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Retracted: Impact of polymorphisms in the oestrogen receptors alpha and beta (ESR1, ESR2) genes on risk of vasculogenic erectile dysfunction

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SUMMARY

Erection is principally a vascular phenomenon. Oestrogen affects the vascular system in various ways. Oestrogen effects are mediated by oestrogen receptors (ERs). We examined the relationship of two polymorphisms in ESR-α (ER-1) (rs2234693 and rs9340799) and two polymorphisms in ESR-β (ER-2) (rs4986938 and rs1256049) with risk of vasculogenic erectile dysfunction (VED). The rs2234693 (ER-α *Pvu*II), rs9340799 (ER-α *Xba*I), rs4986938 (ER-β *Alu*I) and rs1256049 (ER-β *Rsa*I) were genotyped using polymerase chain reaction-restriction fragment length polymorphism technique. Serum levels of sex hormone-binding globulin (SHBG), total testosterone, free testosterone (fT), total oestradiol (E2) and free oestradiol (free E2) were also measured. A total of 266 men with VED and 532 healthy controls were recruited into this study. The ER-α *Pvu*II C allele (OR = 4.2; 95% CI: 2.79–8.43, $p = 0.001$), ER-α *Xba*I A allele (OR = 4.87; 95% CI: 2.75–8.64, $p = 0.001$), ER-β *Rsa*I A allele (OR=0.37; 95% CI: 0.24–0.66, $p = 0.001$) and ER-β *Alu*I A allele (OR = 0.29; 95% CI: 0.16–0.57, $p = 0.001$) were significantly associated with VED. Subjects with ER-α *Pvu*II CC and ER-α *Xba*I AA genotypes had highest serum levels of E2, and subjects with ER-β *Rsa*I AA and ER-β *Alu*I AA genotypes had lowest serum levels of E2. Patients with lower serum levels of E2 had more sever VED and more mixed vascular type VED. In haplotype analysis, *Pvu*II C–*Xba*I A increased the risk of developing VED by more than eightfold, in contrast, *Rsa*I A–*Alu*I A haplotype had protective effect (OR = 0.53; 95% CI: 0.34–0.76, $p = 0.002$). The ER-α and ER-β gene polymorphisms and haplotypes are associated with presence, type and severity of VED.

INTRODUCTION

Erectile dysfunction (ED) adversely affects men's health and quality of life. ED is associated with depression, reduced feelings of general happiness, loss of self-esteem and anxiety (Laumann *et al.*, 1999; Melman & Gingell, 1999). Basic science studies have provided compelling evidence that ED is mainly a disease of vascular aetiology (Kendirci *et al.*, 2005). Despite worldwide popularity of the oral phosphodiesterase 5 (PDE-5) inhibitors for the treatment of ED, these agents only circumvent the problem. We need a therapy to actually cure the ED. As we understand the cellular and genetic mechanisms resulting in ED, we will be able to restore normal erectile function (EF). Oestrogen affects the cardiovascular system in various ways. Oestrogen enhances vascular endothelial function and vasodilatation, and inhibits vascular smooth muscle cell proliferation (Godsland, 2001). Oestrogen effects are mediated by oestrogen receptors (ERs). Two subtypes of ERs exist in humans; ER-1 or alpha (ER-α) (Walter *et al.*, 1985) and ER-2 or beta (ER-β) (Mosselman *et al.*, 1996), which coded by ESR-1 and ESR-2 genes respectively. The ESR-1 gene (140 kb) is located on chromosome 6q25.1 and consists of eight exons and one intron. The ESR-1 gene contains two single-nucleotide polymorphisms (SNPs) named as *Pvu*II (T/C) (rs2234693) and *Xba*I (A/G) (rs9340799) (Aléssio *et al.*, 2007). The ESR-2 gene, resides on chromosome 14 q22-24, comprises eight exons and spans approximately 40 kb (Ponglikitmongkol *et al.*, 1988). The 5′ and 3′ regions of the ER-β gene have two common SNP polymorphisms: G/A exchange at nucleotide 1 730 in the 3′ untranslated region in exon 8 (*Alu*I, rs4986938) and a silent 1082 G/A transition in exon 5 (*Rsa*I, rs1256049) (Rosenkranz *et al.*, 1998). Both receptors are expressed in endothelial cells and vascular smooth muscle cells (Karas *et al.*, 1994). Oestrogen-related vasodilatation and oestrogen's antiatherosclerotic activity are mostly mediated by ER-1 (Hodgin *et al.*, 2001). It has been demonstrated that several conditions which increase the

risk of vascular disease, such as obesity (Rankinen *et al.*, 2006), early-onset coronary artery disease (Mansur *et al.*, 2005), cardiovascular diseases (Schuit *et al.*, 2004), arterial hypertension (Lehrer *et al.*, 1993), venous thromboembolism (Lussana *et al.*, 2006) and increased high-density lipoprotein (HDL) levels (Figtree *et al.*, 2008) are correlated with polymorphisms in the ESR-α or ESR-β genes.

