Transcription factor 7-like 2 polymorphism and context-specific risk of metabolic syndrome, type 2 diabetes, and dyslipidemia

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Background: The transcription factor 7-like 2 gene (TCF7L2) is an element of the Wnt signaling pathway. There is lack of evidence if TCF7L2 has a functional role in lipid metabolism and regulation of the components constitutes the metabolic syndrome (MetSyn). The aims of this study were to evaluate whether the risk allele of TCF7L2 gene polymorphism is associated with dyslipidemia and MetSyn.

Materials and Methods: The MetSyn subjects were participated only based on the National Cholesterol Education Program – Third Adult Treatment Panel criteria. In this case–control study, the DNA from MetSyn patients without (n = 90) and with type 2 diabetes (T2D) (n = 94) were genotyped. Results: The results show that the genotype-phenotype for CC, CT/TT of TCF7L2 gene polymorphism correlated with body mass index and waist circumference in MetSyn and MetSyn + T2D subjects (r = -0.949 and r = -0.963, respectively). The subjects that only possess MetSyn but are not diabetics show the 2 h postprandial glucose and fasting blood glucose, glycated hemoglobin significantly lower (P < 0.05) than those subjects have both abnormality. The level of triglyceride in CT/TT carriers in MetSyn was higher than CC carriers (P = 0.025). A comparison with the controls subjects, the frequencies of the T allele in the groups of MetSyn (46.66%) and MetSyn + T2D (47.34%) show significantly different (P < 0.05). The odds ratios for T allele in (MetSyn)/(normal), (MetSyn + T2D)/(normal), and in (MetSyn + T2D)/(MetSyn) were 3.59 (95% confidence interval [CI], 1.33–9.67, P = 0.0093), 3.76 (95% CI, 1.40–10.07, P = 0.0066), and 1.08 (95% CI 0.55–2.11, P = 0.834), respectively. Conclusion: The results revealed the important insights essential for the role of TCF7L2 that the T allele of TCF7L2 plays a significant role in the susceptibility to dyslipidemia, MetSyn, and T2D.

Key words: Metabolic syndrome, polymorphism, transcription factor 7-like 2 rs7903146, type 2 diabetes

INTRODUCTION

The metabolic syndrome (MetSyn) is a main clinical and public health challenge worldwide. It is considered as the major cause of morbidity and mortality due to genetics, unbalanced diet, and inactive lifestyle. In general, it is defined as a disorder of metabolism (energy utilization and storage). It is suggested that the MetSyn could be presented into clinical practice but with clear and distinct risk factors for health.[1] In recent years, several organizations have defined the MetSyn as interrelated risk conditions for increasing type 2 diabetes (T2D), stroke, and cardiovascular disease (CVD).[1-3]

Nowadays, it is believed that there is evidence for the existence and characteristics of the MetSyn and it is sufficiently criteria developed to support its inclusion in clinical practice. Different definitions for MetSyn in adults were presented by the international scientific communities. The organizations that developed clinical criteria for MetSyn are the International Diabetes Federation, World Health Organization (WHO), the European Group for the Study of Insulin Resistance, and the National Cholesterol Education

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The prevalence of MetSyn in Iran, according to the NCEP-ATP-III definition, was estimated to be 34.7%, which is clearly higher than the average rate mentioned in the USA and Europe. Several studies have shown that people with MetSyn, suffered from heart attacks more than those who have not MetSyn between three and five times. The etiology of MetSyn is complex and determined by the interaction of environmental factors and genetic. Based on the thrifty genotype hypothesis, genes involved in efficiently storing and saving energy could predispose to MetSyn.

Several specific genes are involved in metabolism, such as genes in the systems of energy balance, lipid and insulin metabolism, lipolysis, thermogenesis, fuel oxidation, and glucose uptake in adipocyte tissue and skeletal muscle. Many of these genes have been associated with MetSyn in various ethnic populations. These candidate genes include but are not limited to peroxisome proliferator-activated receptor γ, adiponectin, CD36, β-adrenergic receptors, insulin receptor substrates, 11 β-hydroxysteroid dehydrogenase type 1, C-reactive protein, tumor necrosis factor-α, calpain-10, upstream transcription factor-1, and skeletal muscle glycogen synthase-1.

Transcription factor 7-like 2 gene (TCF7L2), previously known as TCF-4, is a member of the high mobility group box transcription factors. TCF7L2 is a major transcription factor of the canonical Wnt signaling pathway known as β-cat/TCF. This bipartite transcription factor is formed by free β-catenin (β-cat) and a member of the TCF protein family, affecting preadipocyte differentiation, inflammatory status, pancreatic beta-cell function, and cortisol/aldosterone secretion. Impaired Wnt signaling has an important role in generation of ectopic fat, insulin resistance, and elevated plasma lipids.

