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

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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.

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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).

Table 1
Demographic and biochemical characteristics of study subjects.

	Control		T2DM patients		P value
	Non-obese (n = 25)	Obese (n = 15)	Non-obese (n = 40)	Obese (n = 50)	
Age (years)	56.3 ± 3.1	55.8 ± 2.9	56.4 ± 4.2	59.4 ± 4.3	0.4
FBG (mg/dl)	90.7 ± 11.02	91.6 ± 4.4	160.6 ± 24.1	203.5 ± 44.3	0.001*
HBA_1C	4.8 ± 0.6	4.8 ± 0.6	8.2 ± 0.9	8.9 ± 1.3	0.001*
Body mass(kg/m^2)	24.2 ± 1.1	34.1 ± 1.9	26.2 ± 2.1	35.9 ± 2.6	0.001*
TC (mg/dl)	106.4 ± 28.4	214.7 ± 26.7	198.6 ± 25.3	236.1 ± 44.7	0.002*
HDL-C (mg/dl)	46.2 ± 6.2	39.1 ± 7.5	44.8 ± 7.3	38.7 ± 7.6	0.001*
TG(mg/dl)	91.2 ± 7.3	166.9 ± 9.5	201.2 ± 26.8	225.3 ± 41.3	0.001*
LDL-C (mg/dl)	43.1 ± 5.5	142.2 ± 32.8	113.1 ± 29.4	152.4 ± 44.5	0.001*

Data are presented as mean ± SD. Comparisons between groups were analyzed by ANOVA test.
* Statistically highly significant.

Table 2
Allele frequencies and genotypes distribution of ER-α gene PvuII and XbaI polymorphisms in control and T2DM patients.

	Control n (%) (n = 40)	T2DM n (%) (n = 90)	OR (95% CI)	P value
PvuII				
T allele	49(61.3%)	97(53.9%)		
C allele	31(38.7%)	83(46.1%)	1.3(0.5–2.3)	0.02*
TT	15(37.5%)	24(26.7%)	Reference	
TC	19(47.5%)	49(54.4%)	1.3(0.62–2.7)	0.01*
CC	6(15%)	17(18.9%)	1.1(0.47–3.6)	0.04*
XbaI				
A allele	55(68.7%)	107(59.4%)		
G allele	25(31.3%)	73(40.6%)	1.08(0.6–1.9)	0.04*
AA	20(50%)	34(37.8%)	Reference	
AG	15(37.5%)	39(43.3%)	1.01(0.46–2.1)	0.7
GG	5(12.5%)	17(18.9%)	1.6(0.55–4.7)	0.03*

Comparisons were performed by the chi-square test; (CI) = confidence interval; OR = odds ratio. P value of odds ratio.
* Statistically significant. P < 0.05.

3.3. T → C polymorphism (PvuII) (Fig. 1)

Carriers of C allele in the homozygous or heterozygous form (CC or TC genotypes) were associated with highly significant increase in Fasting blood glucose, triglycerides, total cholesterol and LDL-C (p = 0.01), (p = 0.001), (p = 0.002) and (p = 0.01) respectively, but there was a significant decrease in the level of HDL-C (p = 0.002). (Table 3).

3.4. A → G polymorphism (XbaI) (Fig. 2)

Fasting blood glucose, Triglyceride and LDL-C showed an increase among patients who carry G allele in the heterozygous and homozygous (AG, GG genotypes) as there was a highly significant increase in these parameters (p = 0.03), (p = 0.001) and (p = 0.04) respectively while there was no significant difference in total cholesterol and HDL-C (Table 4).

Our results were confirmed by sequencing Fig. 3(A, B).

4. Discussion

Type 2 diabetes mellitus (T2DM) is a public health problem in the world with a high prevalence which is the most noticeable disease in developing countries (Morita et al., 2005) so it is helpful to identify the population with genetic predisposition and to protect them from exposure to environmental risks (Ganasyam et al., 2012). Different studies showed that estrogen can inhibit the deduction of insulin dependent diabetes, modulates insulin secretion, regulates calcium signals through plasma membrane receptors, and regulates K-ATP channel activity (Speer et al., 2001; Zhang et al., 2002). Also, Estrogen has been implicated in the suppression of hepatic glucose production and the protection of B-cell (Matic et al., 2013).

