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Brief Report

Genetic Polymorphisms of Estrogen Receptors in Iranian Women with Diabetes and Coronary Artery Disease

Shekufeh Golkhu[1], Mahboobe Ghaedi[2], Narges Mohammad Taghvaie[2], Mohammad Ali Boroumand[3], Gholamreza Davoodi[3], Alireza Aminzadegan[3], Leila Poorgoli[3], Mahmood Sheikh Fathollahi[3]

[1]Biomedical Laboratory, Women Research Center, Al-Zahra University,
[2]Department of Clinical Biochemistry, Faculty of Medicine, Tarbiat Modarres University,
[3]Research Department, Tehran Heart Center, Tehran University of Medical Sciences, Tehran, Iran.

Correspondence:
Shekufeh Golkhu PhD,
Biomedical Laboratory,
Women Research Center,
Al-Zahra University,
Tehran, Iran.
Tel/Fax: +98 21 88049809
Email: golkhoo@yahoo.com
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Abstract

Estrogen might play an important role in the pathogenesis of diabetes mellitus type 2. Estrogens inhibit diabetes via distinct mechanisms particularly by reducing both hyperglycemia and plasma insulin levels. Estrogen exerts its physiological effects mainly through estrogen receptors including α and β types. Estrogen receptors are found in many tissues that participate in the pathogenesis of type 2 diabetes. Two common polymorphisms, PvuII and XbaI in estrogen receptor α gene, are reported to be associated with decreased receptor activity and increased risk of diabetes. We aimed to investigate the association between estrogen receptor α polymorphisms and diabetes, where a genetic component may be the major risk factor for this disease. One hundred women with diabetes type 2 were compared with one hundred women without diabetes for PvuII and XbaI polymorphisms. Of whom 61% of cases and 29% of controls had coronary artery disease. The participants were genotyped for these polymorphisms using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis. The genotype distribution and frequency of mutated allele showed no significant differences between diabetic and non-diabetic groups in PvuII (χ^2=0.981; P=0.612) and XbaI (χ^2=0.362; P=0.83) polymorphisms. When coronary artery disease as the potential confounding factor was controlled by logistic regression analysis, it was found that the PvuII and XbaI variants were not related to the type 2 diabetes mellitus (P=0.60 and P=0.99, respectively). Neither PvuII nor XbaI genotypes was associated with increased susceptibility to the type 2 diabetes mellitus in selected Iranian women with diabetes and coronary artery disease.

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Keywords ● Estrogen ● PvuII ● XbaI ● diabetes ● coronary artery disease ● estrogen receptor

Introduction

Diabetes mellitus type 2, is a systemic disease characterized by imbalance of energy metabolism mainly caused by inadequate insulin action. The disease is characterized mainly by disrupted glucose homeostasis with deleterious consequences on many organs such as kidneys, eyes, nervous system, and heart and, when untreated, is associated with increased mortality.[1]

The etiology of diabetes mellitus type 2 is a combination of

environmental and genetic factors, however, it is believed that the main factor disrupting glucose homeostasis is insulin resistance.[2] Sex hormones may act on diabetogenic susceptibility in men and women in several ways.[3] Studies have shown that induction of insulin-dependent diabetes can be inhibited by estrogens. Estrogen modulates insulin secretion, regulates *ATP*-sensitive potassium *channel* (*K-ATP channel*) activity, and regulates calcium signals via plasma membrane estrogen receptors. Estrogen can also stimulate liver fatty acid metabolism, suppress hepatic glucose production, reduce both hyperglycemia and plasma insulin levels, protect pancreatic β-cell function/survival, and increase GLUT-4 expression and glucose uptake.[4] Estrogen modulates GLUT-4 expression in tissues through its receptors.

The physiological function of estrogen is mediated via two specific receptors, estrogen receptor-1 (also called ESR1 or ERα) and estrogen receptor-2 (also called ESR2 or ERβ). ERα is a member of the nuclear steroid receptor family and a ligand-inducible transcription factor. Its isoforms are expressed in most tissues of the human body,[5] and were shown to play an important role in prevention of diabetes in both men and women.[6] Thus, the gene encoding ERα gene is a potential candidate for susceptibility to type 2 diabetes.

