

Estrogen receptor alpha gene polymorphisms are associated with type 2 diabetes and fasting glucose in male subjects

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Abstract The PvuII and XbaI polymorphisms of the estrogen receptor α (ER1) gene have been variably associated with type 2 diabetes (T2D) in several populations. However, this association has not been studied in Iranian subjects and we hypothesized that the ER1 variants might be associated with T2D and related metabolic traits in this population. The PvuII and XbaI genotypes were determined by PCR-RFLP in 377 normoglycemic controls and 155 T2D patients. Bonferroni correction was applied for the correction of multiple testing. No significant association was found between the allele and genotype frequencies of PvuII and XbaI variants with T2D in females. In a dominant model (PP vs. Pp + pp), the frequency of the Pp + pp genotype was higher in normoglycemic subjects compared to T2D patients [85.5% vs. 66.7%, OR 0.22 (0.08–0.55), $P = 0.001$]. Four possible haplotypes were observed in the population, whereas haplotype TA had a higher frequency in male T2D subjects than the controls. Furthermore, non-diabetic male subjects carrying the genotype of PP had a higher fasting glucose levels than the individuals with the genotype of Pp + pp ($P = 0.013$).

Multivariate logistic regression analysis showed that PvuII polymorphism was the independent determinants of T2D in males [OR 4.37 (1.61–11.86), $P = 0.004$]. No association was found between the XbaI polymorphism and diabetes in male group. Our results suggest that the ER1 polymorphisms might associate with T2D and fasting glucose among Iranian male subjects.

Keywords Iranian population · Type 2 diabetes · Estrogen receptor · PvuII · XbaI

Introduction

Type 2 diabetes mellitus (T2D) is a common metabolic disorder that affects people all over the world [1]. This disease is characterized by peripheral insulin resistance in the liver, skeletal muscle, and adipose tissues, as well as impaired insulin secretion from the pancreatic beta cells [2]. While resistance to the biological action of insulin is the main feature of most patients with T2D [3], some degrees of insulin resistance has been reported in non-diabetic individuals [4]. It has been proposed that insulin resistance in T2D may result from multiple causes including genetic and acquired factors [5]. General and central obesity, low physical activity, high intake of saturated fat, and low intake of dietary fiber are some of the factors contributing to T2D [2, 3]. Furthermore, genetic factors play a major role in the development of the disease among different populations. Genome-wide scans as well as screening of candidate regions have enabled identification of single nucleotide polymorphisms (SNPs) in various genes, which increase the risk of becoming T2D. Angiotensin-converting enzyme, hepatocyte nuclear factor 4-alpha (HNF4A), human forkhead box O1 (FOXO1), and

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protein tyrosine phosphatase 1B (PTPN1) are some of the genes that have been suggested to link to T2D [6–8].

Estrogen exerts its effect through two transcription factors, estrogen receptors α (ER1), and estrogen receptor β (ER2). It appears that the ER1 is the primary receptor for estrogen. This receptor is located on chromosome 6q25.1, spans >140 kb, and consists of eight exons separated by seven intronic regions [9]. After binding the estrogen to the ERs, a conformational change leads to the homodimerization of the complex, resulting in binding to specific regions of the genome (estrogen response elements) and subsequently altering the expression of target genes [10]. It has been reported that the distribution of ER1 is higher in the reproductive system of women than in that of men; however, its expression in nonreproductive tissues in women and men seems to be similar. The expression of ER1 has been detected in many tissues such as breast, urogenital, gonadal, bone, central nervous system, intestinal, white adipose, skeletal muscle, macrophage, and vascular endothelial [11, 12]. Epidemiological studies have indicated that the estrogen may play a role in the etiology of cardiovascular disease. Specifically, there is some evidence showing a role for ER1 in the development of some cardiovascular risk factors such as insulin resistance, dyslipidemia, hypertension, obesity, and T2D [13–15]. Moreover, it has been suggested that ER1 may play a role in the pathogenesis of some diseases such as breast and prostate cancer, osteoporosis, and Alzheimer [10].