In this study, PvuII polymorphism in 51–70 years' women from Minia city, Egypt was examined. Our data showed that there is an increase in the percentage of heterozygous and homozygous TC and CC alleles in diabetic patients compared to control group suggesting that C allele may increase the risk of incidence of DM.

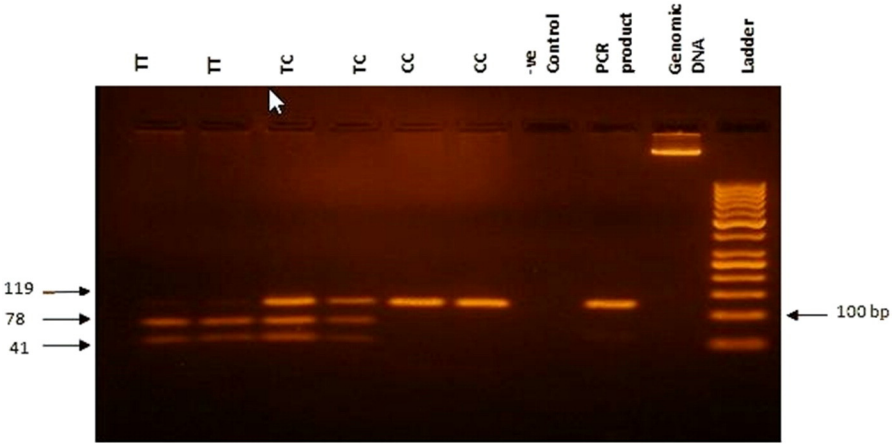

Fig. 1. PCR-RFLP analysis on 2.5% agarose gel representing the PvuII genotypes.

Table 3
The relationship between PvuII genotypes and different clinical parameters among diabetic study population.

	PvuII		
	Carriers of TT Mean ± SD	Carriers of TC Mean ± SD	Carriers of CC Mean ± SD
Age	57.3 ± 3.8	57.6 ± 5	57.6 ± 7.1
FBG (mg/dl)	170.9 ± 21.7	189.5 ± 33.8*	179.8 ± 42*
BMI	28.1 ± 2.2	31.4 ± 2.5	31.2 ± 2.1
Total cholesterol (mg/dl)	185.7 ± 12.5	224.8 ± 36.5**	229.4 ± 33.1**
HDL-C(mg/dl)	46.9 ± 5.4	38.3 ± 7**	39.6 ± 6.5**
Triglycerides (mg/dl)	184.3 ± 11.9	225 ± 33.5**	216 ± 31.6**
LDL-C(mg/dl)	99.7 ± 17.2	139.6 ± 37.7*	146.5 ± 35.1*
HBA_1C (%)	8 ± 0.9	8.5 ± 1.2	8.3 ± 1.4

Data are presented as mean ± SD. comparisons were performed by one way ANOVA test followed by the Turkey's test for multiple comparison.
* Indicates significant difference from carriers of TT at $P < 0.05$.
** Highly significance.

Table 4
The relationship between XbaI genotypes and different clinical parameters among diabetic study population.

	XbaI		
	Carriers of AA Mean ± SD	Carriers of AG Mean ± SD	Carriers of GG Mean ± SD
Age	57.4 ± 4.5	57.5 ± 3.3	58 ± 4.3
FBG (mg/dl)	160.4 ± 25.9	188.7 ± 37*	181 ± 38.7*
BMI	29.6 ± 2.7	31.3 ± 2.2	31.1 ± 2
Total cholesterol (mg/dl)	197.6 ± 22.1	227.1 ± 34.7	216.1 ± 36.6
HDL-C(mg/dl)	46.3 ± 4.9	38.8 ± 6	42.4 ± 6.3
Triglycerides (mg/dl)	181.9 ± 10.5	225 ± 34.1**	216.3 ± 25**
LDL-C(mg/dl)	115.1 ± 33.8	143.1 ± 36*	130.6 ± 34.6*
HBA_1C (%)	8.1 ± 1.1	8.6 ± 1.2	8.1 ± 1.2

Data are presented as mean ± SD. comparisons were performed by one way ANOVA test followed by the Turkey's test for multiple comparison.
* Indicates significant difference from carriers of AA at $P < 0.05$.
** Highly significance.

