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Genetic polymorphism of estrogen receptor alpha gene in Egyptian women with type II diabetes mellitus

Tarek M.K. Motawi [a], Mahmoud A. El-Rehany [b], Sherine M. Rizk [a], Maggie M. Ramzy [c], Doaa M. el-Roby [a,*]

[a] Biochemistry Department, Faculty of Pharmacy, Cairo University, Egypt
[b] Biochemistry Department, Faculty of Pharmacy, Draya University, Egypt
[c] Biochemistry Department, Faculty of Medicine, Minia University, Egypt

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ABSTRACT

Estrogen might play an important role in type 2 diabetes mellitus pathogenesis. A number of polymorphisms have been reported in the estrogen receptor alpha gene including the XbaI and PvuII restriction enzyme polymorphisms. The aim of this study was to determine if ESRα gene polymorphisms are associated with type 2 diabetes mellitus and correlated with lipid profile. Ninety diabetic Egyptian patients were compared with forty healthy controls. ESRα genotyping of PvuII and XbaI was performed using restriction fragment length polymorphism analysis. Our study showed that there is more significant difference in the frequency of C and G polymorphic allele between patients and control groups in PvuII and XbaI respectively. Also carriers of minor C and G alleles of PvuII and XbaI gene polymorphisms were associated with increased fasting blood glucose and disturbance in lipid profile as there is an increase in total cholesterol, triglycerides and Low density lipoprotein. So findings of present study suggest the possibility that PvuII and XbaI polymorphisms in ERα are related to T2DM and with increased serum lipids among Egyptian population.

1. Introduction

Type 2 diabetes mellitus (T2DM) is thought to be a multifactorial disease and both genetic and acquired factors contribute to its pathogenesis. Identification of the susceptibility genes for type 2 diabetes mellitus thus may lead to primary prediction of the disease (Huang et al., 2006). In most patients, T2DM results from genetic changes therefore it is helpful to identify the population with genetic predisposition and to protect them from exposure to environmental risks (Ganasyam et al., 2012) as the environmental factors play an important role in favoring or delaying the expression of the disease.

Estrogen is a steroid hormone that influences many physiological processes, which include female reproduction, cardiovascular control, and bone integrity. Estrogen also exerts beneficial systemic effects on lipoprotein and antioxidant metabolism (Knopp and Zhu, 1997).

Therefore there is some evidence that estrogen plays a role in a combination of physiologic and metabolic disorders including insulin resistance, dyslipidemia, hypertension and excessive fat accumulation (Howard et al., 2003; Lindsay and Howard, 2004).

Due to its lipophilic characteristic, estrogen diffuses through plasma membrane and binds to its receptor (ER), a member of the nuclear receptor superfamily located in the nucleus and cytoplasm forming an estrogen/ER complex. This complex binds to estrogen response element sequences in the promoter region of estrogen responsive genes resulting in recruitment of co regulatory proteins (co-activators or co-repressors) to the promoter and gene expression regulation (Nilsson et al., 2001). Therefore, ERs are key components in the physiological effect of circulating estrogen as well as other metabolic and physiological processes (Casazza et al., 2010).

ESRα gene encompasses 140 kb of DNA composed by eight exons, encoding a 595 amino acids protein with a molecular weight of about 66 KDa. The first intron of a gene, like the promoter, usually contains a larger number of regulatory sequences than other introns. Several single nucleotide polymorphisms (SNPs) have been identified on ESRα and some of them were associated with either an increased or a decreased risk of various diseases (Gennari et al., 2005).

The best characterized SNPs of ESRα are the c454-397T>C and c454-351A>G site polymorphisms, both located in the first intron. These polymorphisms are 397 and 351 bp upstream of exon 2 and have been described by the name of detecting restriction enzyme, PvuII or XbaI, or

Abbreviations: ERα, estrogen receptor alpha; RFLP, restriction fragment length polymorphism; FBG, Fasting blood glucose; SNP, single nucleotide polymorphism.

* Corresponding author at: Department of Biochemistry, Faculty of Pharmacy, Cairo University, Ismail Hamed St., Minia, Egypt.
E-mail addresses: tarek.motawi@pharma.cu.edu.eg (T.M.K. Motawi), elrehany1963@yahoo.com (M.A. El-Rehany), Sherine.abdelaziz@pharma.cu.edu.eg (S.M. Rizk), magymaher@mu.edu.eg (M.M. Ramzy), doaa_mohamed440@yahoo.com (D.M. el-Roby).

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their reference ID numbers, rs2234693 and rs9340799, respectively (Araújo et al., 2011).

The PvuII and XbaI SNPs of the ESRα gene were found to be associated with several estrogen-dependent characteristics such as the onset of menopause (Weel et al., 1999), coronary reactivity (Lehtimäki et al., 2002), lumbar spine bone mineral density (BMD), vertebral bone area and vertebral fracture risk in post-menopausal women (Van Meurs et al., 2003), as well as blood pressure (Peter et al., 2005) and lipid profile (Molvarec et al., 2007a).

Also, various pathological conditions, including cardiovascular disorders (Lawlor et al., 2006), severe pre-eclampsia (Molvarec et al., 2007b) and venous miscarriage (Silva et al., 2010) have been described. A possible functional mechanism attributed to PvuII and XbaI polymorphisms includes a change of ESRα gene expression by altering the binding of transcription factors (Araújo et al., 2011).

The prevalence of T2DM and associated traits such as obesity, dyslipidemias, and hypertension in the overall population has become a worldwide challenge for health care system (Ganasyam et al., 2012).