Several SNPs are located in the ER1 gene; the most frequently analyzed being located in intron 1. The PvuII restriction site polymorphism involves a T-to-C transition (rs2234693), while the XbaI restriction site polymorphism involves an A-to-G transition (rs9340799) [10]. Owing to their physical proximity, they are strongly associated and in linkage disequilibrium with each other. Several human studies have been performed to find the association between the PvuII and XbaI polymorphisms and T2D and related metabolic traits in various populations; however, there has been controversy as to whether these SNPs are correlated with T2D. The purpose of this study was to examine the possible association between the ER1 variants and T2D and its metabolic quantitative traits in a sample of Iranian subjects.

Materials and methods

Subjects

A detailed description of the study population has been previously published [4, 16]. We initially recruited 639 unrelated Iranian individuals ages 23–79 years. After excluding individuals with abnormal glucose tolerance

based on the WHO 1997 criteria [17], 377 normoglycemic and 155 T2D subjects were included in the analyses. 115 diabetic patients were recruited from the Diabetes Clinic of Shariati Hospital, Tehran University of Medical Sciences, whereas 40 diabetic patients were selected after screening of general population from the same area. Written informed consent was obtained from all participants before enrollment in the study.

Screening included standardized questionnaires on personal data and clinical measurements such as age, gender, obesity, drug consumption during the past month, and medical or family history of diabetes. All those who were not taking diabetes medication underwent a 2 h oral glucose tolerance test (OGTT) after an overnight fast. Criteria for control selection were fasting glucose <6.1 mmol/l and 2 h plasma glucose <7.8 mmol/l after OGTT. Diabetes was defined as fasting glucose \geq 7.0 mmol/l, 2 h glucose \geq 11.1 mmol/l after OGTT, or use of hypoglycemic medication. Systolic and diastolic blood pressure was measured twice in the right arm of the subjects who had been resting for at least 10 min in a comfortable position. Body mass index (BMI) was calculated as weight in kilograms divided by the height in meters squared. The waist circumference was taken at the midpoint between the iliac crest and the lower rib margin, and the hip circumference was taken around the maximum circumference of the buttocks posteriorly and the symphysis pubis anteriorly. Insulin resistance was assessed from glucose and insulin concentrations by the use of homeostasis model assessment of insulin resistance (HOMA-IR) equation [18].

Laboratory measurements

Blood samples were taken at 0 and 120 min and plasma preserved with EDTA and serum was separated immediately by centrifugation at 2500 rpm for a period of 10 min. The samples were processed immediately or in the first week following preservation at -20°C . Glucose measurements (intra-assay CV 2.1%, inter-assay CV 2.6%) were carried out using the glucose oxidase method. Total cholesterol and triglyceride were determined using enzymatic methods. High-density lipoprotein (HDL) was measured in the supernatant after precipitation of apolipoprotein B (apoB)-containing lipoproteins using phosphotungstic acid and magnesium chloride. Low-density lipoprotein (LDL) was calculated using the Friedewald formula. All of the measurements were carried out in a Technicon[®] analyzer RA[™] 1000. Serum concentrations of insulin were determined by radioimmunoassay. The intra- and inter-assay coefficients of variation for insulin were 4.8 and 5.5%, respectively. Plasma apoB concentrations were determined using the immunoturbidometric assay.