Although a study of 49 Caucasians with type 2 diabetes mellitus showed no significant difference between type 2 diabetic group and control group in the allelic frequency of PvuII of ESRα (Huang et al., 2006) however African–Americans and European-Americans study (Sale et al., 2004) showed a significant association of ESRα gene PvuII polymorphisms and type 2 diabetes mellitus.

The position of PvuII in an intron, near the gene promoter suggests a possible role in either transcription regulation or mRNA processing and stability (Araújo et al., 2011). So in a previous study it was found that CC genotype at PvuII predicted lower ESRα mRNA levels in patients prefrontal cortex (Weickert et al., 2008). Also PvuII polymorphism may affect the splicing of ESRα mRNA, resulting in the alteration of protein expression (Huang et al., 2006) or production of ESRα isoforms that have different properties than the full-length gene product (Casazza et al., 2010).

The C allele contains a potential binding site for myeloblast transcription factors that can amplify transcription of a downstream reporter as much as tenfold in vitro. The binding site for myeloblastosis expression is responsive to estrogen activation (bound to ER), resulting in a signal-altering system with differential responses (Huang et al., 2006). A previous report confirmed the association between the C allele of the pvuII polymorphism and the absence of expression of the ESRα gene (Li et al., 2012).

It is clear that estrogen affects the lipoprotein metabolism in many potential beneficial ways (Di Croce et al., 1996). Although an Indonesian study in Javanese population showed that PvuII allele C has been associated with lower small LDL concentration also that allele in this study was associated with no increased risk for suffering type 2 diabetes mellitus (Akhmad et al., 2013) however a Chinese study suggested that the PvuII polymorphism of ESRα is associated with type 2 diabetes and related to increased serum lipid levels in postmenopausal women (Huang et al., 2006).

Our study was in agreement with the last one as lipid profile assay shows a significant increase among patients group who carry C allele in the homozygous or heterozygous form (CC or TC genotypes) as there was a highly significant increase in triglycerides, total cholesterol and LDL-C while there was a highly significant decrease in the level of HDL-C, that high serum lipid can increase the risk of T2DM in healthy group (Mohammadi et al., 2013). These results may explain several studies which have shown a positive relationship of ESRα PvuII C allele with a higher risk of atherosclerosis and stroke in men (Lehtimäki et al., 2002; Shearman et al., 2005) and familial hypercholesterolemia (Lu et al., 2002) as ESR-α can indirectly influence many molecular signaling mechanisms related to risk factors for cardiovascular disease (CVD) via co-modulation with important factors such as insulin receptor, insulin-like growth factor receptor, and peroxisome proliferator-activated receptor (Casazza et al., 2010).

For XbaI polymorphism, our study showed that AG and GG genotype was significantly more frequent in type 2 diabetes mellitus women than control representing high risk factor among patients. A previous study

