

Sex-specific associations of *TCF7L2* variants with fasting glucose, type 2 diabetes and coronary heart disease among Turkish adults

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ABSTRACT

Objective: *TCF7L2* is a repressor and transactivator of genes, and its variants are strongly associated with diabetes. This study aimed to evaluate the sex-specific relationship between the most common *TCF7L2* gene variants (-98368G>T, rs12255372 and -47833C>T, rs7903146) with diabetes and coronary heart disease in Turkish Adult Risk Factor (TARF) Study.

Methods: Single nucleotide variants (SNVs) have been genotyped using the TaqMan allelic discrimination assays in 2,024 (51.3% in women, age: 55±11.8) Turkish adults participating in the TARF study. Statistical analyses were used to investigate the association of genotypes with clinical and biochemical measurements.

Results: Among the TARF study participants, 11.7%, 24.3%, 14.1%, and 38.3% had diabetes, hypertension, coronary heart disease (CHD), and obesity, respectively. The frequencies of T allele for -47833C>T and -98368G>T in Turkish adults were determined to be 0.35 and 0.33, respectively. -47833C>T was significantly associated with higher fasting glucose concentrations in all participants, especially in men. Both SNVs were significantly associated with diabetes and CHD in all participants (p<0.05). When study population was stratified according to sex, -98368G>T was associated with diabetes in women (p=0.041) and -47833C>T was associated with diabetes and CHD in men (p=0.018 and p=0.032, respectively). Also, both SNVs and the diplotypes of common haplotype (H1) remained strongly associated with type 2 diabetes after risk factors were adjusted (p<0.05).

Conclusion: T allele homozygosity of two SNVs as well as the diplotype H1-/H1- reflects risk of diabetes primarily in men. Enhanced CHD risk is determined by the presence of diplotype H1-/H1- among nondiabetic participants. (*Anatol J Cardiol* 2020; 24: 326-33)

Keywords: *TCF7L2*, variants, type 2 diabetes, coronary heart disease, TARF study

Introduction

TCF7L2 (transcription factor 7-like 2) gene is localized in 10q25.2-q25.3, and -98386G>T (rs12255372 in intron 4) and -47833C>T (rs7903146 in intron 3) are the most studied polymorphic variants (1-5). *TCF7L2* encodes a transcription factor that affects the Wnt (Wingless-Int) pathway (5). The *TCF7L2* protein has been implicated in blood glucose homeostasis. It has beta-catenin and protein-binding activity (6). It is also an RNA polymerase II transcription factor (7).

Type 2 diabetes is a common disease among adults both in Turkey and other countries (8, 9). Several recent studies have shown that *TCF7L2* gene variants are associated with fasting glucose levels, insulin secretion, and type 2 diabetes among different populations and ethnicities (1-5, 10-16). In contrast, the rs7903146T allele was found to be associated with a higher prevalence and severity of coronary artery disease and cardiovascular events among nondiabetic individuals (17). A recent Turkish study showed six intronic *TCF7L2* variants (including rs7903146 and rs12255372) were genotyped and associated with type 2 dia-

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betes and fasting glucose levels (12). However, the relationship between *TCF7L2* variants and coronary heart disease (CHD) is still unknown in the Turkish population. Additionally, the effects of cardiometabolic traits between men and women in the relationship of genetic variants with diseases were not mentioned in most studies. This study aimed to determine the differences among sexes of the two most studied variants previously associated with diabetes and CHD and to demonstrate the effect of cardiometabolic risk factors in genetic associations with diseases.

This study aimed to determine the sex-specific associations of *TCF7L2* gene variants (-98368G>T, rs12255372 and -47833C>T, rs7903146) and their haplotypes with diabetes and CHD in participants of Turkish Adult Risk Factor (TARF) Study.

Methods

Study sample

The study was designed as a cross-sectional analysis of the Turkish Adult Risk Factor (TARF) follow-up study. The design and methodology of the TARF Study have been previously described. Briefly, participants were randomly selected from the residents of all 7 different regions of Turkey and participated in 6 surveys from 1998 to 2008 (18). The TARF study data were obtained from the history of the past years via a questionnaire, physical examination of the cardiovascular system, and recording of a resting electrocardiogram. Randomly selected 2,024 participants (985 males and 1039 females) were examined for their *TCF7L2* genotype. Study subjects were not related from each other. Written informed consent was obtained from the participants of the study after being informed of its nature. The study protocol was approved by the Ethics Committee of the İstanbul Medical Faculty, İstanbul University.

