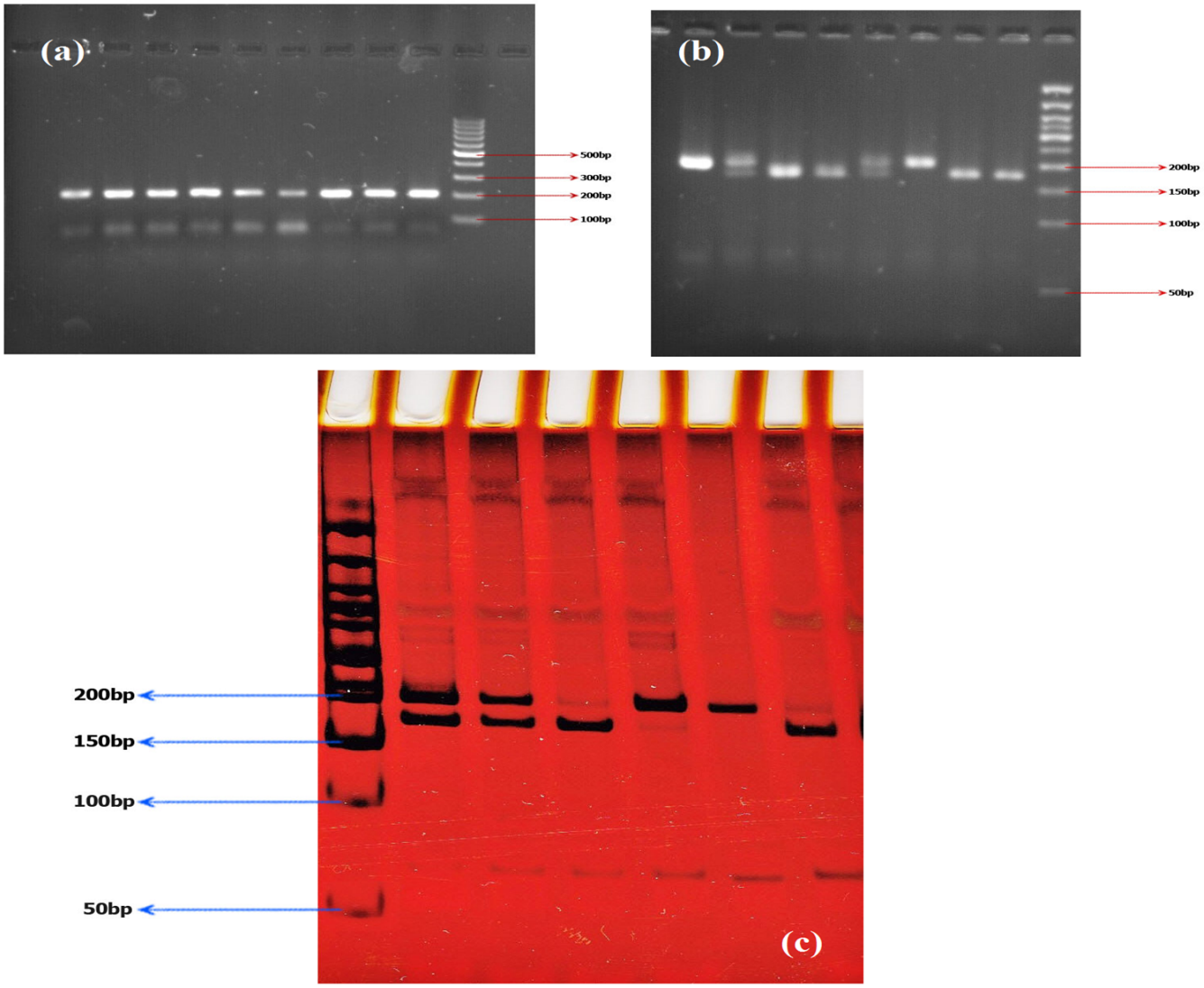

Figure 1. (a) Products of PCR, duplication of a piece containing SNP with length of 201 bp; (b) Products of enzyme digest using enzyme RsaI on Agarose gel 3%; (C) Products of enzyme digest using RsaI restriction enzyme on Polyacrylamide gel electrophoresis 8% with silver staining method

2.5. Statistical analysis

SPSS for Windows, version 16 (SPSS, Chicago, IL), was used to analyze statistical results. P values are two-sided, and the significant level was 0.05 ($P \leq 0.05$). All quantitative values are expressed as mean ± SD. A logistic regression model was performed to analyze the data. The mean of clinical and biochemical characteristics were compared by T-TEST.