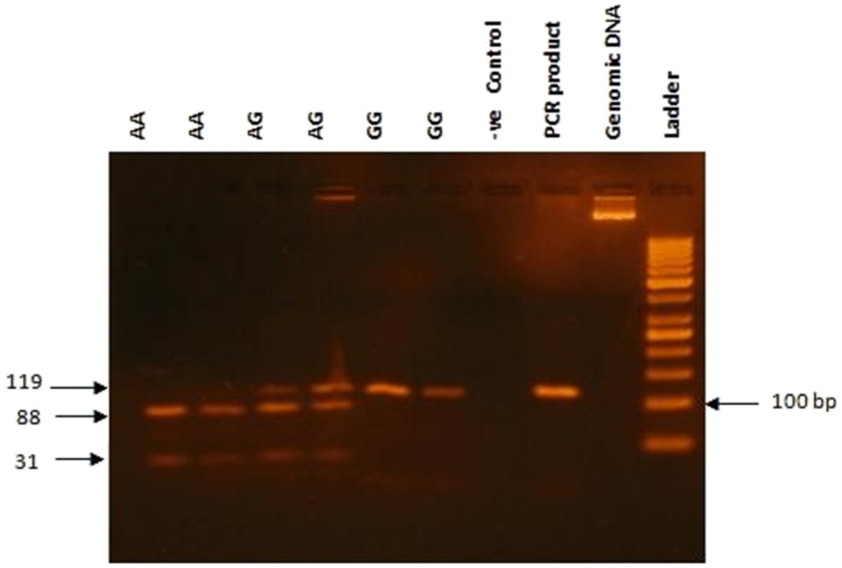

Fig. 2. PCR-RFLP analysis on 2.5% agarose gel representing the XbaI genotypes.

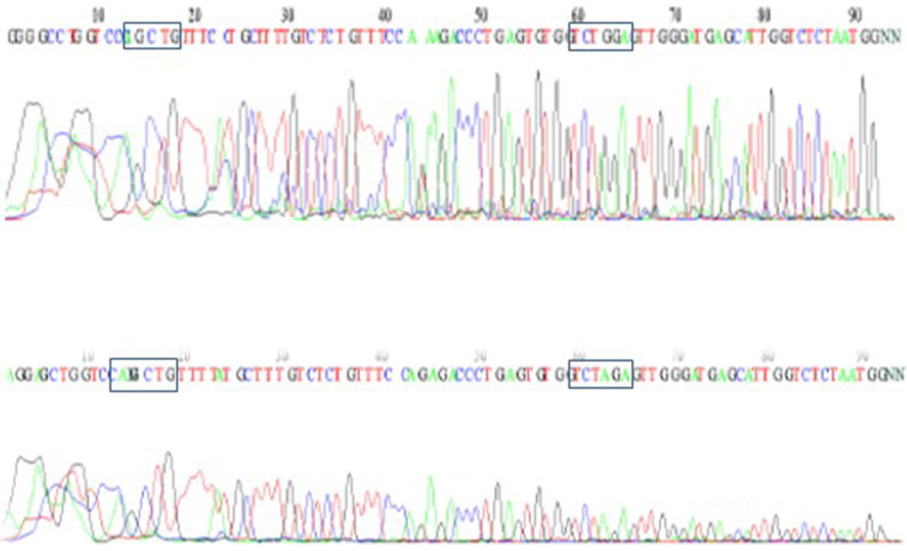

Fig. 3. Sequencing confirmed our result for polymorphism as it shows a different base in the heterozygous and homozygous allele (A sample shows TC and GG allele, B sample shows TT and AG allele). Sequence of restriction cut for PvuII: CAG*CT*G and for XbaII: TCTA*GA*.

of Caucasians patients with type 2 diabetes mellitus showed a significant difference between type 2 diabetes mellitus group and control group of ESRα in the allelic frequency of XbaI (Huang et al., 2006) also Indonesian study in Javanese population showed that XbaI polymorphism is associated with increased susceptibility of type 2 DM in Javanese menopausal women (Akhmad et al., 2013).

A-allele as mayor allele in this study correlated with studies in European-American population (Gallagher et al., 2007). Among Europeans G homozygotes was found to have lower insulin secretory capacity (Speer et al., 2001) leading them to conclude that the G allele may play a role in the pathogenesis of Type 2 diabetes.

Snijder et al. (2003) and his colleagues are in agree with our study that G allele in patients were higher than those in healthy subjects, showed a significant association of ESRα gene XbaI polymorphisms and type 2 diabetes mellitus.

In our study, triglyceride and LDL-C showed significant increase among patients who carry G allele especially in the heterozygous (AG genotypes) while there is no significance difference in total cholesterol and HDL-C and this was in agreement with several studies conducted in Hungary, Japan, the United States and Italy found that the GG genotype was associated with higher total cholesterol and LDL-C and increased risk of CAD and venous thrombosis (Lu et al., 2002; Demissi et al., 2006; Lussana et al., 2006; Molvarec et al., 2007a). However other studies found no association between any cardiovascular risk factor and the G allele this may be due to what previously reported that the G allele may have different consequences depending on the person's age, gender, and hormonal status (Casazza et al., 2010) so in a previous study it was found that among postmenopausal women, the G allele was beneficial, being associated with lower BMI, percentage body fat and waist circumference (Okura et al., 2003).