Genotyping

Genomic DNA was isolated from leukocytes using the commercial DNA isolation kit. The presence of PvuII and XbaI polymorphisms in the ER1 gene was analyzed using PCR-RFLP method. A 1372 bp of ER1 gene was amplified using the forward primer, 5'-CTG CCA CCC TAT CTG-TAT CTT TTC CTATTC TCC-3'; and the reverse primer, 5'-TCTTTC TCT GCC ACC CTG GCG TCG ATT ATC TGA-3'. PCR was carried out in a final volume of 25 μ l containing 100 ng genomic DNA, 1.5 mmol/l MgCl₂, 0.5 mmol/l of each dNTPs, 0.5 pmol of each primer. After an initial denaturation of 2 min at 94°C, the samples were subjected to 30 cycles at 94°C for 1 min, 62°C for 40 s, and 72°C for 60 s, with a final extension of 10 min at 72°C. The 1372 bp product was digested with *Avall* for 2 h at 37°C. The undigested 1372 bp product represents the pp genotype; the PP genotype produced two fragments with 982 and 390 bp, while heterozygote Pp produced three fragments of 1372, 982, and 390 bp. For XbaI polymorphism, the XX genotype produced two fragments of 936 and 436 bp whereas heterozygote Xx produced three fragments of 1327, 936, and 436 bp. The xx genotype had one fragment of 1327 bp. The genotypes were scored after running on a 2.5% agarose gel and staining with ethidium bromide.

Statistical analysis

All statistical analyses were carried out using the statistical program SPSS (version 13, SPSS, Chicago, IL, USA); *P* values are two-sided throughout, and *P* < 0.05 was considered significant. Genotypic and allelic frequencies were compared using the χ^2 or Fisher's exact test. Odds ratios (ORs) and 95% CI, with adjustment for age and BMI were calculated by logistic regression analysis. We used the χ^2 test to evaluate deviation from Hardy–Weinberg equilibrium. Baseline quantitative results are expressed as mean \pm SD; the continuous variables that failed the normality test were logarithmically transformed before analysis. The variables transformed were triglyceride, insulin, and HOMA-IR. Statistical differences are based on analyses of log-transformed data, but the means of untransformed data are presented in table. We used general linear model analysis to assess the relationship between the genotypes of ER1 SNPs with clinical and biochemical parameters. Covariates included age and BMI. We used the genotype data for each of the two SNPs to infer the haplotype alleles present in the population by using the program PHASE [19]. For the purpose of correcting for multiple testing, the statistical significance was specified by means of Bonferroni correction. Consequently, difference was considered significant when the *P* value was less than 0.0125.

Results

All clinical and metabolic characteristics differed significantly between T2D and normoglycemic groups. T2D subjects had significantly higher values for systolic and diastolic blood pressure, BMI, waist and waist to hip ratio, glucose, cholesterol, triglyceride, LDL, apoB, insulin, and HOMA-IR, and lower levels of HDL than the normoglycemic subjects (data not shown) (All *P* values < 0.0001). The mean age of men and women were 45.21 \pm 12.9 and 45.89 \pm 13.5 years old, respectively.

We evaluated the genotype and allele frequency of the PvuII and XbaI variants in the case–control samples. The genotype frequencies of both SNPs did not differ significantly from those expected under Hardy–Weinberg equilibrium. No significant differences in PvuII and XbaI genotype and allele frequencies were noted between T2D and normoglycemic subjects. However, when stratified by gender, we noticed a significant difference for PvuII allele frequency between the T2D and controls only in male group. The P allele of the ESR-1 PvuII polymorphism was more prevalent in the T2D group than in the controls in male group (*P* = 0.024) (Table 1), but this result did not remain statistically significant after Bonferroni correction. In a dominant model (PP vs. Pp + pp), the frequency of the Pp + pp genotype was higher in normoglycemic subjects compared to T2D patients [85.5% vs. 66.7%, OR 0.22 (0.08–0.55), *P* = 0.001] and these results were still statistically significant after Bonferroni correction for multiple testing (Table 1). However, no significant association was found between the allele and genotype frequencies of PvuII variant with T2D in females. For XbaI polymorphism, no statistically significant difference was detected for allele or genotype frequencies between T2D and control in female group. However, in a dominant model, the XX + xx genotype was more frequent in male subjects with normoglycemic than the T2D patients [78.9% vs. 62.5%, OR 0.39 (0.17–0.89), *P* = 0.026] (Table 2), but this data did not remain statistically significant after Bonferroni correction. Four possible haplotypes were observed in the population. Logistic regression analyses showed no significant association of haplotypes with T2D in females. In men, a significant association was found between the frequency of haplotypes and T2D after adjustment for age and BMI and these results remain statistically significant after Bonferroni correction. Haplotype TA had a higher distribution in T2D than the controls [35.5% vs. 18.6%, OR 1.49 (1.03–2.34), *P* = 0.03], this was not statistically significant following Bonferroni correction (Table 3).