The network of Wnt signaling pathways and β-cat/TCF7L2 bipartite transcription factor in obesity, insulin resistance, insulin secretion, lipoprotein metabolism, and inflammation is important to understand for prevention and treatment of MetSyn, diabetes, and CVD. Many studies confirmed the association between the single-nucleotide polymorphism (SNP) (rs7903146) in the TCF7L2 and T2D in population of different ethnic origins and also Persian.

Involvement of the TCF7L2 rs7903146 (C/T) gene polymorphism in regulating essential or major components of the MetSyn such as hypertension, dyslipidemia, insulin sensitivity, and obesity is not well understood. Researchers are still investigating to find the relationship between the frequency of a number of genes and their association with increased risk of T2D and MetSyn components. There is lack of evidence if TCF7L2 has a functional role in lipid metabolism and has a direct functional role in the regulation of the components constituted the MetSyn. Therefore, the aims of this study were to evaluate if the risk allele of TCF7L2 (rs7903146C/T) gene is associated with dyslipidemia and MetSyn and T2D.

MATERIALS AND METHODS

Study subjects
The patient population consist of a total of 184 subjects, MetSyn (n = 90) and MetSyn + T2D (n = 94). All participants were well informed the purposes of the study, and hopefully, all were happy to make positive contribution. Written informed consent from patients and participants in the study were also obtained to register for a confidential barcode. All subjects with self-reported history of disease and registered data for treatments that confound the study were excluded from the study. A group of unrelated healthy subjects from healthy population was used as control to test TCF7L2 genotypes for departure from Hardy–Weinberg equation (HWE). All peripheral blood samples of patients were collected from patients from the Diabetes Clinic Centers/Isfahan City, Iran. The MetSyn subjects diagnosed based on the definition provided by the NCEP-ATP-III. The MetSyn subjects were encounter in this study if they had the four previously mentioned criteria of the NCEP-ATP-III definition. The MetSyn + T2D were included in this study according to the NCEP-ATP-III to include MetSyn plus WHO criteria to enclose the T2D subjects.
Genotyping of single-nucleotide polymorphism rs7903146 (C/T) polymorphism

Peripheral blood samples of the patient were collected in ethylenediaminetetraacetic acid anticoagulated tubes. Then, DNA was extracted from whole-blood samples using the QIAGEN DNA purification kit (QIAGEN Ltd., UK). DNA integrity was checked by ultraviolet spectrophotometer at 260/280 nm and by agarose electrophoresis to visualize the purity. The genotyping was performed by mismatched polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The following primers were used for the experiment.

Forward: 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 determine the allele differences of the rs7903146 variant. The PCR was carried out on an Eppendorf thermal cycler (AG2231, Eppendorf, Germany) under the following conditions for PCR-RFLP: 94°C for 4 min by 35 cycles of 94°C for 30 min, 61.1°C for 1 min, 72°C for 20 s, and a final extension of 72°C for 5 min.

Finally, the PCR-RFLP products were then digested with 0.5 unit of RsaI restriction enzyme (Promega UK Ltd.). After 4-h incubation at 37°C, the digestive products were visualized by 3% agarose and/or polyacrylamide gel electrophoresis.

Statistical analysis

SPSS for Windows, version 22 (SPSS, Chicago, IL, USA), was used for statistical analysis. For all statistical analyses, P < 0.05 was considered as statistically significant. All quantitative values are expressed as mean ± standard deviation HWE for the distribution of genotypes was estimated by the Chi-square test. The odds ratios (ORs) with 95% confidence interval (CI) to understand the different association were calculated by multiple logistic regressions with confounders determined by a backward conditional elimination method to find a significance level below 0.05. The logistic regression analysis was also performed with and without adjustment for age and sex as well as body mass index (BMI). STATA/SE 10 program was used for power calculation. The mean of clinical and biochemical characteristics was compared by post hoc and Mann–Whitney tests.