This study found that that PvuII and XbaI polymorphism of ESRα increases susceptibility to type 2 diabetes mellitus in Egyptian postmenopausal women. ESRα variants may also affect serum lipid metabolism, which might provide a mechanism connecting ESRα to type 2diabetes so further studies with larger sample sizes are required to replicate these findings. Also investigation of SNPs of ESRα polymorphisms other than PvuII and XbaI should also be addressed in future studies.

Conflicts of interest

None of authors have any conflicts of interest.

All authors contributed in the design of the study, analysis and interpretation of data, and in drafting or revising it. Also they have read and agreed to the publication of the manuscript, and the manuscript has not been submitted elsewhere.

References

Akhmad, S.A., Madiyan, M., Hastuti, P., Sinorita, H., 2013. Estrogen receptor alpha (Esr) gene polymorphism as risk factor for type 2 diabetes mellitus (T2DM) in javenese menopause women of Indonesia. J. Med. Sci. 12 (02), 171.

Araújo, K.L., Cunha, L., Rezende, D.D., Souza, L.S., Daltoé, R.D., Madeira, K.P., et al., 2011. Prevalence of estrogen receptor alpha PvuII (c454-397T>C) and XbaI (c454A>G) polymorphisms in a population of Brazilian women. Braz. Arch. Biol. Technol. 54, 1151–1157.

Bittencourt-Oliveira, M.C., Cunha, M.C.C., Moura, A.N.M., 2009. Genetic polymorphism in Brazilian microcystis spp. (cyanobacteria) toxic and Non-toxic through RFLP-PCR of the cpcBA-IGS. Braz. Arch. Biol. Technol. 54 (4), 901–909.

Casazza, K., Page, G.P., Fernandez, J.R., 2010. The association between the rs2234693 and rs9340799 estrogen receptor gene polymorphisms and risk factors for cardiovascular disease. Biol. Res. Nurs. 12 (1), 84–97.

Demissi, S., Cupples, L.A., Shearman, A.M., et al., 2006. Estrogen receptor-alpha variants are associated with lipoprotein size distribution and particle levels in women: the Framingham heart study. Atherosclerosis 185, 210–218.

Di Croce, L., Bruscalupi, G., Trentalance, A., 1996. Independent behavior of rat liver LDL receptor and HMGCoA reductase under estrogen treatment. Biochem. Biophys. Res. Commun. 224, 345–350.

Friedewald, W.T., Levy, R.I., Fredrickson, D.S., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem. 18, 499–502.

Gallagher, C.J., Keene, K.L., Mychaleckyj, J.C., Langefeld, C.D., Hirschhorn, J.N., Henderson, B.E., Gordon, C.J., Freedman, B.I., Rich, S.S., Bowden, D.W., Sale, M.M., 2007. Investigation of the estrogen receptor- alpha gene with type 2 diabetes and/or nephropathy in African-American and European-American populations. Diabetes 56 (3), 675–684.

Ganasyam, S.R., Rao, T.B., Murthy, Y.S.R., Jyothy, A., Sujatha, M., 2012. Association of estrogen receptor-gene & metallothionein-1 gene polymorphisms in type 2 diabetic women of Andhra Pradesh. Indian J. Clin. Biochem. 27 (1), 69–73.

Gennari, L., Merlotti, D., De Paola, V., Calabrò, A., Becherini, L., Martini, G., Nuti, R., 2005. Estrogen receptor gene polymorphisms and the genetic of osteoporosis: a huge review. Am. J. Epidemiol. 161, 307–320.

Howard, B.V., Criqui, M.H., Curb, J.D., Rodabough, R., Safford, M.M., Santoro, N., Wilson, A.C., Wylie-Rosett, J., 2003. Risk factor clustering in the insulin resistance syndrome and its relationship to cardiovascular disease in postmenopausal White, Black, Hispanic,and Asian/Pacific Islander women. Metabolism 52, 362–371.

Huang, Q., Wang, T.H., Lu, W.S., Mu, P.W., Yang, Y.F., Liang, W.W., Li, C.X., Lin, G.P., 2006. Estrogen receptor alpha gene polymorphism associated concentration in Chinese women in Guangzhou. Chin. Med. J. 119 (21), 1794–1801.