The vascular effects of E2 have been attributed to alteration of circulating lipoproteins (Gerhard & Ganz, 1995), inhibition of intravascular increase of collagen (Wolinsky, 1972), changes in blood coagulation (Bing & Conforto, 1992) and genomic and non-genomic impacts on the vascular bed (Serock *et al.*, 2008). It is well documented that both ER-α and ER-β are expressed in many circulating and resident vascular wall cell types (Deroo & Korach, 2006). Intronic polymorphisms of ER-α have been associated with increased susceptibility to atherosclerosis in men (Sudhir *et al.*, 1997). The proliferation of vascular smooth muscle cells is associated with vascular proliferative disorders such as atherosclerosis (Ross, 1993). It has been shown that ER-β mediates the inhibitory effect of E2 on vascular smooth muscle cell proliferation (Watanabe *et al.*, 2003). The growth inhibitory effect of oestrogen can be abolished by the oestrogen receptor antagonist tamoxifen (Morey *et al.*, 1997). Endothelial ER-α plays a central role in the E2-induced prevention of endothelial dysfunction (Favre *et al.*, 2010) after ischaemia/reperfusion. Calcium and plaque formation are main components of atherosclerosis, and calcification of plaque is an actively regulated process (Doherty *et al.*, 2003). It has been demonstrated that increased ER-β expression is linked to advanced atherosclerosis and calcification (Christian *et al.*, 2006). The associations between ER-α polymorphisms and cardiovascular diseases have been well established in various studies (Evangelopoulos *et al.*, 2003; Shearman *et al.*, 2003; Pollak *et al.*, 2004). ER-α is necessary for oestrogens to inhibit neointima formation (Pelzer *et al.*, 2005). ERs also regulate several vasodilatory and vasoconstrictor proteins, such as some components of the renin–angiotensin and nitric oxide systems (Villablanca *et al.*, 2004). The heat shock protein 27 is an ER-β-associated protein, which demonstrates decreased expression with coronary atherosclerosis and modulates oestrogen signalling (Miller *et al.*, 2005).

Based on the effects of ER-α and ER-β polymorphisms on oestrogen actions, and on the clinical associations that have been reported in the above mentioned studies, it is rational to assume that ER-α and ER-β polymorphisms might be independently associated with a multifactorial disease such as VED. To the best of our knowledge, there are no study available examining the association between ER-α and ER-β polymorphisms and vasculogenic erectile dysfunction (VED).

The aim of this study was to examine the association between ESR-α (rs2234693 and rs9340799) ESR-β (rs4986938 and rs1256049) polymorphisms and the presence of VED.

MATERIALS AND METHODS

SUBJECTS

The cases for the study were 344 consecutively selected and unrelated Iranian men aged between 39 and 61 years old with VED. These men had been attending our outpatient office of Urology and Andrology Clinic. To establish the diagnosis of ED the definition of the National Institutes of Health statement on ED was used (NIH Consensus Conference, 1993). A total of 684 unrelated healthy volunteers, aged between 40 and 60 years old, were recruited from blood donors who served as controls. All of the subjects were of Caucasian origin and recruited from the city of Tehran. All participants gave informed consent and the study was carried out in accordance with the International Conference on Harmonization-Good Clinical Practice (ICH-GCP) guidelines and the principles of the Declaration of Helsinki. The study protocol was approved by Medical Ethics Committee at the study site.

Inclusion and exclusion criteria

None of the participants had a psychotic/affective disorder on the basis of the Diagnostic and Statistical Manual, 4th edition criteria (American Psychological Association, 2000). Participants were excluded if they had a history of endocrinopathy, neurogenic or psychogenic origin of ED, Peyronie's disease, penile anatomical abnormality, drug or substance abuse; use of psychotropic or neuroleptic medication; and of any medication known to cause ED such as antiandrogenic, and anticholinergic agents and histamine receptors blockers (H1 or H2). Exclusion criteria also included relationship problems; smoking more than five cigarettes per day; major physical illness and renal or liver function impairment.