This study aimed both to evaluate the ESRα gene polymorphisms (PvuII and XbaI) in type 2 diabetic Egyptian women and to correlate the lipid profile (serum cholesterol, triglycerides, LDL and HDL) changes with ESRα gene polymorphism.

2. Patients and methods

2.1. Study population

This study includes ninety (obese and non-obese) postmenopausal women with T2DM and forty non diabetic (obese and non-obese) controls. All subjects were Egyptians of the same ethnic group selected from outpatient clinic in Minia Hospital University aged 51–70 years, non-smokers, not consanguineous, and had no significant liver damage or renal dysfunction. Diagnosis of type 2 diabetes mellitus was based on WHO criteria (1999). Individuals with fasting blood glucose (FBG) ≥ 125.9 mg/dl were considered as having diabetes, while individuals with FBG < 100.7 mg/dl were considered non-diabetic. Others with borderline values (125.9 mg/dl > FBG ≥ 100.7 mg/dl) were excluded from the study. Patients and control subjects have no history of a sex-hormone-dependent disease and never received hormone replacement therapy.

The present study was conducted according to the principles of the Declaration of Helsinki, and all the participants provided written informed consent following a protocol approved by Minia University Research Ethics Committee. Body mass index (BMI) was measured for all the subjects.

2.2. Biochemical analysis

Peripheral blood was collected from the patients and control after a 12-h fasting on plain tubes, fluoride tube for fasting blood glucose and ethylenediaminetetraacetic acid (EDTA) tubes. The portion collected on EDTA tubes were divided into two aliquots; the first one was used for estimation of glycated hemoglobin (HBA_1C) and the later were stored at-20 °C for DNA extraction. (HBA_1C) was measured by TC MATREX analyzers using the kit supplied from TECO DIAGNOSTICS (USA) using (HBA_1C) reagent 1, 2a, 2b and two hemolysis liquid reagents. The serum was separated and used for assessment of the following parameters: FBG was assayed enzymatically using kit supplied by (Biomed, Germany). Total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), and triglyceride (TG) levels were determined by standard methods using commercial kits from (Biomed, Germany). Low density lipoprotein-cholesterol (LDL-C) was calculated according to the Friedewald formula (Friedewald et al., 1972).

2.3. Genomic DNA extraction and genotyping

Genomic DNA was extracted from whole blood using the established protocol for DNA extraction from blood cells (Medrano et al., 1990).

The PvuII and XbaI polymorphisms of ERα were analyzed by polymerase chain reaction restriction fragment length Polymorphism (PCR-RFLP) (Bittencourt-Oliveira et al., 2009).

A 119 bp DNA fragment that contains two polymorphic sites was amplified using forward and reverse primers 5′-CTGTGTTGTCCATCAC TTCATC-3′ and 5′-CCATTAGAGACCAATGCTCATC-3′. PCR was performed through 30 cycles by the following steps: denaturation at 95 °C for 60 s; annealing at 52 °C for 30 s; and extension at 72 °C for 30 s. The PCR product was digested with restriction enzyme PvuII, Xba1 (BioLabs, USA) at 37 °C for 16 h. The digested PCR products applied to a 2.5% agarose gel and stained with ethidium bromide. For PvuII, the homozygous variant TT produced two fragments 78 and 41 bp (indicate the presence of restriction site) while heterozygote TC produced three fragments of 119 and 78 bp and 41. The homozygous CC produced one fragment of 119 bp (represent the absence of restriction site). For XbaI the homozygous variant AA produced two fragments 88 and 31 bp (indicate the presence of restriction site) when heterozygote AG produced three fragments of 119, 88, and 31 bp and GG produced one fragment of 119 bp (represent the absence of restriction site). Representative samples were confirmed by sequencing. Samples were sequenced in MACROGEN lab technology.

2.4. Statistical analysis

Data entry and analysis were all done using software SPSS version 13. Quantitative data were presented by mean and standard deviation, while qualitative data were presented by frequency distribution. Chi Square used to test the significant difference for proportion and calculation of Odds ratio, and one way ANOVA test followed by the Tukey's test for multiple comparison. The genotypic and allelic frequencies were assessed using the Hardy–Weinberg equilibrium. The probability of less than 0.05 was used as a cut off point for all significant tests.

3. Results

3.1. Demographic and clinical variables

The demographic and biochemical parameters of the T2DM patients and their age-matched controls are summarized in (Table 1). The average age of obese and non-obese patients with diabetes were 59.4 ± 4.3 and 56.4 ± 4.2, respectively and in controls were 55.8 ± 2.9 and 56.3 ± 3.1, no significant difference between the four groups was found when analyzed by ANOVA. In comparison with the control groups and T2DM patients showed a significant increase in serum TG, TC, LDL-C, fasting blood glucose and HBA_1C levels.

3.2. Genotypes and allele frequencies

The genotype distribution and the allele frequencies among the control and T2DM patients are shown in (Table 2). The frequencies of TT, TC, and CC genotypes in control group were 37.5%, 47.5%, 15%, while in T2DM patients were 26.7%, 54.4%,18.9% respectively showing presence of significant difference and represent high risk factor among patients who carry heterozygous and homozygous polymorphic gene TC, CC (OR = 1.3, CI: 0.62–2.7; OR = 1.1, CI: 0.47–3.6; P = 0.01,0.04). The frequencies of AA, AG, and GG genotypes in control group were 50%, 37.5%, 12.5%, while in T2DM patients were 43.3%, 37.8%, 18.9% respectively showing a risk factor among patients who carry G allele in homozygous and heterozygous form (OR = 1.6, CI: 0.55–4.7; OR = 1.01, CI: 0.46–2.1; p = 0.03,0.7).