RESULTS

The biochemical factors of the subjects were measured to distinguish the MetSyn and diabetics conditions in individuals. Subjects were characterized and grouped according to the essential components for MetSyn and T2D. The main features of subjects with regard to cutoff levels provided by NCEP-ATP-III and WHO to distinguish the MetSyn + T2D from the MetSyn were measured. The characteristics in subjects were measured and the results are summarized in Table 1. The biochemical factors in the MetSyn were compared with

| Table 1: The clinical and biochemical characteristics of the metabolic syndrome subject |
| MetSyn core components | Characteristics | MetSyn subjects without T2D | MetSyn subjects with T2D | P |
|------------------------|----------------|-----------------------------|--------------------------|---|
| Age (year)             | 59.8±7.95      | 57.9±11.76                  | 0.393                    |
| Gender (male/female)   | 24/66          |                             |                          |
| Obesity                |                |                             |                          |
| BMI (kg/m²)            | 32.0±4.5       | 29.3±4.48                   | 0.008*                   |
| Waist (cm)             | 102.7±8.79     | 101.7±8.65                  | 0.623                    |
| Blood pressure         |                |                             |                          |
| SBP (mmHg)             | 132.4±12.66    | 130.8±27.52                 | 0.740                    |
| DBP (mmHg)             | 78.3±9.61      | 78.4±9.27                   | 0.955                    |
| Glucose regulation     |                |                             |                          |
| FBG (mg/dL)            | 95.6±8.84      | 175.3±59.70                 | 0.001*                   |
| 2HPPG (mg/dL)          | 132.5±38       | 206.6±90.08                 | 0.008*                   |
| HbA1c (%)              | 5.3±0.56       | 7.5±1.69                    | 0.000*                   |
| Lipids                 |                |                             |                          |
| TG (mg/dL)             | 203.8±78.99    | 209.3±137.81                | 0.816                    |
| HDL (mg/dL)            | 38.0±13.33     | 42.2±11.53                  | 0.030*                   |
| LDL (mg/dL)            | 108.4±39.45    | 96.4±40.20                  | 0.079                    |
| LDL/HDL                | 3.0±1.73       | 2.1±1.31                    | 0.000*                   |
| Cholesterol (mg/dL)    | 189.8±45.26    | 182.4±45.81                 | 0.429                    |
| Other biochemical factors |              |                             |                          |
| BUN (mg/dL)            | 24.8±10.20     | 24.0±13.45                  | 0.770                    |
| Creatine (mg/dL)       | 0.897±0.18     | 1.06±0.64                   | 0.093                    |
| AST (U/L)              | 24.4±12.83     | 22.3±6.29                   | 0.454                    |
| ALT (U/L)              | 28.7±20.66     | 25.1±9.36                   | 0.416                    |

Data analyzed by one way ANOVA/post hoc test. Means in same row with star are shown significantly (P<0.05). Data are presented as mean±SD. BMI = Body mass index; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; FBG = Fasting blood glucose; 2HPPG = 2 h postprandial glucose; HbA1c = Glycated hemoglobin; TG = Triglyceride; HDL = High density lipoprotein; LDL = Low density lipoprotein; BUN = Blood urea nitrogen; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; MetSyn = Metabolic syndrome; T2D = Type 2 diabetes; SD=Standard deviation
the MetSyn + T2D subjects. The comparison revealed that there are significant differences ($P < 0.05$) in BMI, HDL, and low-density lipoprotein/HDL ratio. As shown in Table 1, FBG, 2 h postprandial glucose (2HPPG), and glycated hemoglobin (HbA1c) in the MetSyn subjects which have T2D are significantly higher ($P < 0.05$) than those subjects without T2D.

The clinical and biochemical characteristics of the subjects based on the rs7903146(C/T) genotypes (CC and CT/TT) were analyzed, and the results are shown in Table 2. The results revealed that there is a genotype-phenotype correlation as shown in Figure 1. The CC, CT/TT genotype frequencies in MetSyn and MetSyn + T2D with BMI, and waist circumference were correlated with coefficient correlations of $r = -0.949$ and $r = -0.963$, respectively. TG in MetSyn group with CT/TT genotype is higher than the CC genotype ($P > 0.05$), [Table 2].

The genotype distribution at this SNP did not obey the HWE within both groups of the MetSyn and MetSyn + T2D subjects ($P > 0.05$).