The ERα gene is located on chromosome 6. Five polymorphisms in the ERα gene have been reported in the genomic DNA extracted from human breast tumors or normal human peripheral blood leukocytes.[7] The first polymorphism, PvuII caused by a C/T transition (P1/P2) in intron 1 is located 0.4 kb upstream from exon 2.3. The second polymorphism XbaI caused by a G/A transition (X1/X2) is located 50 bp downstream from the PvuII polymorphic site. Recent studies have suggested that the two polymorphisms might be associated with breast cancer, postmenopausal osteoporosis, repeated abortion, arterial hypertension, altered serum lipid levels, coronary artery disease, and diabetes mellitus.[8]

We speculated that possible link might exist between PvuII and XbaI polymorphisms and the occurrence of diabetes. Therefore, the present study was designed to examine the prevalence of PvuII and XbaI mutation to assess the association between these polymorphisms and diabetes in a sample of Iranian women with diabetes.

Material and Methods

Subjects

One hundred women with diabetes type 2 were compared with 100 non-diabetic ones (controls). All participants were consecutively selected from patients underwent coronary angiography who admitted to Tehran Heart Center between June 2004 and June 2005. A single physician visited and examined all the participants.

Diagnosis of type 2 diabetes mellitus was based on WHO criteria (1999).[9] Individuals with fasting blood glucose ≥ 7.0 mmol/L or plasma blood glucose≥ 11.1 mmol/L were considered as having diabetes while individuals with fasting blood glucose < 5.6 mmol/L and 2-hour plasma blood glucose < 7.8 mmol/L were considered as those without diabetes. Others with borderline values (7.0 mmol/L > fasting blood glucose ≥ 5.6 mmol/L or 11.1 mmol/L > plasma blood glucose ≥ 7.8 mmol/L) were excluded from the study.

Patient and control individuals had no history of a sex-hormone-dependent disease, never received hormone replacement therapy, and had no significant liver damage or renal dysfunction. Informed consent was obtained from the participants according to the guidelines of our ethics committee.

Biochemical Analyses

Blood samples were collected from the patients after a 12-hour fasting. Total cholesterol, HDL-cholesterol, and triglyceride levels were determined by standard methods using commercial kits from (Pars Azmon, Iran). LDL-cholesterol was calculated according to the Friedewald formula.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using standard salting out method. The presence of PvuII and XbaI polymorphism within estrogen receptor-1 gene was analyzed using polymerase chain reaction–based restriction fragment length polymorphism (PCR-RFLP). The oligonucleotide primers used to determine the PvuII and XbaI polymorphisms were: forward, 5'-CTG CCA CCC TAT CTG TAT CTT TTC CTA TTC TCC-3'; reverse, 5'-TCT TTC TCT GCC ACC CTG GCG TCG ATT ATC TGA- 3'. The PCR was carried out in a total volume of 50 µ1. Reaction mixtures consisted of 100 ng template DNA, 50 ng of the forward primer and 50 ng of the reverse primer, 200 µM each dNTP, 10 mM Tris-Hcl (pH 8.3), 50 mM KCl, 3 mM MgCl2 and 1 unit Taq DNA polymerase. PCR parameters for detection of these transitions were as follows: an initial denaturation step of 5 min at 95 ˚C followed by 32 cycle of 95 ˚C/50s (denaturation), 62 ˚C/50s (annealing) and 72 ˚C/50s (extension) and final extension for 7 min at 72 ˚C to

ensure a complete extension of all PCR products. To distinguish c.454-397 T > C (PvuII) and c.454-351A > G (XbaI) polymorphism, the amplified PCR fragment of 1372 bp was digested with restriction enzyme XbaI and PvuII separately, followed by electrophoresis on 3% agarose gel. For PvuII, the mutated homozygous variant CC produced two fragments 982 and 390 bp when heterozygote CT produced three fragments of, 1372 and 982 bp and 390. Wild-type CC produced one fragment of 1372 bp. For XbaI the mutated homozygous variant AA produced two fragments 936 and 436 bp when heterozygote AG produced three fragments of 1327, 936, and 436 bp and GG wild-type produced one fragment of 1327 bp.