Knopp, R.H., Zhu, X., 1997. Multiple beneficial effects of estrogen on lipoprotein metabolism. J. Clin. Endocrinol. Metab. 82, 3952–3954.

Lawlor, D.A., Timpson, N., Ebrahim, S., 2006. The association of estrogen receptor alpha-haplotypes with cardiovascular risk factors in the British Women's Heart and Health Study. Eur. Heart J. 27, 1597–1604.

Lehtimäki, T., Kunnas, T.A., Mattila, K.M., Perola, M., Penttila, A., Koivula, T., Karhunen, P.J., 2002. Coronary artery wall atherosclerosis in relation to the estrogen receptor 1 gene polymorphism: An autopsy study. J. Mol. Med. 80, 176–180.

Li, Y., Liu, F., Tan, S.Q., Wang, Y., Li, S.W., 2012. Estrogen receptor-alpha gene PvuII (T/C) and XbaI (A/G) polymorphisms and endometriosis risk: a meta-analysis. Gene 508, 41–48.

Lindsay, R.S., Howard, B.V., 2004. Cardiovascular risk associated with the metabolic syndrome. Curr. Diabetes Rep. 4, 63–68.

Lu, H., Higashikata, T., Inazu, A., Nohara, A., Yu, W., Shimuzu, M., Mabuchi, H., 2002. Association of estrogen receptor-alpha gene polymorphisms with coronary artery disease in patients with familial hypercholesterolemia. Arterioscler. Thromb. Vasc. Biol. 22, 817–823.

Lussana, F., Faioni, E.M., Mavilia, C., Bucciarelli, P., Bran-di, M.L., Cattaneo, M., 2006. Association of estrogen receptor-alpha gene polymorphisms with venous thrombosis. Haematologica 91, 279–280.

Matic, M., Bryzgalova, G., Goa, H., Antonson, P., Humire, P., Omoto, Y., Portwood, N., Pramfalk, C., Efendic, S., Berggren, P.O., Gustafsson, J.A., Dahlman-Wright, K., 2013. Estrogen signaling and the metabolic syndrome: targeting the hepatic estrogen receptor alpha action. PLoS ONE 8 (2), e57458.

Medrano, J.F., sAasen, E., Sharrow, L., 1990. DNA extraction from nucleated red blood cells. Biotechniques 8 (1), 43.

Mohammadi, F., Pourahmadi, M., Mosalanejad, M., Jamali, H., Ghobadifar, M.A., Erfanian, S., 2013. Association of estrogen receptor α genes PvuII and XbaI polymorphisms with type 2 diabetes mellitus in the inpatient population of a hospital in southern Iran. Diabetes Metab. J. 37, 270–277.

Molvarec, A., Nagy, B., Kovács, M., Walentin, S., Imreh, E., Rigó Jr., J., Szalay, J., Füst, G., Prohászka, Z., Karádi, I., 2007a. Lipid, haemostatic and inflammatory variables in relation to the estrogen receptor α (ESR1) PvuII and XbaI gene polymorphisms. Clin. Chim. Acta 380, 157–164.

Molvarec, A., Ver, A., Fekete, A., Rosta, K., Derzbach, L., Derzsy, Z., Karádi, I., Rigó Jr., J., 2007b. Association between estrogen receptor α (ESR1) gene polymorphisms and severe preeclampsia. Hypertens. Res. 30 (3), 205–211.

Morita, T., Tabata, S., Mineshita, M., Mizoue, T., Moore, M.A., Kono, S., 2005. The metabolic syndrome is associated with increased risk of colorectal adenoma development: the Self-Defense Forces health study. Asian Pac. J. Cancer Prev. 6, 485–489.

Nilsson, S., Makela, S., Treuter, E., Tujague, M., Thomsen, J., Andersson, G., Enmark, E., Pettersson, K., Warner, M., Gustafsson, J.A., 2001. Mechanisms of estrogen action. Physiol. Rev. 81 (4), 1535–1565.