Definitions

Metabolic syndrome (MetS) was identified when 3 out of the 5 criteria of the National Cholesterol Education Program (ATP III), modifying for prediabetes (fasting glucose 100-125 mg/dL) and abdominal obesity (cut point ≥ 95 cm in men) according to evaluations in the Turkish Adult Risk Factor study, are present (10). Atherogenic dyslipidemia (or simply dyslipidemia) was referred to as the combined presence of high triglyceride (≥ 150 mg/dL) and low HDL-C (< 40 mg/dL for men and < 50 mg/dL for women) values defined by the ATP III (19). Individuals with diabetes were diagnosed based on the American Diabetes Association criteria (20), namely, when fasting plasma glucose level was 126 mg/dL (or 2-h postprandial glucose > 200 mg/dL and/or the current antidiabetic medication use). Individuals with coronary heart disease (CHD) were defined as in a previously published study (21). Obesity was defined as a body mass index of ≥ 30 kg/m². Hypertension was defined as blood pressure of ≥ 140 mm Hg and/or ≥ 90 mm Hg and/or antihypertensive medication use.

Measurement of risk factors

Cigarette smoking was classified into two categories: current smokers and nonsmokers. An alcohol user was defined as anyone consuming alcohol once a week or more. Physical activity was classified into two categories: low physical activity, which includes white-collar worker, sewer-knitter, repair worker, house worker, and walking with a maximum of 2 km daily; and high physical activity, which includes mason, carpenter, truck driver, and floor and window cleaners and heavy labor, farming, walking for 2 and more km daily, and regular sports activity. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m²). Waist circumference was measured using a tape (Roche LI95 63B 00), with the subject standing and wearing only underwear, at the level midway between the lower rib margin and the iliac crest. Blood pressure was measured with an aneroid sphygmomanometer (Erka, Bad Tölz, Germany) on the right arm with the subject in the sitting position. Moreover, the mean of two recordings, 5 minutes apart, was recorded. Blood samples were collected after an 11-hour or longer fasting. Samples were shipped within a few hours on cooled gel packs stored at -75°C and analyzed at the Yıldız Technical University. Serum concentrations of total cholesterol, fasting triglycerides, glucose, and HDL-C (directly without precipitation) were determined using enzymatic kits from Roche Diagnostics with a Hitachi 902 autoanalyzer. Concentrations of apolipoprotein B and apolipoprotein A1 were measured using Behring kits and nephelometry (BN Prospec, Behring Diagnostics, Westwood, MA) (22). The insulin resistance of participants was assessed using the homeostatic assessment (HOMA) index calculated as fasting glucose (mmol/L) \times fasting insulin (micro units/mL)/22.5.

DNA isolation and genotyping of the *TCF7L2* variants

Genomic DNA was extracted from the peripheral blood leukocytes using a QIAmp_DNA Maxi Kit (Qiagen, Hilden, Germany). *TCF7L2* gene variants -47833C>T (rs7903146, intron 3) and -98368G>T (rs12255372, intron 4) were genotyped using the ABI prism 7900HT Sequence Detection System for both PCR and allelic discrimination with TaqMan technology (Applied Biosystems, Foster City, CA). The single nucleotide variants were genotyped using Assays-by-Design by Applied Biosystems under standard conditions. Genotyping quality for each variant was controlled using blind DNA duplicates for some samples. Genotyping accuracy was 100% for each duplicate.

Statistical analysis

Chi-square test was used in comparing genotypic and allelic. Hardy-Weinberg equilibrium was computed for the expected genotype distribution. Kolmogorov-Smirnov test ($p > 0.05$) was used to assess the normality of the continuous variables. Two-tailed t-test for normally distributed parameters and Mann-Whitney U test for not normally distributed parameters were used to compare continuous variables and were expressed as means and standard deviation (SD), while chi-square test was