Statistical Analysis

Numerical variables were presented as mean ± standard deviation while categorical variables were summarized by absolute frequencies and percentages. Allele frequencies were calculated for each genotype by allele counting. Continuous variables were compared using independent two-sample *t* test, and categorical variables were compared using Chi-square test. Two separate logistic regression models were established to evaluate the relation between the PvuII and XbaI variants and the type 2 diabetes mellitus when controlling for coronary artery disease as the potential confounder. For the statistical analysis, the SPSS software version 13.0 for windows (SPSS Inc., Chicago, IL, USA) was used. All P values were 2-tailed with statistical significance defined as P≤0.05.

Results

One hundred patients with diabetes and 100 controls without diabetes were genotyped for the two common polymorphisms, XbaI and PvuII, in estrogen receptor-1 gene. All participants were women aged 30 to 70 years. The mean age of the patients with diabetes and controls were 60.19 ± 8.07 years and 56.84 ± 10.08 years (t=2.59; P=0.01), respectively. Sixty one percent of the patients with diabetes (n=61) and 29% of controls (n=29) had coronary artery disease (χ^2=20.69; P<0.001). The blood LDL, HDL, total cholesterol, and triglyceride levels of the participants were measured to determine their relations with coronary artery disease. Clinical data of the participants are summarized in table 1.

PvuII and XbaI Polymorphism

The frequencies of CC, CT, and TT genotypes in diabetic and control groups were 21%, 53%, and 26%, and 24%, 46%, and 30%, respectively. The frequencies of mutated allele (C) and normal allele (T) in patients with diabetes were 48% and 52% while in control group were 47% and 53%, respectively.

There was no significant difference in prevalence of PvuII genotype in either groups (χ^2=0.981; P=0.612). The frequency of PvuII polymorphisms in patients with diabetes compared with non-diabetic group is shown in table 2.

Table 1: Mean age and laboratory data of the participants

Characteristics	Women with diabetes (n=100)		Women without diabetes (n=100)		P value
	Mean ± SD	median	Mean ± SD	median	
Age (year)	60.19 ± 8.07	59.50	56.84 ± 10.08	57.00	0.01
TG (mg/dl)	208.76 ± 112.94	173.00	175.55 ± 89.47	157.50	0.02
TCH (mg/dl)	209.65 ± 51.67	207.00	209.43 ± 47.71	206.00	0.97
HDL (mg/dl)	43.04 ± 12.27	43.00	41.64 ± 10.49	41.50	0.37
LDL (mg/dl)	126.63 ± 42.46	124.00	134.12 ± 4.40	132.00	0.22

TCH: Total cholesterol, TG: Triglycerides, LDL: Low-density lipoprotein cholesterol, HDL: High-density lipoprotein cholesterol, SD: Standard deviation

Table 2: Distribution of PvuII and XbaI genotype and alleles' frequency in women with and without diabetes

Genotype / Patients	TT (%)	CT (%)	CC (%)	T (%)	C (%)
with diabetes	26	53	21	52	48
without diabetes	30	46	24	53	47
Genotype / Patients	**GG (%)**	**AG (%)**	**AA (%)**	**G (%)**	**A (%)**
with diabetes	27	46	27	50	50
without diabetes	26	50	24	51	49

For PvuII χ^2=0.981; P=0.612, Heterozygous T/C, Homozygous mutant C/C, Normal T/T, For XbaI χ^2=0.362; P=0.83, Heterozygous A/G, Homozygous mutant A/A, Normal G/G

When the effect of coronary artery disease was controlled by logistic regression analysis, it was revealed that there was no association between PvuII variant and type 2 diabetes mellitus (P=0.60). The frequencies of AA, AG, and GG genotypes were 27%, 46%, and 27%, and also 24%, 50%, and 26% in women with and without diabetes, respectively. The distribution of mutated allele (A) and normal allele (G) in diabetic and non-diabetic groups were 50%, and 50%, and 49%, and 51%, respectively. The difference between the two groups was not significant (χ^2=0.362; P=0.83).