Evaluation

All of the participants were asked regarding their age, educational level, smoking status, surgical and medical history, sexual history and medication used. A complete physical examination was performed and a five-item abridged form of the International Index of Erectile Function (IIEF), the Sexual Health Inventory for Men (SHIM) (Rosen *et al.*, 1999) was administered by physician. For study purposes, VED was categorized as mild SHIM score 11–21, moderate 8–10 and severe 7 or less. Laboratory examination included measuring the serum glucose, creatinine, luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), total testosterone (T), free testosterone (fT), total oestradiol (E2), free oestradiol (fE2), thyroid-stimulating hormone (TSH), free thyroxin (FT4), free triiodothyronin (FT3), liver function tests and lipid profile [cholesterol, low-density lipoprotein, HDL and triglyceride]. Before going on to sophisticated and invasive tests to establish the diagnosis of VED, psychological status of all patients were assessed using the Dissociative Experiences Scale, the Hamilton Rating Scale for Anxiety and the Liebowitz Social Anxiety Scale. Patients with normal psychiatric examinations and normal serum hormonal profile were further evaluated for presence VED. To document the diagnosis and types of VED, all cases underwent penile colour duplex Doppler ultrasonography before and after injection with 20 μg prostaglandin E_1. The haemodynamics of the left and right cavernous arteries were calculated by measuring peak systolic velocity (PSV), end-diastolic velocity (EDV) and resistivity index (RI). The following criteria were used to differentiate different types of ED from each other: pure arterial insufficiency (AI), a PSV of <25 cm/sec; borderline AI, PSV 25–30 cm/sec; pure veno-occlusive dysfunction (VOD), PSV >30 cm/sec + EDV >5 cm/sec + RI <0.8; mixed vascular disorder, PSV <25 cm/sec + EDV >5 cm/sec; and non-vascular disorder, PSV >30 cm/sec + EDV $\leq$5 cm/sec + RI >0.8. To establish the diagnosis of VED, pudendal nerve conduction tests and impaired sensory-evoked potential studies were performed when necessary.

The following predisposing risk factors for VED were determined accurately. Hypertension was defined as a repeatedly increased blood pressure exceeding 140 over 90 mmHg – a systolic pressure above 140 mmHg with a diastolic pressure above 90 mmHg or the use of antihypertensive drugs. Two fasting blood glucose measurements above 126 mg/dL, non-fasting blood glucose $\geq$200 mg/dL, or the use of antidiabetic medications were considered diagnostic for diabetes mellitus. Hyperlipidaemia was defined as a total cholesterol concentration above 200 mg/dL, or LDL cholesterol level above 120 mg/dL or triglyceride levels higher than 150 mg/dL, or hypolipidaemic drug treatment. A body mass index (BMI) $\geq$30 kg/m^2 was considered obesity. Of recruited subjects 266 patients and 532 controls (two controls for each case) met study criteria and were genotyped.

Genotyping

Genotyping was performed exactly as per the methods we used in our previous study (Safarinejad *et al.*, 2012).

Statistical analysis

Results are expressed as the means $\pm$ SD. The statistical power was calculated using the PS software (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize). Our study had an 85% power for the lowest detectable OR of 1.58 with an exposure frequency of 20% under the study sample size (the inputted parameters of PS software were as follows: $\alpha = 0.05$, $p_0 = 0.20$, $n = 266$, $m = 1.00$). The normality of the populations was tested by Kolmogorov–Smirnov test. Departures from the Hardy–Weinberg equilibrium (HWE) and differences in allele and genotype distributions between the two groups were tested by the chi-squared test with 1 and 2 d.f. respectively. To compare categorical variables, contingency tables and the Pearson's chi-squared test were used. For comparison of continuous variables linear ANOVA was used. Associations between VED and genotype were tested with chi-squared test. Multiple logistic regression analysis was applied to examine the effect of genotype on the development of VED. Following confounding factors were put in multivariate analysis: daily smoking (yes/no), age, BMI, serum concentrations of cholesterol, triglycerides, HDL, blood glucose, occupational status, diabetes mellitus, diastolic and systolic blood pressure, years of education (primary or secondary) and college or university (yes/no). Logistic regression model was used to calculate odds ratio (OR) with 95% confidence interval (CI) for the association between VED and the genotype. The haplotype frequencies were calculated from the genotype data using Haploview software version 3.2 developed by The International HapMap Project available at http://www.hapmap.org.. Pair-wise linkage disequilibrium (LD) between the individual SNPs was estimated using the LD-plot function of this software. Ten thousand permutations were carried out with Haploview software to adjust *p* values for multiple testing. The *p* value less than 0.05 was considered statistically significant. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 17.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

Population characteristics

The clinical and demographic characteristics of study groups are presented in Table 1. No statistically significant differences were noted between two groups with regard to age, occupational status, education level, serum hormones and BMI. Diabetes mellitus, arterial hypertension, dyslipidaemia, serum total cholesterol, triglycerides and HDL were significantly higher in patient group than in controls. The effects of confounding factors in developing VED are separately shown in Table 2.