We used general linear model analysis to assess the relationship between the genotypes of PvuII and XbaI polymorphisms with anthropometrical and biochemical parameters. Covariates in the analysis were age, BMI, and

Table 1 Genotype and allele frequency of the PvuII variant

Variant	Normoglycemic subjects (%)	T2D subjects (%)	OR (95%CI)	<i>P</i> value
<i>PvuII</i>				
Men				
Genotype				
PP	22 (14.5)	24 (33.3)	–	
Pp	88 (57.9)	33 (45.8)	0.76 (0.53–2.36)	0.535
pp	42 (27.6)	15 (20.8)	0.26 (0.09–0.79)	0.017
Allele				
<i>P</i>	43.8%	56.6%	–	
<i>p</i>	56.2%	43.4%	0.54 (0.32–0.92)	0.024
Dominant model				
PP	22 (14.5)	24 (33.3)	–	
Pp + pp	130 (85.5)	48 (66.7)	0.22 (0.08–0.55)	0.001 ^a
Recessive model				
PP + Pp	110 (72.4)	57 (79.2)	–	
pp	42 (27.6)	15 (20.8)	0.80 (0.34–1.87)	0.618
Women				
Genotype				
PP	42 (28.6)	28 (35.0)	–	
Pp	85 (57.8)	40 (50.0)	0.88 (0.57–2.93)	0.971
pp	20 (13.6)	12 (15.0)	0.63 (0.25–1.6)	0.336
Allele				
<i>P</i>	57.5%	60.0%	–	
<i>p</i>	42.5%	40.0%	0.91 (0.57–1.44)	0.705
Dominant model				
PP	42 (28.6)	28 (35.0)	–	
Pp + pp	105 (71.4)	52 (65.0)	0.78 (0.31–1.82)	0.338
Recessive model				
PP + Pp	127 (86.4)	68 (85.0)	–	
pp	20 (13.6)	12 (15.0)	1.01 (0.94–1.82)	0.734

Data are presented as *n*(%). All *P* values and odds ratios are adjusted for age, BMI, and family history of diabetes

^a *P* values less than 0.0125 are considered significant after Bonferroni correction

family history of diabetes. Among T2D patients, when the data were analyzed in male and female groups separately, no significant difference in anthropometric and biochemical parameters was seen between the genotypes of PvuII (PP vs. Pp + pp) and XbaI (XX vs. Xx + xx) in both genders (data not shown).

Among non-diabetic group, no significant difference in anthropometrical and biochemical parameters was observed between the genotypes of PvuII (PP vs. Pp + pp) and XbaI (XX vs. Xx + xx) in female group. In non-diabetic male individuals, we only observed significantly higher fasting glucose levels in the PP carriers compared to the individuals carrying the Pp + pp genotype of the PvuII polymorphism (5.23 ± 0.46 vs. 4.98 ± 0.49 , $P = 0.13$) after Bonferroni correction (Table 4).

We further assessed the relation between PvuII and XbaI variants and T2D by multiple logistic regression analysis, including the PvuII variant and several clinical

and biochemical features as independent variables (Table 5). In addition to age and triglycerides and after bonferroni correction, the PP genotype was associated with a significantly increased risk of T2D [OR 4.37 (1.61–11.86), $P = 0.004$], suggesting that the PP genotype is an independent risk factor for T2D in male group, with the *P* allele increasing the risk. Replacing the XbaI variant (XX vs. Xx + xx) with the PvuII in the model resulted in age, triglyceride and XX genotype [OR 2.72 (1.09–6.73), $P = 0.012$] as independent risk factors of T2D and this result remained significant after bonferroni correction.