used to compare categorical variables. One-way covariance analyses (ANCOVA) was performed using fasting glucose levels as dependent variables and the following covariates as independent variables, which are adjusted for the following: age, cigarette smoking, physical activity, and obesity. A p-value of 0.05 was considered statistically significant. Maximum likelihood estimates of odds ratios (OR) and associated 95% confidence intervals (CI) were calculated using binary logistic regression models, and a 95% CI not overlapping 1 was considered statistically significant. All statistical analyses were performed using Windows SPSS version 14.0 Software (SPSS Inc., Chicago, IL, USA) (21). Haplotypes were estimated from the two variants using the PHASE v2.0.2 program, which uses a Bayesian statistical method for reconstructing haplotypes from population genotype data and lists the most probable haplotype pairs (Hp) for each individual. Individuals with -98368G>T and -47833C>T variant

genotyping results were included in the haplotype estimation procedure (n=2024). Haplotype alleles were coded as haplotype numbers from 1 to 4 according to the frequency in all participants. Results are shown in the order as -98368 and -47833; haplotype 1 (H1) is G-C, haplotype 2 is T-T, haplotype 3 is G-T, and haplotype 4 is T-C. Linkage disequilibrium between sites was estimated using Haploview version 4.0 (www.broad.mit.edu/mpg/haploview), with data being presented as D'.

Results

Study population characteristics

To investigate the association of *TCF7L2* variants and type 2 diabetes, 2,024 subjects (985 males and 1,039 females) from the TARF study population have been analyzed. Table 1 shows the

Table 1. Demographic and biochemical characteristics of the participants

Characteristics	Men (n)	Women (n)	P-value
Age, years	55.3±12.1 (985)	55.0±11.8 (1039)	0.706
BMI, kg/m ²	27.8±4.4 (965)	30.6±5.7 (1016)	0.001
Total cholesterol, mg/dL	190.1±39.9 (983)	202.5±40.8 (1039)	<0.001
HDL-C, mg/dL	40.1±10.9 (977)	47.4±11.8 (1036)	<0.001
LDL-C, mg/dL	115.8±33.5 (950)	123.9±35 (1017)	<0.001
Fasting glucose, mg/dL	101.7±38.85 (968)	99.4±35.2 (1028)	0.371
Fasting triglycerides, mg/dL	147.9±1.74 (942)	134.9±1.62 (1009)	<0.001
HOMA index	2±2.29 (654)	2±2.09 (742)	0.201
Insulin, mIU/L	8.32±2.04 (658)	8.51±1.86(744)	0.075
Lipoprotein(a), mg/dL	8.13±3.09(398)	11.48±3.02 (398)	<0.001
CRP, mg/dL	1.05±4.57 (588)	1.23±4.37 (620)	0.037
Apolipoprotein A1, mg/dL	132.53±24.28 (550)	146.4±27.60 (583)	0.001*
Apolipoprotein B, mg/dL	99.7±26.23 (555)	103.06±26.64 (591)	0.068
Systolic BP, mm Hg	123.9±20.46 (978)	128.9±23.39 (1030)	<0.001
Diastolic BP, mm Hg	78.5±11.67 (978)	79.9±12.4 (1030)	0.008
Waist-to-hip ratio	0.97±0.37 (976)	0.86±0.08 (1032)	0.001*
Waist circumference, cm	96.3±11.18 (978)	93.1±12.56 (1034)	<0.001
Testosterone, ng/dL	7.59±2.34 (454)	3.16±3.47 (457)	<0.001
SHBG, nmol/L	40.7±1.62 (441)	50.1±1.67 (423)	<0.001
Prevalences			
Type 2 diabetes, % (n)	13.4 (132)	10.1 (105)	0.021
Hypertension, % (n)	34.9 (344)	47 (488)	<0.001
Metabolic syndrome, % (n)	39.1 (380)	45.4 (468)	0.004
Coronary heart disease, % (n)	15.3 (151)	13 (135)	0.129
Obesity, % (n)	26.9 (260)	50.8 (516)	<0.001
Dyslipidemia, % (n)	32.9 (308)	30.6 (308)	0.286
Current smoking, % (n)	38.7 (375)	14.5 (149)	<0.001
Alcohol usage, % (n)	26.9 (261)	1.9 (19)	<0.001
High physical activity, % (n)	50.6 (489)	27.1 (277)	<0.001

Continuous variables are presented as mean±SD and dichotomous variables as percentages. A two-tailed t-test* and Mann-Whitney U test were used for comparison of means, and X²-test was used for percentages. BP - blood pressure; CRP - C-reactive protein; HDL-C - high-density lipoprotein cholesterol; LDL-C - low-density lipoprotein cholesterol; HOMA - homeostatic assessment; SHBG - sex-hormone binding globulin

characteristics of the participants in the TARF study. Results revealed that men had slightly higher fasting serum glucose levels than women. Prevalences of type 2 diabetes and CHD were 13.4% and 15.3% in men and 10.1% and 13% in women ($p=0.021$ and $p=0.129$, respectively).