Table 1 Baseline characteristic and clinical data in cases and control subjects

Characteristics	Cases (n = 266)	Controls (n = 532)	p value
Age (year)	51.8 $\pm$ 11.4	52.1 $\pm$ 10.7	0.64
Duration of VED (year)	4.6 $\pm$ 2.3	–	NA
BMI (kg/m^2)	27.2 $\pm$ 2.6	27.4 $\pm$ 2.7	0.71
Systolic BP (mmHg)	137.2 $\pm$ 10.6	112.0 $\pm$ 6.7	0.027
Diastolic BP (mmHg)	85.0 $\pm$ 6.4	73.3 $\pm$ 6.2	0.014
Occupational status no. (%)			
Employed	220 (82.7)	437 (82.1)	0.74
Unemployed	41 (17.3)	95 (17.9)	0.82
Educational level			
None	0	0	–
Primary school	18 (6.8)	38 (7.2)	0.62
High school	181 (68.0)	364 (68.4)	0.78
Graduate	67 (25.2)	130 (24.4)	0.54
Serum hormones			
Sex hormone binding globulin	24.8 $\pm$ 4.4	24.2 $\pm$ 4.2	0.51
Total testosterone (ng/mL)	5.7 $\pm$1.6	5.9 $\pm$ 1.7	0.82
Free testosterone (pg/mL)	148 $\pm$ 42	151 $\pm$ 44	0.83
Estradiol (pg/mL)	18.2 $\pm$ 6.4	17.9 $\pm$ 6.1	0.26
Free estradiol (pg/mL)	0.35 $\pm$ 0.04	0.34 $\pm$ 0.05	0.43
LH (IU/L)	6.7 $\pm$ 2.3	6.8 $\pm$ 2.1	0.76
FSH (IU/lL)	7.2 $\pm$ 2.3	7.0 $\pm$ 2.1	0.66
PRL (pmol/Ls)	354 $\pm$ 127	365 $\pm$ 124	0.47
TSH (mIU/mL)	2.2 $\pm$ 1.4	2.2 $\pm$ 1.3	0.91
Free thyroxine (pmol/L)	14.4 $\pm$ 2.7	14.2 $\pm$ 2.4	0.74
Free triiodothyronine (pmol/L)	3.4 $\pm$ 1.1	3.6 $\pm$ 1.2	0.68
Serum lipid profile			
Total cholesterol (mg/dL)	251 $\pm$ 18	172 $\pm$ 16	0.022
HDL (mg/dL)	39 $\pm$ 15	57 $\pm$ 10	0.015
LDL (mg/dL)	148 $\pm$ 38	114 $\pm$ 43	0.026
Triglycerides (mg/dL)	191 $\pm$ 74	134 $\pm$ 59	0.013
Associated comorbidities n (%)			
Arterial hypertension	31 (11.6)	31 (5.8)	0.020
Diabetes mellitus	32 (12.0)	42 (7.9)	0.024
Dyslipidemia	82 (30.8)	79 (14.8)	0.011
Smoking status n (%)			
Never	130 (48.9)	267 (50.2)	0.31
Former	26 (9.7)	53 (10.0)	0.82
Current	109 (41.0)	208 (39.1)	0.47
Types of ED, n (%)			
Pure arterial insufficiency	52 (19.5)	NA	
Borderline arterial insufficiency	50 (18.8)	NA	
Pure veno-occlusive dysfunction	54 (20.3)	NA	
Mixed vascular disease	13 (4.9)	NA	
Nonvascular status	97 (36.5)	NA	

NA: not applicable, LH: luteinizing hormone, FSH: follicle stimulating hormone, PRL: prolactin, HDL: high density lipoprotein, LDL: low density lipoprotein, ED: erectile dysfunction.

ER-α genotypes

The ER-α genotype and allele frequency distributions by case–control status are shown in Table 3. The *Pvu*II and *Xba*I polymorphisms of the ESR-α gene show strong significant linkage disequilibrium with each other ($D' = 0.984$). The *Pvu*II TC polymorphism was in HWE ($\chi^2 = 0.18$, $p = 0.80$), but the *Xba*I AG polymorphism demonstrates slight deviation from HWE ($\chi^2 = 3.61$, $p = 0.044$). The frequency of *Pvu*II CC genotype of ER-α gene was significantly lower in controls (0.280) when

Table 2 Relationship of VED with medical conditions, sociodemographic characteristic and toxic habits