Discussion

Many studies in different populations have been conducted to find an association between the PvuII and XbaI variants

Table 2 Genotype and allele frequency of the XbaI variant

Variant	Normoglycemic subjects (%)	T2D subjects (%)	OR (95%CI)	P value
<i>XbaI</i>				
Men				
Genotype				
XX	32 (21.1)	27 (37.5)	–	
Xx	87 (57.2)	30 (41.7)	0.58 (0.22-1.52)	0.292
xx	33 (21.7)	15 (20.8)	1.05 (0.91-1.95)	0.049
Allele				
X	49.7%	58.3%	–	
x	50.3%	41.7%	0.71 (0.42-1.20)	0.207
Dominant model				
XX	32 (21.1)	27 (37.5)	–	
Xx + xx	120 (78.9)	45 (62.5)	0.39 (0.17-0.89)	0.026
Recessive model				
XX + Xx	119 (78.3)	57 (79.2)	–	
xx	33 (21.7)	15 (20.8)	1.13 (0.47-2.15)	0.775
Women				
Genotype				
XX	44 (29.9)	30 (37.5)	–	
Xx	80 (54.4)	38 (47.50)	0.675 (0.27-1.68)	0.677
xx	23 (15.6)	12 (15.0)	1.17 (0.66-2.21)	0.329
Allele				
X	57.1%	61.2%	–	
x	42.9%	38.8%	0.83 (0.52-1.32)	0.447
Dominant model				
XX	44 (29.9)	30 (37.5)	–	
Xx + xx	103 (70.1)	50 (62.5)	0.607 (0.30-1.20)	0.154
Recessive model				
XX + Xx	124 (84.4)	68 (85.0)	–	
xx	23 (15.6)	12 (15.0)	1.17 (0.49-1.96)	0.719

Data are presented as *n*(%). All *P* values and odds ratios are adjusted for age, BMI, and family history of diabetes

Table 3 Analysis of ESR1 haplotypes in male group

Haplotypes	Normoglycemic (%)	T2D (%)	OR (95%CI)	P value	Total P value
CG	24.1	22.1			
CA	2.1	1.6	0.89 (0.66–1.56)	0.43	
TG	55.2	41.0	0.64 (0.39–1.06)	0.08	
TA	18.6	35.3	1.49 (1.03–2.34)	0.03	0.001 ^a

All *P* values and odds ratios are adjusted for age, BMI, and family history of diabetes

^a *P* values less than 0.0125 are considered significant after Bonferroni correction

of the ER1 gene and CVD risk factors such as T2D, dyslipidemia, hypertension, and insulin resistance. Conflicting results have been reported about the effect of the ER1 PvuII and XbaI variants on the risk of developing T2D and its related metabolic traits. In this study, it appears that there is an association between the ER1 PvuII and XbaI variants and T2D and fasting glucose only in male subjects. The frequency of T allele of PvuII and A allele of XbaI

variants significantly were higher in male T2D patients compared to normoglycemic subjects. Furthermore, we found that male normoglycemic subjects carrying the AA genotype of PvuII polymorphism had higher fasting plasma glucose levels in comparison with subjects with the Pp + pp genotype. In addition, logistic regression analysis showed an increased risk of T2D for both PvuII and XbaI variants. We also observed a TA haplotype that increases

Table 4 Clinical and biochemical parameters of PvuII genotypes in male normoglycemic subjects