Allele and genotype frequencies of TCF7L2 variants

In this study population, both of the variants' (-98368G>T and -47833C>T) genotype distributions were shown in Hardy-Weinberg equilibrium ($p>0.05$). Table 2 shows the genotype and allele frequencies of -47833C>T and -98368G>T variants and diplotype frequencies of haplotype 1 (H1) in study participants. In the study population, allele frequencies for -47833T and -98368T were 0.345 and 0.331, respectively. Haplotype analysis revealed four possible haplotypes; two of them being more common. Haplotype 1 (GC), haplotype 2 (TT), haplotype 3 (GT), and haplotype 4 (TC) distributions were 0.624 ($n=2526$), 0.301 ($n=1218$), 0.044 ($n=179$) and 0.031 ($n=125$), respectively. In the study population, -98368G>T and -47833C>T variants were strongly linked with the D' value of 0.847.

Association between fasting glucose levels and TCF7L2 variants

In ANCOVA, among subjects not using antidiabetic medication, the distribution of mean fasting glucose levels by genotypes of variants and diplotypes of the haplotype 1 (H1), adjusted for age, cigarette smoking, physical activity, and obesity, is shown in Table 3. The -98368G>T variant was not significantly associated with fasting glucose levels in the study population. The -47833TT genotype was associated with higher glucose levels by 6 and 5 mg/dL in men and in all participants ($p=0.018$ and $p=0.029$, respectively) compared with C-allele carriers. Similarly, among men and all

participants, homozygous noncarriers of H1 had highly increased glucose levels (by 5.4–7.6 and 3.1–4.4 mg/dL, $p=0.037$ and 0.054, respectively) compared with carriers of diplotype H1. Glucose

Table 2. Genotype and allele frequencies of TCF7L2 variants and diplotypes in all participants, men and women

	All participants	Men	Women
-47833C>T, rs7903146			
Genotype frequency*			
CC, % (n)	42.2 (840)	40.6 (393)	43.7 (447)
CT, % (n)	46.6 (927)	46.0 (445)	47.2 (482)
TT, % (n)	11.2 (222)	13.3 (129)	9.1 (93)
Allele frequency			
G, %	65.5	63.7	67.3
A, %	34.5	36.3	32.7
-98368G>T, rs12255372			
Genotype frequency**			
GG, % (n)	44.0 (885)	42.0 (410)	46.0 (475)
GT, % (n)	45.7 (918)	46.6 (455)	44.8 (463)
TT, % (n)	10.3 (207)	11.5 (112)	9.2 (95)
Allele frequency			
G, %	66.9	65.3	68.4
A, %	33.1	34.7	31.6
Diplotypes of H1			
Diplotype frequency***			
H1-/H1-	13.2 (267)	14.8 (146)	11.6 (121)
H1-/H1+	48.8 (988)	48.8 (481)	48.8 (507)
H1+/H1+	38.0 (769)	36.3 (358)	39.6 (411)

The association between the genotype frequencies and sex was compared using the χ^2 -test (* $P=0.010$, ** $P=0.096$, *** $P=0.073$)

Table 3. Comparisons of multi-adjusted fasting glucose levels by TCF7L2 variants and diplotypes for all participants, men and women