	Odds ratio (95% CI)		
	Crude	Age adjusted	*p* value
Medical conditions			
Diabetes mellitus	6.74 (4.21–9.28)	3.41 (2.54–5.83)	0.0001
Hypertension	3.31 (2.33–4.27)	1.75 (1.38–2.59)	0.01
Dyslipidemia	2.79 (1.83–4.16)	1.67 (1.18–2.54)	0.02
Occupational status			
Employed	1	1	NA
Unemployed	4.45 (2.27–8.21)	3.38 (1.32–6.74)	0.0001
Educational level			
None	3.54 (2.12–7.4)	2.14 (1.77–4.42)	0.001
Primary school	1.71 (0.74–2.25)	1.41 (1.11–1.89)	0.04
High school	1.22 (0.57–2.14)	0.76 (0.44–1.65)	0.08
Graduate	1	1	NA
Body habits			
Height (cm)	0.84 (0.72–0.91)	0.87 (0.76–1.14)	0.08
Weight (kg)	1.16 (0.92–1.04)	1.08 (0.91–1.01)	0.2
BMI	1.17 (1.03–1.46)	1.06 (0.93–1.22)	0.2
Toxic habits			
Current smoker	2.51 (1.66–3.36)	2.38 (1.57–3.27)	0.001
Ex–smoker	2.17 (1.52–3.11)	2.11 (1.34–3.8)	0.01
None	1	1	NA

NA: not applicable, VED: Vasculogenic erectile dysfunction, OR: Odds ratio, CI: Confidence interval.

Table 3 Frequency distribution of the ER-α, and ER-β alleles, genotypes and haplotypes, and their associations with the risk of VED

Genotype frequency	Controls (*n* = 532)	Cases (*n* = 266)	Adjusted OR[a] (95% CI)	*p* value
ER-α *Pvu*II				
TT	0.199	0.071	1.0 (Referent)	
TC	0.521	0.538	1.26 (0.84–2.21)	0.36
CC	0.280	0.391	4.64 (2.72–8.31)	0.002
T	0.460	0.340	1.0 (Referent)	
C	0.540	0.660	4.82 (2.79–8.43)	0.001
ER-α *Xba*I				
GG	0.173	0.120	1.0 (Referent)	
AG	0.577	0.583	1.16 (0.82–1.76)	0.037
AA	0.250	0.297	3.65 (2.42–5.69)	0.004
G	0.461	0.412	1.0 (Referent)	
A	0.539	0.588	4.87 (2.75–8.64)	0.001
ER-β *Rsa*I				
GG	0.925	0.962	1.0 (Referent)	
GA	0.062	0.030	0.78 (0.62–0.87)	0.02
AA	0.013	0.008	0.54 (0.37–0.75)	0.002
GA + AA	0.075	0.038	0.32 (0.21–0.62)	0.001
G	0.956	0.977	1.0 (Referent)	
A	0.044	0.023	0.37 (0.24–0.66)	0.001
ER-β *Alu*I				
GG	0.491	0.571	1.0 (Referent)	
AG	0.382	0.403	1.36 (0.79–2.24)	0.31
AA	0.127	0.026	0.56 (0.38–0.72)	0.002
G	0.681	0.773	1.0 (Referent)	
A	0.319	0.227	0.29 (0.16–0.57)	0.001
ER-α haplotypes				
*Pvu*II T–*Xba*I A	0.460	0.340	1.0 (Referent)	
*Pvu*II C–*Xba*I A	0.079	0.248	8.26 (4.72–18.27)	0.0004
*Pvu*II C–*Xba*I G	0.461	0.412	0.47 (0.31–0.76)	0.002
*Pvu*II T–*Xba*I G	0 (0.0)	0 (0.0)	NA	
ER-β haplotypes				
*Rsa*I G–*Alu*I A	0.275	0.205	1.0 (Referent)	
*Rsa*I G–*Alu*I G	0.681	0.773	3.84 (2.72–6.41)	0.003
*Rsa*I A–*Alu*I A	0.044	0.022	0.53 (0.34–0.76)	0.002
*Rsa*I A–*Alu*I G	0 (0.0)	0 (0.0)	NA	

VED: vasculogenic erectile dysfunction, OR: odds ratio, CI: confidence interval, ER: estrogen receptor, NA: not applicable. [a]Odds ratios were obtained from a logistic regression model with adjusting for age; body mass index; serum total cholesterol, triglyceride, LDL and HDL; smoking status, associated comorbidities, occupational status, and educational level.

compared with the cases (0.391) resulting in an increased risk of VED more than 4.5-fold (OR = 4.64; 95% CI: 2.72–8.31, $p = 0.002$). The frequency of the *Xba*I AA genotype of ER-α was found to be higher in cases (0.297) when compared with the controls (0.250), and resulted in a significant increased risk to VED in the cases (OR = 3.65; 95% CI: 2.42–5.69, $p = 0.004$). Multivariate analysis with the logistic regression model demonstrated that the presence of *Pvu*II C and *Xba*I A alleles was independent predisposing factor for developing VED (by 4.82 and 4.87 folds respectively (Table 3).