### Table 2: The clinical and biochemical characteristics of the subjects based on rs7903146 (C/T) genotypes

| MetSyn core components | Characteristics | MetSyn subjects without T2D | MetSyn subjects with T2D | $P$ |
|------------------------|----------------|-----------------------------|--------------------------|-----|
|                        |                | CC ($n=30$)                 | CT/TT ($n=60$)          |     |
| Age (year)             |                | 60.57±7.19                  | 59.38±8.44              | 0.658 |
| Subjects               |                | 30                          | 60                       | 0.703 |
| Obesity                | BMI (kg/m$^2$) | 34.50±5.52                  | 30.64±3.14              | 0.008* |
| waist (cm)             |                | 105.50±8.97                 | 101.2±8.48              | 0.145 |
| Blood-pressure         | SBP (mmHg)     | 132.86±15.28                | 132.2±11.28             | 0.878 |
|                       | DBP (mmHg)     | 79.29±9.17                  | 77.8±10.00              | 0.649 |
| T2D factors            | FBG (mg/dL)    | 96.60±8.16                  | 95.13±9.26              | 0.605 |
|                       | 2HPPG (mg/dL)  | 125.33±46.69                | 134.55±37.64            | 0.734 |
|                       | HbA1c (%)      | 6.05±0.49                   | 5.12±0.39               | 0.025* |
| Lipids                 | TG (mg/dL)     | 167.00±45.86                | 222.26±86.01            | 0.025* |
|                       | HDL (mg/dL)    | 39.40±11.14                 | 36.27±14.11             | 0.460 |
|                       | LDL (mg/dL)    | 105.67±29.83                | 111.21±46.21            | 0.677 |
|                       | LDL/HDL        | 2.95±1.55                   | 3.23±1.55               | 0.627 |
|                       | Cholesterol (mg/dL) | 177.73±32.99  | 196.13±49.8            | 0.204 |
| Other chemical factors | BUN (mg/dL)    | 23.38±9.37                  | 25.55±10.68             | 0.535 |
|                       | Creatine (mg/dL) | 0.8429±0.031            | 0.94±0.2                | 0.106 |
|                       | AST (U/L)      | 18.50±2.51                  | 27.85±14.31             | 0.100 |
|                       | ALT (U/L)      | 18.38±1.87                  | 34.71±23.87             | 0.703 |

Data analyzed by one way ANOVA/post hoc test. Means in same row with star are shown significantly ($P<0.05$). Data are presented as mean±SD. BMI = Body mass index; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; FBG = Fasting blood glucose; 2HPPG = 2 h postprandial glucose; HbA1c = Glycated hemoglobin; TG = Triglyceride; HDL = High density lipoprotein; LDL = Low density lipoprotein; BUN = Blood urea nitrogen; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; MetSyn = Metabolic syndrome; T2D = Type 2 diabetes; SD = Standard deviation

![Figure 1: The calculator for finding the correlation coefficient (r) values which represent the relationship between (a) body mass index and (b) waist circumference with genotypes. Pearson’s formula is used for this calculation](image-url)
The genotype and allele frequency data are shown in Table 3. The analysis of the data shows that the frequency of the T risk allele was approximately equal in two groups, i.e., MetSyn (46.66%) and MetSyn + T2D (47.34%). In other words, the frequency of T allele is not meaningfully different (P = 0.884). The genotypes frequency of CC, CT, and TT in MetSyn subjects are 31.11%, 44.44%, and 24.44%, respectively. That is likely similar to the genotypes frequency in MetSyn + T2D subjects for CC, CT, and TT which are 29.79%, 45.74%, and 24.47%, respectively. The ORs of the presence of T allele frequency together with the presence of T2D and MetSyn are presented in Table 4. Logistic regression analysis and Chi-square test indicated that the OR for the T risk allele for rs7903146 C/T in (MetSyn)/(normal), (MetSyn + T2D)/(normal), and (MetSyn + T2D)/(MetSyn) are 3.59 (95% CI, 1.33–9.67 P = 0.0093), 3.76 (95% CI, 1.40–10.07 P = 0.0068), and 1.08 (95% CI: 0.55–2.11 P = 0.834), respectively, as shown in Table 4.

DISCUSSION

The association between a common variant of TCF7L2 polymorphisms (rs7903146) and T2D mellitus has been widely investigated in Iranian and many other populations. Up to now, no significant function for TCF7L2 is being well documented. The MetSyn with several biochemical components causes many chronic diseases such as diabetes and CVD. There is no report related to the association between dyslipidemia and MetSyn with TCF7L2 polymorphisms. As it was expected, subjects with MetSyn + T2D had FBG, 2HPPG, and HbA1c significantly higher (P < 0.05) than those subjects without T2D. This was consistence with WHO definition for T2D. In this study, there is a genotype-phenotype correlations. The CC, CT/TT genotype frequencies in MetSyn and MetSyn + T2D with BMI and waist circumference with coefficient correlations of r = −0.949 and r = −0.963, respectively. Therefore, in this investigation, the genotyping in the MetSyn subjects which carrying the rs7903146 T allele (risk allele) was significantly associated with decreasing BMI and waist circumference.