Okura, T., Koda, M., Ando, F., Niino, N., Ohta, S., Shimokata, H., 2003. Association of polymorphisms in the estrogen receptor alpha gene with body fat distribution. Int. J. Obes. Relat. Metab. Disord. 27, 1020–1027.

Peter, I., Shearman, A.M., Zucker, D.R., Vasan, R.S., Schmid, C.H., Demissie, S., Cupples, L.A., Larson, M.G., D'Agostino, R.B., Karas, R.H., Mendelsohn, M.E., Housman, D.E., Levy, D., 2005. Variation in estrogen-related genes and crosssectional and longitudinal blood pressure in the Framingham Heart Study. J. Hypertens. 23, 2193–2200.

Sale, M.M., Freedman, B.I., Langefeld, C.D., Williams, A.H., Hicks, P.J., Colicigno, C.J., Beck, S.R., Brown, W.M., Rich, S.S., Bowden, D.W., 2004. A genome-wide scan for type 2 diabetes in African–American families reveals evidence for a locus on chromosome 6q. Diabetes 53, 830–837.

Shearman, A.M., Cooper, J.A., Kotwinski, P.J., 2005. Estrogen receptor alpha gene variation and the risk of stroke. Stroke 36, 2281–2282.

Silva, I.V., Rezende, L.C.D., Lanes, S.P., Souza, L.S., Madeira, K.P., Cerri, M.F., Paes, M.F., Daltoé, R.D., Chambô-Filho, A., Guimarães, M.C., Graceli, J.B., Rangel, L.B., 2010. Evaluation of PvuII and XbaI polymorphisms in the estrogen receptor alpha gene (ESR1) in relation to menstrual cycle timing and reproductive parameters in post-menopausal women. Maturitas 67 (4), 363–367.

Snijder, M.B., Dekker, J.M., Visser, M., Yudkin, J.S., Stehouwer, C.D., Bouter, L.M., Heine, R.J., Nijpels, G., Seidell, J.C., 2003. Larger thigh and hip circumferences are associated with better glucose tolerance: the horn study. Obes. Res. 11 (1), 104–111.

Speer, G., Cseh, K., Winkler, G., Vargha, P., Braun, E., Takacs, I., Lakatos, P., 2001. Vitamin D and estrogen receptor gene polymorphisms in type 2 diabetes mellitus and in android type obesity. Eur. J. Endocrinol. 144 (4), 385–389.

Van Meurs, J.B., Schuit, S.C., Weel, A.E., van der Klift, M., Bergink, A.P., Arp, P.P., Colin, E.M., Fang, Y., Hofman, A., van Duijn, C.M., van Leeuwen, J.P., Pols, H.A., Uitterlinden, A.G., 2003. Association of 5′ estrogen receptor alpha gene polymorphisms with bone mineral density, vertebral bone area and fracture risk. Hum. Mol. Genet. 12, 1745–1754.

Weel, A.E., Uitterlinden, A.G., Westendorp, I.C., Burger, H., Schuit, S.C.E., Hofman, A., Helmerhorst, T.J., van Leeuwen, J.P., Pols, H.A., 1999. Estrogen receptor polymorphism predicts the onset of natural and surgical menopause. J. Clin. Endocrinol. Metab. 84 (9), 3146–3150.

Weickert, C.S., Miranda-Angulo, A.L., Wong, J., Perlman, W.R., Ward, S.E., Radhakrishna, V., Straub, R.E., Weinberger, D.R., Kleinman, J.E., 2008. Variants in the estrogen receptor alpha gene and its mRNA contribute to risk for schizophrenia. Hum. Mol. Genet. 17 (15), 2293–2309.

World Health Organization, 1999. Consultation Definition. World Health Organization Consultation. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications, Part 1: Diagnosis and classification of Diabetes Mellitus. Report of a WHO Consultation. World Health Organization, Geneva.

Zhang, Y., Howard, B.V., Cowan, L.D., Yeh, J., Schaefer, C.F., Wild, R.A., Wang, W., Lee, E.T., 2002. The effect of estrogen use on levels of glucose and insulin and the risk of type 2 diabetes in american Indian postmenopausal women: the strong heart study. Diabetes Care 25, 500–504.