3. RESULTS AND DISCUSSION

This study investigated the relationship between rs7903146 with Metabolic Syndrome components and CVD risk. The biochemical factors of the normal subjects were measured to compare Metabolic Syndrome subjects. The clinical and biochemical characteristics of the study participants are presented in Table 1. The data were analyzed and the results revealed that the mean of factors such as BMI, SBP, DBP, FBS, TG and HDL with P value ≤0.05 were significantly different between normal and MetS subjects.

Table 2. The Clinical and biochemical characteristics of the subjects based on rs7903146(C/T) genotypes

Genotypic groups	Normal subjects(80)			MetS subjects(92)		
	CC (n=32)	CT+TT (n=48)	P value	CC (n=30)	CT+TT (n=62)	P value
Age(y)	53.8±7.5	52.1±6.4	0.52	60.57±7.19	59.38±8.44	0.658
BMI(kg/m²)	25.2±3.1	25.75±3.1	0.76	32.50±5.52	29.64±3.14	0.002*
SBP(mmHg)	112.3±17.68	116.42±14.96	0.12	134.2±17.03	130.01±23.72	0.312
DBP(mmHg)	73.13±7.5	73.19±8.34	0.44	80±8.21	77±9.70	0.307
FBS(mg/dl)	95.6±8.1	93±7.65	0.33	132.60±44.16	134.13±55.26	0.000
CHO(mg/dl)	190.3±33.6	185.65±69.2	0.52	182.73±32.99	186.13±49.8	0.204
TG(mg/dl)	163.3±89.5	160.7±95.1	0.99	186.00±120	206±109	0.000*
HDL(mg/dl)	44.23±11.13	43.19±13.13	0.83	42.40±11.14	39.27±12.11	0.003
LDL(mg/dl)	102.61±25.1	105.65±33.87	0.66	104.67±28.83	101.21±48.21	0.677
Waist(cm)	NA	NA	NA	104±9	101±7	0.71

ıBody mass index (BMI), Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Fasting blood sugar (FBS), Triglyceride (TG), High density lipoprotein (HDL), Low density lipoprotein (LDL). Data analyzed by one way ANOVA/T-test. Means in same row with star are shown significantly (P < 0.05).

The clinical and biochemical characteristics of the subjects based on rs7903146(C/T) genotypes are shown in Table 2. The data showed that BMI (P=0.002) and HDL (P=0.003) in MetS subjects were noticeably dependent to CC genotype. The results also showed that TG and FBS level in CT+TT genotype in MetS subjects was higher than CC genotype (P=0.000) (Figure 2).

Table 3. Frequency of the rs7903146(C/T) polymorphism in Normal subjects and Metabolic Syndrome subjects

Genotype Rs7903146	Normal subjects(n=80)	Metabolic Syndrome Subjects(n=92)	Odds ratio (95 % CI) P	Relative risk(95 % CI) P
CC	32(40%)	30(32.608%)	1.3778 (0.7378 - 2.5729) P = 0.3146	1.1648 (0.8585 - 1.5805) P = 0.3271
CT+TT	48(60%)	62(67.39%)		

OR= Odds Ratio, 95%CI = 95% confidence interval (from minimum to maximum). *P-values calculated by Logistic regression analysis. The result is significant at p < 0.05 means it is dealing with dependent variables.

The results of logistic regression analysis, the odds ratios (OR) and relative risk of the existence CT+TT genotypes in connection with the development of the metabolic syndrome are shown in Table 3. The odds ratio of CT+TT genotypes for (rs7903146 C/T) in Normal subjects versus Metabolic Syndrome Subjects was (OR: 1.3778; 95 % CI: 0.7378 - 2.5729; P = 0.3146). Relative risk in Normal subjects / Metabolic Syndrome Subjects was (RR: 1.1648; 95 % CI: 0.8585 - 1.5805; P = 0.3271). Odds ratio and risk ratio were not significant between the two groups.

Table 4. Frequency of the rs7903146(C/T) polymorphism and association of this variant with Cardiovascular disease risk in Metabolic Syndrome Subjects

	SNP	Genotype	Percent of without CVD Subjects	Percent of CVD Subjects	Odds ratio (95 % CI) P	Relative risk (95 % CI) P
Metabolic Syndrome Subjects(n=92)	rs7903146	CC(n=30)	24 (80%)	6 (20%)	2.8889(1.0344 - 8.0680) P = 0.0429	1.3542(1.0386 - 1.7656) P = 0.0251
		CT+TT(n=62)	36 (58.06%)	26 (41.93%)		

OR= Odds Ratio, 95%CI = 95% confidence interval (from minimum to maximum). *P-values calculated by Logistic regression analysis. The result is significant at p < 0.05 means it is dealing with dependent variables.