The prevalence of XbaI polymorphism in patients with diabetes in comparison with non-diabetic group is shown in table 2. Again, when the effect of coronary artery disease was controlled by logistic regression model, it was found that the XbaI variant was not related to type 2 diabetes mellitus (P=0.99).

Upon adjustment for age and triglyceride, as potential confounders, we found no association between PvuII, and XbaI polymorphisms and type 2 diabetes mellitus (table 3).

Table 3: Association between PvuII, and XbaI polymorphisms and diabetes analyzed by using logistic regression in women with and without diabetes

Type of polymorphism	OR	95% CI for OR	P value
PVUII			
+/- *v* -/-	1.224	0.621-2.413	0.559
+/+ *v* -/-	0.959	0.427-2.156	0.919
Xbal			
+/- *v* -/-	0.836	0.418-1.675	0.613
+/+ *v* -/-	1.313	0.650-2.656	0.448

OR: Odds ratio; CI: Confidence interval, * ORs are adjusted for age and triglycerides

Discussion

Type 2 diabetes mellitus is a heterogeneous disorder caused by a combination of genetic and acquired abnormalities that affect insulin sensitivity and insulin secretion.[10] Identification of the susceptibility genes for type 2 diabetes mellitus; thus, may lead to primary prevention of the disease.

Sex steroids clearly have an impact on insulin resistance risk. Recent data have revealed a surprising role of estradiol in regulating energy metabolism, which opened new insights into the role of the two estrogen receptors.[11] Therefore, estrogen receptors seem to play a role in the prevention or in the occurrence of diabetes type 2.[12]

ERα polymorphisms have attracted great interest in the last few years and the PvuII and XbaI are the most extensively investigated issues. However, we do not know whether polymorphic alterations in the genes are responsible for ERα function and, in particular, the alterations analyzed in the present study are responsible for higher or lower receptor expression. Also, it is not known how and to what extent the polymorphisms of the ERα gene may act as genetic markers of diabetes.

PvuII and XbaI polymorphisms may be different in their effects on ERα. There are a few studies on the relationship of ERα polymorphisms and diabetes. A study of 49 Caucasians with type 2 diabetes mellitus and a control group indicated that ERα may be associated with type 2 diabetes. But in our study population, genotype distribution in the healthy individuals was different from that of other Caucasians and Chinese.[13] The genotype related to type 2 diabetes mellitus may be the reason why the cases group did not match with Hady-Weinberg equilibrium.

In another study in China, Qin and coworkers reported that PvuII polymorphism in ERα is associated with type 2 diabetes. In the present study, XbaI genotype was not related to type 2 diabetes mellitus. Our results indicated that, for the two single nucleotide polymorphism, difference in the locations might lead to difference in the roles. However, the different roles need to be shown in further studies.[14] The statistical power that can be seriously affected by small sample size might be a reason for reaching non-significant genetic associations in our study.

Because of the receptor-mediated effects of estradiol on inhibition of insulin-dependent diabetes and decreasing diabetes complications, we hypothesized that PvuII and XbaI polymorphisms might be associated with higher risk of diabetes.

The association between PvuII and XbaI polymorphisms in ERα gene and diabetes was studied in our selected participants who were divided into diabetic and non-diabetic groups. The genotype distribution of PvuII and XbaI polymorphisms in women with diabetes were compared with non-diabetic ones. We did not observe any association between PvuII polymorphism and diabetes or significant relation between XbaI polymorphism and diabetes. The genotype distribution and frequency of mutated alleles in both polymorphisms showed no significant difference in women with or without diabetes. When the effect of coronary artery disease was controlled by logistic regression, it was found that the PvuII and XbaI variants were not related to type 2 diabetes mellitus.

The present findings are limited in the way that they were obtained from a relatively small study population and our non-significant findings may be caused by the poor statistical power. The

results of our study should be considered exploratory and confirmed by additional studies, which include larger sample size and other polymorphisms in estrogen receptor. Investigation of these polymorphisms in other ethnic groups and comparing premenopausal with postmenopausal women are recommended. The molecular mechanism of type 2 diabetes pathogenesis mediated by PvuII and XbaI polymorphism should also be elucidated in experimental animals.

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Conflict of Interest: None declared

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