	PP 22	Pp + pp 130	<i>P</i> value
Systolic blood pressure (mmHg)	116.50 ± 16.85	115.20 ± 17.48	0.494
Diastolic blood pressure (mmHg)	77.40 ± 10.01	76.42 ± 10.31	0.509
Body mass index (kg/m ²)	26.15 ± 4.48	25.90 ± 4.27	0.685
Waist circumference (cm)	86.9 ± 11.7	86.4 ± 18.3	0.571
Waist to hip ratio	0.86 ± 0.08	0.85 ± 0.13	0.578
Glucose (mmol/l)	5.23 ± 0.46	4.98 ± 0.49	0.013 ^a
Cholesterol (mmol/l)	5.11 ± 1.04	5.15 ± 1.25	0.524
Triglyceride (mmol/l)	1.59 ± 0.99	1.50 ± 0.89	0.509
HDL (mmol/l)	1.05 ± 0.23	1.03 ± 0.22	0.501
LDL (mmol/l)	3.80 ± 1.00	3.75 ± 1.18	0.734
Apolipoprotein B (mmol/l)	1.05 ± 0.27	1.04 ± 0.34	0.790
Insulin (μU/ml)	9.31 ± 4.22	9.59 ± 4.43	0.640
HOMA-IR	2.22 ± 1.01	2.08 ± 0.98	0.310

All *P* values are adjusted for age, BMI, and family history of diabetes

^a *P* values less than 0.0125 are considered significant after Bonferroni correction

Table 5 Multiple logistic regression analysis of determinants of T2D in male group

Variables	OR	OR (95%CI)	<i>P</i> value
Age	1.164	1.111–1.146	0.000
Systolic blood pressure (mmHg)	0.987	0.966–1.188	0.225
Diastolic blood pressure (mmHg)	1.013	0.980–1.048	0.441
Body mass index (kg/m ²)	1.004	0.945–1.079	0.887
Cholesterol (mmol/l)	0.945	0.725–1.455	0.569
Triglyceride (mmol/l)	1.869	1.145–4.441	0.008
HDL (mmol/l)	0.896	0.389–2.078	0.624
LDL (mmol/l)	1.125	0.848–3.215	0.242
PvuII (PP vs. Pp + pp)	4.37	1.61–11.86	0.004 ^a
XbaI (XX vs. Xx + xx)	2.72	1.09–6.73	0.012 ^a

^a *P* values less than 0.0125 are considered significant after Bonferroni correction

the risk of developing T2D in the population. Taken together, our results suggest that the T allele of PvuII and A allele of XbaI increase the risk of developing T2D in male individuals of this population. A gender-specific association has also been reported from Chinese [20] and Hungarian [21] populations. To our knowledge, this study is the first report on the association of ER1 variation and diabetes in males only. In contrast, a study in African- and European-Americans showed no association between the PvuII and XbaI SNPs and T2D [22]. In another study in Swedish population, although SNPs of the ER1 gene were significantly associated with T2D and fasting plasma glucose, but PvuII and XbaI SNPs were not found to be associated with T2D and fasting glucose [23]. It is worth noting that no difference in the allele frequency of both ER1 SNPs was observed in a study from Iran among female subjects [24], supporting our study that demonstrated no association between PvuII and XbaI SNPs and T2D in female group. The reasons for these discrepancies are unknown and differences in the genetic and/or environmental background as well as in recruitment procedures of the studied populations may have played a role.

It may be surprising that the findings of this study are among men only and that there is no evidence of association in women. The explanation for such an association in this study is unclear. Previous studies have demonstrated that normal ER α function is needed for normal cardiovascular development in males [25, 26] and a modest effect of ESR1 polymorphisms on ER α function may have larger clinical importance under conditions of very low levels of circulating estrogens, such as in males [27]. It is important to consider that an association has been reported between the ESR1 PvuII variation and myocardial infarction [28], coronary artery disease [29], and stroke [30] in males. In contrast to our negative result in women, it has been reported that PvuII variation might be associated with diabetes in post-menopause women [31]. To rule out the possibility that we recruited more pre-menopause than post-menopause women in the study sample, we analyzed the association of ER1 variation with menopause status in women. Our data showed that there is no difference in the PvuII and XbaI allele and genotype frequencies between pre- and post-menopause women (data not shown). In addition, as our negative results in women, we do not

completely exclude the possibility that the reported findings may be due to a smaller effect size among women.