	All Participants		Men		Women	
	Glucose, mg/dL (n)	P-value	Glucose, mg/dL (n)	P-value	Glucose, mg/dL (n)	P-value
Genotypes of -47833C>T						
CC	92.6±0.86 (727)	0.029	93.4±1.31 (339)	0.018	91.9±1.11 (388)	0.810
CT	93.4±0.83 (783)		94.1±1.25 (377)		92.9±1.09 (406)	
TT	97.9±1.70 (186)		100.7±2.30 (111)		93.0±2.53 (75)	
Genotypes of -98368G>T						
GG	92.4±0.83 (774)	0.244	93.5±1.28 (357)	0.261	91.7±1.07 (417)	0.147
GT	94.4±0.83 (765)		94.9±1.41 (380)		93.9±1.11 (385)	
TT	94.3±1.75 (175)		97.9±2.46 (97)		89.3±2.47 (78)	
Diplotypes of H1						
H1-/H1-	96.6±1.54 (225)	0.054	99.6±2.15 (126)	0.037	92.6±2.19 (99)	0.660
H1-/H1+	93.6±0.80 (828)		94.3±1.19 (407)		93.0±1.06 (421)	
H1+/H1+	92.3±0.89 (671)		93.1±1.37 (308)		91.6±1.15 (363)	

Subjects using antidiabetic medication were excluded from the analysis. Data is expressed as mean±SE. P-values are adjusted for the following covariates: age, cigarette smoking, physical activity, and obesity

levels were not significantly associated with -47833TT, -98368TT genotypes, and H1-/H1- diplotype among women.

Basic associations between type 2 diabetes and TCF7L2 variants

The allele frequencies of variants showed a significant association between diabetic and nondiabetic groups ($p < 0.001$ for -47833C>T and $p = 0.003$ for -98368G>T). TT genotype frequencies were 7.2% for -47833C>T variant and 3.3% for -98368G>T variant, higher in diabetic than nondiabetic individuals. The genotype frequencies of -47833C>T and -98368G>T variants showed statistically significant differences between diabetic and nondiabetic groups ($p = 0.002$ and $p = 0.007$, respectively) (Table 4).

When the study population was stratified according to sex, -47833C>T genotypes were associated significantly with diabetes in men ($p = 0.018$), but no significant difference was found in women ($p = 0.071$). The genotypic frequencies in men with diabetes were 21.3% for -47833TT, 43.3% for -47833CT, and 35.4% for -47833CC. The frequencies in nondiabetic men were 12.1%, 46.4%, and 41.4%, respectively. On the other hand, the -98368G>T variant was associated significantly with the presence of type 2 diabetes in women ($p = 0.041$), but no significant association was found in men ($p = 0.081$). The genotypic frequencies in women with diabetes were 9.6% for -98368TT, 55.8% for -98368GT, and 34.6% for -98368GG. The frequencies in nondiabetic women were 9.1%, 43.6%, and 47.3%, respectively.

Multi-adjusted association between type 2 diabetes and TCF7L2 variants

Multiple logistic regression analyses, adjusted for age, cigarette smoking, physical activity, and obesity, showed that both variants strongly affected the risk for type 2 diabetes in all participants and in men (Table 5). The logistic regression model comprised 933 men, with 121 cases of diabetes, and 991 women, with 100 cases of diabetes. Carriers of -47833TT were more likely to have diabetes

with an OR of 2.17 (95% CI: 1.42–3.32, $p < 0.001$). While male carriers had a similar OR of 2.16 (95% CI: 1.24–3.75, $p = 0.006$), female carriers had borderline association with diabetes (OR=1.94; 95% CI: 0.94–3.90, $p = 0.060$). Carriers of -98368TT had significant ORs for diabetes in men (OR=1.95; 95% CI: 1.08–3.53, $p = 0.026$) and all participants (OR=1.80, 95% CI: 1.14–2.84, $p = 0.012$), but not in women in whom heterozygote genotype for -98368G>T was associated with type 2 diabetes risk (OR=1.70; 95% CI: 1.08–2.67, $p = 0.021$). Similarly, -98368GT genotype increased the likelihood for diabetes in all participants (OR=1.44; 95% CI: 1.06–1.96, $p = 0.021$).

H1-/H1- diplotype was significantly associated with participants with diabetes at ORs of 1.90 (95% CI: 1.24–2.89, $p = 0.003$). Diabetes was, furthermore, associated with H1-/H1- diplotype in men (OR=1.91; 95% CI: 1.10–3.31, $p = 0.021$) and with H1+/H1- diplotype in women (OR=1.77; 95% CI: 1.10–2.84, $p = 0.018$), after being adjusted for age, cigarette smoking, physical activity, and obesity (Table 4).