The study on the MetSyn + T2D subjects carrying the rs7903146 T allele, the BMI, and waist circumference in this group was also slightly decreased but was not significantly different. It is known that the TCF7L2 plays a critical role in the Wnt signaling pathway to regulate adipose cell differentiation and adipogenesis.[26,27] This phenomena could be associated with minor increase in TCF7L2 expression in adipose tissue.[26,27] HbA1c is formed by long-term exposure of normal hemoglobin to plasma glucose in a nonenzymatic glycation pathway. Therefore, HbA1c level is proportional to average blood glucose, and it is a useful factor for diabetes treatment monitoring. HbA1c in MetSyn is significantly lower than MetSyn + T2D. The results were expected to be associated to phenotype. HbA1c levels in the MetSyn groups with the rs7903146 C allele are higher than T allele carriers. There are no differences between the HbA1c levels in both allele in the MetSyn + T2D. Although the C allele is good factor to prevent MetSyn, HbA1c concentration might not be correlated to TCF7L2 genotype. There is one report suggest that there is an association between the T risk allele and the levels of HbA1c.[28]

The variations in TCF7L2 gene polymorphism particularly rs7903146 could be a genetic risk factor for T2D.[29,30] The beta-cell dysfunction is also present in MetSyn patients, affecting dynamic pathways of glucose metabolism.[31] The alterations in glucose metabolism cause by these gene variations have been investigated largely. The T allele of TCF7L2 is a risk factor regulates insulin secretion. The TCF7L2 is transcription factor of Wnt signaling involves in several biological processes, from the differentiation of adipocytes, and the regulation of adipokines to pancreatic beta cell function.[32] The presence of the T allele of TCF7L2 rs7903146 gene variant could be linked to other diseases.

| Genotype | Normal subjects (n=80), n (%) | T2D subjects (n=110) n (%) | MetSyn subjects (n=90) n (%) | MetSyn + T2D subjects (n=94) n (%) |
|----------|-------------------------------|-----------------------------|-----------------------------|----------------------------------|
| rs7903146 (C/T) | | | | |
| TT | 7 (8.7) | 26 (23.6) | 22 (24.44) | 23 (24.47) |
| CT | 41 (51.3) | 52 (47.3) | 40 (44.44) | 43 (45.74) |
| CC | 32 (40.0) | 32 (29.1) | 28 (31.11) | 28 (29.79) |

Frequency of T allele (%) = 34.34

MetSyn = Metabolic syndrome; T2D = Type 2 diabetes
such as the incidence of cancer or the severity of coronary artery disease.\(^{30}\) The OR and the logistic regression analysis for T allele indicated that the common variant of TCF7L2 rs7903146 could be associated with MetSyn and the T allele of TCF7L2 rs7903146 gene polymorphism is a risk allele for MetSyn as well as type-2 diabetes mellitus. The variants of the TCF7L2 gene polymorphism could likely predict the development of T2D mellitus and abnormalities introduce MetSyn components such as dyslipidemia.

**CONCLUSIONS**

The present study demonstrated that TCF7L2 plays a significant role in the regulation of lipid and glucose metabolism, wherein the changes in its biological function could cause alteration in the susceptibility to dyslipidemia, MetSyn, and T2D. Our finding related to T allele, which plays as a risk allele in diabetes and metabolic syndrome, opens a narrow window to find the etiology of these diseases with important insights necessary for evaluating the role of TCF7L2 polymorphism in metabolic pathways.

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**Conflicts of interest**

There are no conflicts of interest.

**AUTHORS’ CONTRIBUTIONS**

- AP designed and contributed in the conception of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.
- MR carried out the molecular biology experiments and participated in revising the draft and agreed for all aspects of the work, read and approved the final draft of manuscript.
- HK participated in molecular biology experiments.
- MF agreed for all aspects of the work.

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**Table 4: The odds ratio of the presence of T allele frequency associated with the presence of type 2 diabetes and metabolic-syndrome**

| Genotype rs7903146 (C/T) | OR (95% CI) | P |
|--------------------------|------------|---|
| TT                       | 3.71 (1.43–9.56)\(^*\) | 0.008 |
| CT                       | 1.26 (0.67–2.39) | 0.465 |
| CC                       | Reference    | - |

\(^*\)=values calculated by Chi-square test. The result is significant at P<0.05 means it is dealing with dependent variables. OR = Odds ratio; 95% CI = 95% confidence interval (from minimum to maximum); MetSyn = Metabolic syndrome; T2D = Type 2 diabetes
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