The odds ratios (OR) of the existence CT+TT genotypes with risk of Cardiovascular disease are presented in Table 4. Logistic regression analysis indicated that the odds ratio of CT+TT genotypes for (rs7903146 C/T) in (MetSyn-with CVD)/ (MetSyn-without CVD) was (OR: 2.8889; 95 % CI: 1.0344 - 8.0680; P = 0.0429). Relative risk was significantly higher for the CT+TT genotypes versus CC genotype (RR: 1.3542; 95 % CI: 1.0386 - 1.7656; P = 0.0251) (Figure 3).

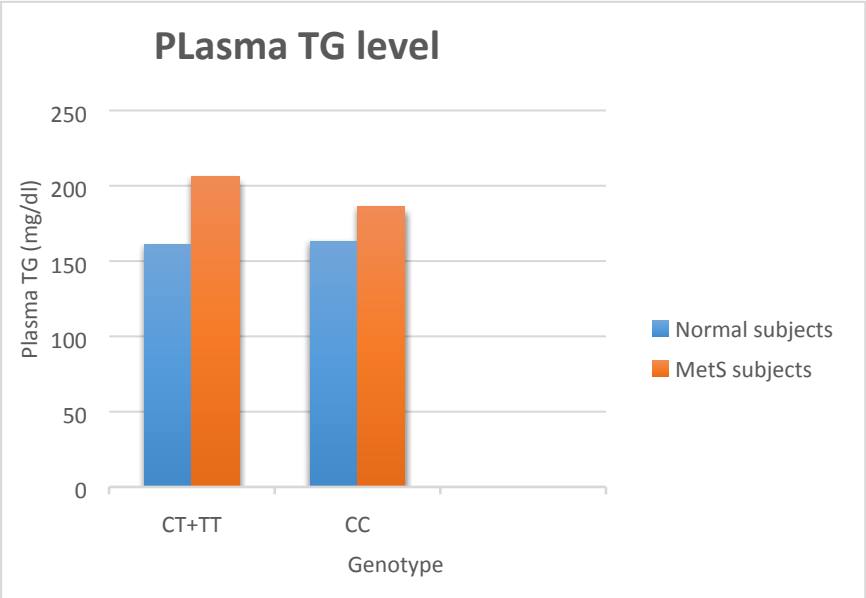

Figure 2. Comparison of plasma TG levels in two groups according to genotypes of TCF7L2 gene. TG level in CT+TT genotype in MetS subjects was significantly higher than CC genotype (P= 0.000)

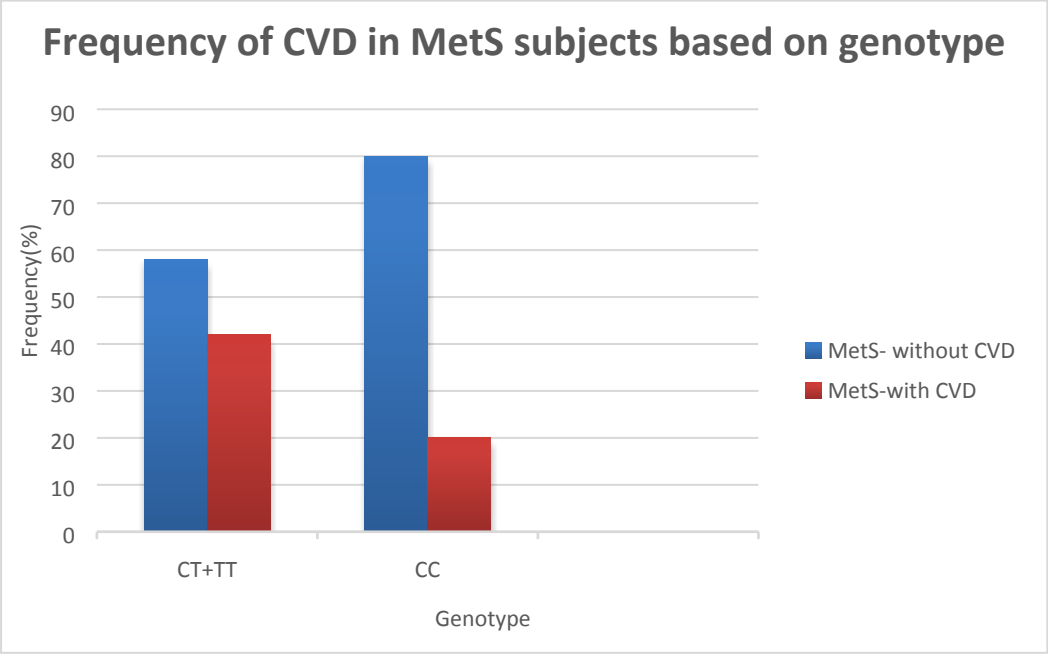

Figure 3. Comparison of genotype frequencies among people with Cardiovascular disease and people without Cardiovascular disease in MetS subjects. CT+ TT genotypes associate significantly with the risk of cardiovascular disease