Basic associations of TCF7L2 variants with CHD

A significant association was found between both variants and CHD in all participants. The T allele frequencies were 39% for -47833C>T ($p = 0.030$) and 38% for -98368G>T ($p = 0.010$) in subjects with CHD and 34% and 32% in subjects without CHD, respectively (Table 4). The frequency of -47833TT, CT, and CC genotypes was 20.1%, 43.8%, and 36.1%, respectively, in men with CHD ($n = 144$) and 12.2%, 46.4%, and 41.5%, respectively, in men without CHD ($n = 822$) ($p = 0.032$). However, no significant association was found between the -98368G>T variant and CHD in women (data not shown).

Multi-adjusted association of TCF7L2 variants with CHD

In multiple logistic regression analyses for the likelihood of CHD risk (Table 5), after being adjusted for age, cigarette smoking, physical activity, and obesity, H1-/H1- diplotype was associated with a 1.6-fold OR (95% CI: 1.02–2.49, $p = 0.040$) in all participants; the genotype -47833TT was associated in men with a 1.88-fold

Table 4. Frequencies of TCF7L2 variants in all participants with type 2 diabetes and coronary heart disease

Clinical status of subjects		Genotype frequencies, % (n)			P-value	Rare allele frequency	P-value
Diabetic	-47833C>T	CC	CT	TT	0.002	T allele	<0.001
	Nondiabetic	34.6 (79)	47.8 (109)	17.5 (40)		0.41	
Diabetic	-98368G>T	GG	GT	TT	0.007	T allele	0.003
	Nondiabetic	43.2 (761)	46.5 (818)	10.3 (182)		0.36	
With CHD	-47833C>T	CC	CT	TT	0.022	T allele	0.030
	Non-CHD	34.6 (81)	52.1 (122)	13.2 (31)		0.32	
With CHD	-98368G>T	GG	GT	TT	0.031	T allele	0.010
	Non-CHD	45.3 (804)	44.8 (796)	9.9 (176)		0.39	
With CHD	-47833C>T	CC	CT	TT	0.022	T allele	0.030
	Non-CHD	38.9 (107)	45.1 (124)	16.0 (44)		0.39	
With CHD	-98368G>T	GG	GT	TT	0.031	T allele	0.010
	Non-CHD	42.8 (733)	46.8 (802)	10.4 (178)		0.34	
With CHD	-47833C>T	CC	CT	TT	0.022	T allele	0.030
	Non-CHD	38.6 (110)	47.4 (135)	14 (40)		0.38	
With CHD	-98368G>T	GG	GT	TT	0.031	T allele	0.010
	Non-CHD	45 (775)	45.4 (782)	9.7 (167)		0.32	

The association between the genotype/allele frequencies and clinical status was compared using the χ^2 -test. CHD - coronary heart disease

Table 5. Association of *TCF7L2* variants and diplotypes with diabetes and coronary heart disease, by logistic regression analysis

	All Participants			Men			Women		
	P-value	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI
Type 2 diabetes									
-47833C>T CT	0.170	1.25	0.91-1.72	0.850	1.05	0.67-1.64	0.080	1.52	0.96-2.43
TT	<0.001	2.17	1.42-3.32	0.006	2.16	1.24-3.75	0.060	1.94	0.97-3.90
-98368G>T GT	0.021	1.44	1.06-1.96	0.410	1.20	0.78-1.85	0.021	1.70	1.08-2.67
TT	0.012	1.80	1.14-2.84	0.026	1.95	1.08-3.53	0.340	1.44	0.68-3.07
H1-/H1-	0.003	1.90	1.24-2.89	0.021	1.91	1.10-3.31	0.140	1.67	0.84-3.30
H1+/H1-	0.058	1.37	0.99-1.88	0.810	1.06	0.68-1.65	0.018	1.77	1.10-2.84
Coronary heart disease*									
-47833C>T CT	0.650	1.08	0.77-1.52	0.91	1.03	0.63-1.69	0.600	1.14	0.71-1.81
TT	0.055	1.59	0.99-2.54	0.043	1.88	1.02-3.48	0.720	1.15	0.53-2.49
-98368G>T GT	0.200	1.24	0.89-1.72	0.40	1.22	0.77-1.95	0.360	1.25	0.78-1.99
TT	0.068	1.58	0.97-2.56	0.31	1.41	0.72-2.75	0.120	1.75	0.86-3.58
H1-/H1-	0.040	1.60	1.02-2.49	0.06	1.79	0.98-3.28	0.390	1.34	0.69-2.63
H1+/H1-	0.830	1.04	0.74-1.46	0.73	1.09	0.67-1.78	0.950	0.99	0.62-1.58
Coronary heart disease†									
-47833C>T CT	0.830	1.04	0.74-1.46	0.880	1.04	0.64-1.7	0.910	1.03	0.43-1.64
TT	0.060	1.58	0.98-2.57	0.070	1.83	0.96-3.49	0.530	1.27	0.60-2.69
-98368G>T GT	0.370	1.16	0.84-1.62	0.500	1.18	0.74-1.88	0.600	1.13	0.71-1.81
TT	0.090	1.53	0.93-2.52	0.550	1.24	0.61-2.55	0.080	1.88	0.93-3.76
H1-/H1-	0.035	1.62	1.04-2.54	0.090	1.72	0.91-3.24	0.220	1.50	0.78-2.85
H1+/H1-	0.870	0.97	0.69-1.36	0.780	1.07	0.66-1.75	0.550	0.86	0.54-1.39