ER-β genotypes

The observed genotype frequencies for the ER-β *Rsa*I and ER-β *Alu*I polymorphisms were in HWE ($\chi^2 = 0.22$, $p = 0.78$). The frequency of ER-β *Rsa*I AA genotype was significantly higher in controls (0.013) when compared with the cases (0.008) resulting in a reduced risk of about 50% (OR = 0.54; 95% CI: 0.37–0.75, $p = 0.002$). The frequency of the ER-β *Alu*I AA genotype was found to be higher in controls (0.127) when compared with the cases (0.26), and resulted in a significant diminished risk to VED in the cases (OR = 0.32; 95% CI: 0.21–0.62, $p = 0.001$). Subjects with ER-β *Rsa*I A and ER-β *Alu*I A alleles had 63 and 71% decreased risk for VED respectively.

Association of combined ER-α *Pvu*II /ER-α*Xba*I and ER-β *Rsa*I/ER-β*Alu*I polymorphisms with VED

Gene–gene interactions was assessed considering only genotypic combinations for each polymorphism pairs (Table 4). The frequency of combined ER-α *Pvu*II TT/ER-α*Xba*I GG variant was significantly higher in the controls (0.055) than the cases (0.019), which decreased more than 70% the risk of developing VED (OR = 0.26; 95% CI: 0.17–0.42, $p = 0.0004$). When we used ER-α *Pvu*II TT/ER-α*Xba*I AA variant as the reference, we found that ER-α *Pvu*II TC/ER-α*Xba*I AA, ER-α *Pvu*II CC/ER-α*Xba*I AG and ER-α *Pvu*II CC/ER-α*Xba*I GG variants were associated with a higher risk of VED (OR = 4.63; 95% CI: 2.74–9.67, $p = 0.001$; OR = 6.46; 95% CI: 4.74–12.65, $p = 0.0001$ and OR = 2.33; 95% CI: 1.78–3.77, $p = 0.022$ respectively; Table 4). These ER-β *Rsa*I/ER-β *Alu*I polymorphisms combinations only showed predisposing effects. Combined ER-β *Rsa*I GG/ER-β *Alu*I AG and ER-β *Rsa*I GG/ER-β *Alu*I GG variants were associated with a higher risk of VED (OR = 2.86; 95% CI: 1.74–4.72, $p = 0.014$ and OR = 5.64; 95% CI: 3.73–12.42, $p = 0.0003$ respectively; Table 4).

ESR-α *Pvu*II -*Xba*I haplotype analysis

There was strong linkage disequilibrium between ESR-α *Pvu*II T/C and *Xba*I A/G polymorphisms ($D' = 0.970$) which results in three common and one rare haplotypes (for details see Table 3). The *Pvu*II C-*Xba*I A haplotype occurred at greater frequencies in cases (0.248) vs. controls (0.079), suggesting that it predisposes for VED (OR = 8.26; 95% CI: 4.72–18.27, $p = 0.0004$).

ESR-β *Rsa*I-*Alu*I haplotype analysis

Similarly, *Rsa*I G-*Alu*I G haplotype was also at higher frequency in cases (OR = 3.84; 95% CI: 2.72–6.41, $p = 0.003$; for details see Table 3). On contrary, individuals with *Rsa*I A-*Alu*I A

Table 4 Combined genotype frequency for ER-α *Pvu*II /ER-α *Xba*I, and ER-β *Rsa*I/ER-β *Alu*I polymorphisms in all cases and controls

ER genotypes	Cases (n = 266)	Controls (n = 532)	Adjusted OR[a] (95% CI)	p value
ER-α *Pvu*II /ER-α *Xba*I				
TT + AA	0.026	0.068	1.0 (Referent)	
TT + AG	0.026	0.077	0.37 (0.22–0.76)	0.001
TT + GG	0.019	0.055	0.26 (0.17–0.42)	0.0004
TC + AA	0.271	0.182	4.63 (2.74–9.67)	0.001
TC + AG	0.252	0.295	0.81 (0.77–2.67)	0.07
TC + GG	0.015	0.043	0.48 (0.33–0.69)	0.004
CC + AA	0 (0.0)	0 (0.0)	NA	
CC + AG	0.305	0.205	6.46 (4.74–12.65)	0.0001
CC + GG	0.086	0.075	2.33 (1.78–3.77)	0.022
ER-β *Rsa*I/ER-β *Alu*I				
GG + AA	0.026	0.129	1.0 (Referent)	
GG + AG	0.395	0.368	2.86 (1.74–4.72)	0.014
GG + GG	0.541	0.428	5.64 (3.73–12.42)	0.0003
GA + AA	0 (0.0)	0 (0.0)	NA	
GA + AG	0 (0.0)	0 (0.0)	NA	
GA + GG	0.030	0.062	0.48 (0.31–0.72)	0.004
AA + AA	0	0 (0.0)	NA	
AA + AG	0.008	0.013	0.85 (0.76–2.14)	0.072
AA + GG	0	0 (0.0)	NA	