The former study has reported that the TCF7L2 gene (rs7903146 SNP) was associated strongly with type 2 diabetes in Persian population (26, 27). In this study, the relationship between TCF7L2 polymorphism rs7903146 with Metabolic Syndrome and so CVD risk was evaluated. The results of previous studies demonstrated that the TCF7L2-rs7903146 polymorphism in addition to type 2 diabetes risk is related to other factors which are linked to diabetes like plasma concentrations (19, 28, 29), metabolic lipid syndrome (20, 27), and even with increased cardiovascular disease risk and atherosclerosis (25, 30, 31), but the findings of different studies are inconsistent (18, 27, 32, 33). The findings of this study revealed that the CT+TT genotypes carriers had greater levels of FBS and TG, these results are in agreement with previous study that shown that rs7903146 in TCF7L2 predicts the components of MetS (21). In this study, the risk allele carriers genotypes are related with concentrations of TG and cardiovascular disease risk. Plasma concentrations of TG in CT+TT subjects were higher than the CC subjects. The present finding seems to be consistent with previous study that the minor T allele of TCF7L2 (rs7903146 and rs12255372) were linked with raised TG in participants with familial combined hyperlipidemia (34). This also accords with another observation, which showed that there was a relationship between this TCF7L2 variant with the risk of MetS, the most powerful association being found with hypertriglyceridemia (18). Perez-Martinez et al argued that the minor allele carriers of TCF7L2 rs7903146 have an interrupted lipid metabolism (29). Interference in function of beta-cell, could influence markers of glucose metabolism among MetS patients (20). Moreover, the involvement of the TCF7L2 pathway has been observed in several other biological processes, for instance, the regulation of adipokines and the differentiation of adipocytes (12, 20). The results of logistic regression analysis indicated that there is a strong influence of CT+TT genotypes on the risk of cardiovascular disease (OR: 2.8889; 95 % CI: 1.0344 - 8.0680; P = 0.0429). Additionally, the findings of the current study suggest that Relative risk for CT+TT genotypes carriers for CVD risk was remarkable (RR: 1.3542; 95 % CI: 1.0386 - 1.7656; P = 0.0251). This conclusion is consistent with previous study that shown T allele of rs7903146 was associated with a higher incidence and harshness of coronary atherosclerosis (25). The findings of previous studies about association between TCF7L2 polymorphisms and cardiovascular risk, are inconsistent (25, 30, 31, 35, 36). Therefore, more studies are necessary to confirm this association. A previous study in a meta-analysis showed that TG was an independent risk factor for CVD (37). As mentioned by Stalenhoef et al, the principle abnormality that is responsible for this relationship, is the presence of prominent concentrations of atherogenic TG (38). Increasing levels of plasma TG have been observed clearly among people who have similar background such as type 2 diabetes (39), the metabolic syndrome (40) and familial combined hyperlipidemia (FCHL) (41). Since, the chance of dying from coronary heart disease or stroke among people with Metabolic Syndrome is more as compared with people without the syndrome, recognition of the metabolic syndrome is important (1). In addition, patients with MetS are disposed to develop type 2 diabetes and consequently CVD (42). Interestingly, results indicated that HDL level in CT+TT genotypes was lower than CC genotype (P=0.003). It is possible that the reduced HDL level in CT+TT genotypes be associated with the increasing TG in these genotypes. Patients with high TG levels recurrently display additional risk factors, such as insulin resistance that may influence their susceptibility to athero-sclerosis. Finally Isomaa et al, stated that raised plasma TG levels are powerfully connected with low

HDL-C levels (43). Since the carriers of CT+TT genotypes have higher TG level than CC genotypes, therefore it is may be rs7903146 polymorphism (C/T) affected the cardiovascular disease risk through increasing plasma triglyceride levels. The mechanism in which rs7903146 polymorphism (C/T) is influenced the developing of the Metabolic Syndrome and Cardiovascular disease is unknown. The presence of risk alleles of TCF7L2 gene in adipocytes causes to creating a proinflammatory phenotype, supporting lipolysis, decreasing adiponectin, and increasing resistin gene transcription (23). It is also worth mentioning that, TCF modifies TG metabolism and adipokine secretion in adipocytes by three key genes involved in adipogenesis and lipid metabolism which are included, regulating peroxisome proliferator-activated receptor gamma, CCAAT/enhancer binding proteinα, and lipoprotein lipase gene transcription (44). Considerably more work will need to be done to understanding the clear fact.

4. CONCLUSION

The present study demonstrated that TCF7L2 plays a significant role in regulation of glucose and lipid metabolism, so results revealed that the T allele carriers of rs7903146 variation could be predisposed to CVD risk. T allele carriers increased risk of developing CVD through increased TG levels.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

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