Adjusted for age, cigarette smoking, physical activity and obesity. P-values were obtained with logistic regression analysis. *Subjects using antidiabetic medication were excluded from the analysis. †Subjects with type 2 diabetes were excluded from the analysis. Significant ORs are highlighted in boldface. OR - odds ratio; CI - confidence interval

OR (95% CI: 1.02–3.48, p=0.043) when subjects using antidiabetic medication were excluded. This logistic regression model comprised 854 men, with 108 cases of CHD, and 890 women, with 99 cases of CHD.

After subjects with type 2 diabetes were excluded, the association between CHD and -47833TT genotype in men (p=0.070) and also between CHD and -98368TT genotype in women (p=0.080) was attenuated, but H1-/H1- diplotype persisted to be associated with a 1.62-fold OR (95% CI: 1.04–2.54, p=0.035) in all participants (Table 5). This model comprised 805 men, with 99 cases of CHD, and 881 women, with 96 cases of CHD.

Discussion

In this cross-sectional study, the associations of -47833C>T and -98368G>T variants with fasting glucose levels, type 2 diabetes, and CHD in a representative sample of Turkish adults were analyzed. Compared with common homozygotes, the T homozygotes of both variants and their diplotypes exhibited a significant multi-adjusted elevation of fasting glucose levels in men and approximately twofold higher risk of diabetes in all participants and

particularly in men. In women, contrary to men, with -98368GT genotype and diplotype H1+/H1-, fasting glucose level tended to be higher, and a significant adjusted OR of 1.7 was observed for diabetes. The noncarriage of haplotype 1 was associated with CHD in all participants.

SNVs in the *TCF7L2* gene were previously found to be strongly associated with type 2 diabetes and glucose levels among different populations (1-5, 10-17). The *TCF7L2* gene affects the Wnt pathway and beta-cell signaling. The mechanisms by which the *TCF7L2* affects type 2 diabetes are still not fully understood, but its influence on glucose levels has been confirmed. The Wnt signaling pathway plays important functions in different cell lineages and organs (23). Transcription factor β -cat/TCF is the main effector of the canonical Wnt signaling, which is formed by β -catenin and a member of the TCF family (TCF7, TCF7L1, *TCF7L2*, and LEF-1) (24). Several studies have shown that many elements of the Wnt pathway are involved in beta-cell activation (25), cholesterol metabolism, and glucose-induced insulin secretion (26). Wnt signaling through the *TCF7L2* receptor is important for GLP-1 secretion. GLP-1 hormone was shown to induce Wnt signaling in adult mouse pancreatic cells, and both *TCF7L2* and β -cat are necessary for GLP-1-stimulated proliferation of the rat pancreatic cell line INS-

1 (27). *TCF7L2* gene is also expressed in human pancreatic cells and affects normal beta-cell insulin secretion, beta-cell growth, and differentiation from precursor cells (28). Most SNVs related to diabetes, including genotyped intronic variants in this study, suggest that they are linked to functional variants in the *TCF7L2* gene, which may affect glucose metabolism and insulin secretion.