OR: odds ratio, CI: confidence interval, ER: estrogen receptor, NA: not applicable. [a]Odds ratios were obtained from a logistic regression model with adjusting for age; body mass index; serum total cholesterol, triglyceride, LDL and HDL; smoking status, associated comorbidities, occupational status, and educational level.

haplotype had about 50% increased risk for developing VED (OR = 0.53; 95% CI: 0.34–0.76, p = 0.002).

ER-α and ER-β gene polymorphisms and VED severity

In multivariate analysis (Table 5), the two ER-α *Pvu*II CC genotype was associated with 8.6-fold increased risk of severe VED compared with the reference genotype (ER-α *Pvu*II TT) (OR = 8.62; 95% CI: 4.65–17.62; p = 0.0001). After adjusting for confounding factors, a significant increased risk of 6.7-fold for severe VED was also found for carriers of the ER-α *Xba*I AA genotype (OR = 6.76; 95% CI: 4.78–14.64, p = 0.0004). There was no significant association between ER-β *Rsa*I polymorphism and severity of VED. But, in a multivariate analysis, the ER-β *Alu*I AA genotype was associated with a more than 70% decreased risk of severe VED compared with the ER-β *Alu*I GG genotype (OR = 0.28; 95% CI: 0.12–0.48, p = 0.0001) (for details see Table 5).

Association between ER-α and ER-β gene polymorphisms and type of VED

ER-α and ER-β gene polymorphisms and haplotypes had significant impact on the type of VED (Table 6). For example, carriers of the ER-α *Pvu*II CC genotype had 72% decreased risk for AI (OR = 0.28, 95% CI: 0.14–0.57, p = 0.0004) and 6.6-fold increased risk for non-vascular status (OR = 6.66; 95% CI: 4.72–12.69,

Table 5 Adjusted odds ratios for relation between ER-α, and ER-β alleles, genotypes and haplotypes, and severity of VED in all patients