The molecular mechanism by which the PvuII and XbaI variants might affect glucose homeostasis has not yet been completely understood. It has been suggested that the C allele of PvuII produces a binding site for B-myb transcription factor. B-myb that its expression is induced by estrogen, can augment transcription of a downstream reporter construct tenfold [32]. This suggests that the presence of the C allele might amplify the ER1 transcription or produce ER1 isoforms that have different properties than the full-length gene product. Similarly, the A-to-G transition of XbaI may also alter transcription and have an effect on the expression of the ER1.

Estrogen affects glucose homeostasis in humans and animals. Estrogen deficiency in postmenopausal women may contribute to the risk of visceral obesity, insulin resistance, and T2D [33]. Hormone replacement therapy reduces the incidence of T2D [34] and may have a beneficial effect on insulin sensitivity and glucose homeostasis in healthy and T2D postmenopausal women [35], although this effect has not been observed in all studies [36]. Studies in experimental animals support that ESR1 mediates the impact of estrogens on glucose homeostasis. ESR1 deficient (ERKO), but not ESR2 deficient (BERKO), mice exhibit insulin resistance, impaired glucose tolerance, and obesity [37]. Studies in ERKO mice have further revealed that estrogens regulate glucose homeostasis mainly by modulating hepatic insulin sensitivity [37]. Furthermore, the Aromatase knockout (ArKO) mice cannot produce estradiol [38], and both male and female ArKO mice have reduced glucose oxidation, increased adiposity, and insulin levels [39] that might lead to T2D. One study has shown that male ArKO mice develop glucose intolerance and insulin resistance that can be reversed by estradiol treatment [40]. In addition, in vitro studies have shown that ER1 can modulate many factors involved in the molecular mechanism of T2D such as Akt/PKB [41], insulin receptor substrate 1 (IRS1) [41], islet amyloid polypeptide (IAPP) [42], peroxisome-proliferator activated receptor (PPAR) [43], and caveolin-3 (Cln-3) [44]. These factors affect insulin secretion, insulin resistance, and insulin signaling. Furthermore, it has been suggested that the ER1 variations may affect the level of adipokines and cytokines in the plasma. In one study, it was shown that the T allele of PvuII polymorphism may associate with low circulating levels of adiponectin, an antidiabetic adipokine, in postmenopausal women with T2D [31]. Therefore, it can be concluded that the ER1 SNPs such as PvuII and XbaI, as described above, may affect some factors in the insulin signaling and insulin secretion leading to hyperglycemia and T2D in this population. However, further studies are

needed to reveal the mechanism by which PvuII and XbaI SNPs affect glucose homeostasis in different tissues.

Our results did not show any association of the PvuII and XbaI SNPs with the cardiovascular risk factors such as obesity, hypertension, insulin resistance, and dyslipidemia. The A allele of XbaI and T allele of PvuII SNP has been reported to have adverse and beneficial effect on health outcomes [45–49]. Several other studies have also shown no association between any cardiovascular factors and ER1 SNPs in various populations [50–53]. The reason for the apparent discrepancies between the studies, including ours, can be attributed to several factors such as the study design, population heterogeneity, sample size, and gene-environment interactions [54].

Our study had some strengths and limitations. We collected a homogeneous sample of well-characterized cases and controls, which increases sensitivity for detecting the associations. Most of the studies performed on the ER1 variants did not include normoglycemic subjects and individuals with impaired glucose tolerance were included in the samples analyzed. The healthy subjects included in the analyses of this study were all normal glucose tolerance and normal fasting glucose. This was due to the characteristic of the subjects with abnormal glucose tolerance that manifest the dual defects of T2D, reduced insulin sensitivity and impaired β -cell function [47]. We acknowledge that the number of female subjects might be small in the population and this does not allow drawing any definitive conclusions in this group. Therefore, a larger population is required to establish a definitive role for these variations in Iranian female population.

In conclusion, the findings of our study revealed that the ER1 PvuII and XbaI SNPs seem to influence the risk of T2D in Iranian male individuals. However, drawing any definitive conclusion on the effect of the ER1 variants on T2D needs to study the association in larger and gender-specified groups in other populations.

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