This study showed similar independent risk factors of T alleles of -47833C>T and -98368G>T variants for diabetes to a previously published study by Erkoç Kaya et al. (12) including 288 Turkish adults. Similar findings were found by Erkoç Kaya et al. (12) showing a significant association between these variants and type 2 diabetes and between -47833C>T variant and fasting glucose levels. However, in this study, both variants were shown to be associated with glucose levels, especially in men, and the effect of genotypes on diabetes in both sexes was different. These results suggest that *TCF7L2* SNVs play a role in the gender-specific differences of diabetes risk.

Similar findings were found in a meta-analysis conducted by Peng et al. (14), which revealed that -47833C>T and -98368G>T variants are associated with higher type 2 diabetes risk. Similarly, meta-analysis results reported by Liu et al. (15) and Dou et al. (16) showed a significant association between -47833C>T and type 2 diabetes susceptibility in different populations. Our findings are in agreement with those reported by Chandak et al. (29), who showed that -98368TT genotype increases fasting plasma glucose levels in the Indian population and that both of the most common variants are associated with type 2 diabetes in diabetic and nondiabetic individuals. Our results are also parallel to those reported by Kimber et al. (30), who showed that -47833C>T and -98368G>T variants significantly increase the risk of type 2 diabetes when adjusted for age, sex, and obesity.

A multi-ethnic study of determined genetic variations genome-wide found that the -47833C>T variant in *TCF7L2* gene locus was one of the variants that showed a very significant association with both type 2 diabetes and CHD (31). There are limited studies on this gene's variants and CHD in the literature. One study showed that no association was found between *TCF7L2* genotypes and coronary atherosclerotic lesions in diabetic patients (17). However, Sousa et al. (17) found that nondiabetic individuals carrying the -47833 T-allele were associated with 2.32-fold risk of coronary artery disease than noncarriers of the T-allele (adjusted for multiple confounders such as sex, BMI, smoking, hypertension, glycemia, total cholesterol, LDL-c, HDL-c, and triglycerides and the use of statins, aspirin, and beta-blockers). In this study (17), sexes were not analyzed separately. However, almost similar to their findings, the H1-/H1- diplotype in nondiabetic Turkish adults was associated with a 1.6-fold risk of CHD compared with common diplotypes of haplotype 1 (H1+/H1+). Also this study showed that the -47833TT genotype was associated in men with CHD (non-using antidiabetic drugs). These results show that cardiometabolic risk factors such as cigarette smoking, physical activity, and obesity affect T-allele homozygosity of both variants in all participants. In men, compared to all participants, with -47833TT genotype, a sig-

nificant adjusted OR of 1.88 was observed for CHD. This is the first study to report an association between sexes and the rare allele of these variants and/or the haplotype 1 carriage with respect to fasting glucose levels and risk for diabetes and CHD in the Turkish population. Underlying causes of sex-specific associations are unclear; an enhanced pro-inflammatory state may be a prominent pathophysiologic factor in women (32). Lifestyle characteristics and hormonal differences between sexes may play important roles in modulating these relationships.

Study limitations

This study has certain limitations. First, the haplotype analysis including all SNVs of the *TCF7L2* gene has not been completed. Second, the functional effect of these intronic variants has not been explained. In this study, participants constituting about 75% of the TARF cohort were genotyped. Detailed statistical analysis would have been possible if all contributors of the cohort could be included in the study. Furthermore, the generalizability of the present findings may be limited, considering the high prevalence of MetS in the study population. Nonetheless, most of the findings were similar with those observed from some other populations.

Conclusion

Compared with common homozygotes, the T homozygotes of each of the *TCF7L2* -47833C>T (rs7903146) and -98368G>T (rs12255372) variants as well as the diplotype H1-/H1- are significantly associated with elevated fasting glucose levels and risk of diabetes primarily among men. The -98368GT genotype and the diplotype H1+/H1- were associated with an increased risk of diabetes among women. Enhanced CHD risk was determined by the presence of diplotype H1-/H1- in all study participants without type 2 diabetes. As a result, in this cross-sectional study, sex-specific associations of the two variants in *TCF7L2* gene with diabetes and coronary heart disease in Turkish population were revealed. The current study results suggested that rare diplotype was an independent risk factor for the presence of CHD in nondiabetic adults. These variants might be strong candidates in determining Turkish adults' susceptibility to diabetes and CHD. However, the effects of *TCF7L2* SNVs and haplotypes on pathogenesis of diabetes and CHD with respect to sex differences require more extensive investigations in the future.

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