	Severity of VED								
ER-α, and ER-β polymorphism	Mild (n = 65)	OR[a] (95% CI)	p value	Moderate (n = 109)	OR[a] (95% CI)	p value	Severe (n = 92)	OR[a] (95% CI)	p value
ER-α *Pvu*II									
TT	0.526	1.0 (Referent)		0.263	1.0 (Referent)		0.211	1.0 (Referent)	
TC	0.280	0.38 (0.26–0.71)	0.002	0.517	4.28 (2.72–7.48)	0.001	0.203	0.86 (0.62–1.78)	0.08
CC	0.144	0.29 (0.14–0.56)	0.0001	0.288	1.34 (0.79–2.71)	0.071	0.568	8.62 (4.65–17.62)	0.0001
T	0.331	1.0 (Referent)		0.464	1.0 (Referent)		0.205	1.0 (Referent)	
C	0.199	0.28 (0.16–0.52)	0.0001	00.382	0.64 (0.47–0.82)	0.01	0.419	8.74 (4.46–18.25)	0.0001
ER-α *Xba*I									
GG	0.500	1.0 (Referent)		0.250	1.0 (Referent)		0.250	1.0 (Referent)	
AG	0.258	0.55 (0.32–0.73)	0.003	0.503	4.61 (2.46–8.75)	0.001	0.239	0.79 (0.61–2.32)	0.07
AA	0.114	0.22 (0.12–0.51)	0.0001	0.291	1.67 (0.84–2.78)	0.07	0.595	6.76 (4.78–14.64)	0.0004
G	0.329	1.0 (Referent)		0.429	1.0 (Referent)		0.242	1.0 (Referent)	
A	0.185	0.21 (0.16–0.52)	0.0001	0.396	0.76 (0.61–1.67)	0.08	0.419	6.72 (4.34–12.62)	0.004
ER-β *Rsa*I									
GG	0.242	1.0 (Referent)		0.410	1.0 (Referent)		0.348	1.0 (Referent)	
GA	0.250	1.16 (0.82–1.62)	0.76	0.375	0.78 (0.67–2.24)	0.07	0.375	1.74 (0.69–2.31)	0.08
AA	0.500	0 (0.0)	NA	0.500	0 (0.0)	NA	0 (0.0)	NA	
G	0.242	1.0 (Referent)		0.410	1.0 (Referent)		0.348	1.0 (Referent)	
A	4–0.333	1.82 (0.84–2.73)	0.31	0.417	1.22 (0.77–2.24)	0.26	0.250	0.74 (0.64–2.31)	0.08
ER-β *Alu*I									
GG	0.204	1.0 (Referent)		0.441	1.0 (Referent)		0.355	1.0 (Referent)	
AG	0.280	2.65 (1.84–4.67)	0.01	0.374	0.74 (0.51–0.88)	0.02	0.346	0.87 (0.68–2.24)	0.07
AA	0.571	6.64 (4.87–14.65)	0.002	0.286	0.52 (0.31–0.84)	0.006	0.143	0.28 (0.12–0.48)	0.0001
G	0.224	1.0 (Referent)		0.423	1.0 (Referent)		0.353	1.0 (Referent)	
A	0.314	4.82 (2.51–7.67)	0.006	0.363	0.84 (0.68–2.34)	0.07	0.323	0.82 (0.64–2.36)	0.07
ER-α haplotypes									
*Pvu*II T–*Xba*I A	0.116	1.0 (Referent)		0.541	1.0 (Referent)		0.343	1.0 (Referent)	
*Pvu*II C–*Xba*I A	0.091	0.87 (0.72–1.76)	0.27	0.470	0.52 (0.31–0.74)	0.003	0.439	4.74 (2.64–7.38)	0.003
*Pvu*II C–*Xba*I G	0.443	7.47 (4.61–16.62)	0.0001	0.265	0.46 (0.32–0.75)	0.001	0.292	0.35 (0.24–0.67)	0.0006
*Pvu*II T–*Xba*I G	0 (0.00)	NA		0 (0.00)	NA			0 (0.00)	NA
ER-β haplotypes									
*Rsa*I G–*Alu*I A	0.505	1.0 (Referent)		0.385	1.0 (Referent)		0.110	1.0 (Referent)	
*Rsa*I G–*Alu*I G	0.165	0.27 (0.11–0.42)	0.0001	0.421	2.42 (0.72–3.71)	0.06	0.414	7.65 (4.29–14.61)	0.0002
*Rsa*I A–*Alu*I A	0.583	2.47 (1.64–4.66)	0.02	0.250	0.48 (0.34–0.72)	0.003	0.167	1.68 (0.84–2.68)	0.07
*Rsa*I A–*Alu*I G	0 (0.00)	NA		0 (0.00)	NA	0 (0.00)	NA		

VED: vasculogenic erectile dysfunction, OR: odds ratio, CI: confidence interval, ER: estrogen receptor, NA: not applicable. [a]Odds ratios were obtained from a logistic regression model with adjusting for age; body mass index; serum total cholesterol, triglyceride, LDL and HDL; smoking status, associated comorbidities, occupational status, and educational level. All p values are two-sided (χ^2 test).

Table 6 Association of ER-α, and ER-β alleles, genotypes and haplotypes, with the type of VED in patients

Polymorphism	Types of erectile dysfunction								
	AI (n = 102)	OR[a] (95% CI)	p value	VOD (n = 54)	OR[a] (95% CI)	p value	MVS (n = 97)	OR[a] (95% CI)	p value
ER-α *Pvu*II									
TT	0.530	1.0 (Referent)		0.294	1.0 (Referent)		0.176	1.0 (Referent)	
TC	0.588	2.34 (0.76–3.73)	0.074	0.140	0.38 (0.22-0.61)	0.001	0.272	2.82 (1.68-4.74)	0.01
CC	0.130	0.28 (0.14–0.57)	0.0004	0.300	1.22 (0.74-2.65)	0.072	0.570	6.68 (4.72-12.69)	0.0002
T	0.576	1.0 (Referent)		0.171	1.0 (Referent)		0.253	1.0 (Referent)	
C	0.315	0.28 (0.14–0.54)	0.0001	0.235	2.32 (0.87-3.46)	0.075	0.450	7.24 (4.67-14.41)	0.0001
ER-α *Xba*I									
GG	0.414	1.0 (Referent)		0.241	1.0 (Referent)		0.345	1.0 (Referent)	
AG	0.557	2.52 (0.84–1.71)	0.068	0.168	0.81 (0.66–1.72)	0.074	0.275	0.74 (0.61–2.38)	0.072
AA	0.093	0.21 (0.11–0.52)	0.0001	0.294	1.62 (0.82–2.73)	0.072	0.613	4.57 (2.61–7.62)	0.004
G	0.517	1.0 (Referent)		0.188	1.0 (Referent)		0.295	1.0 (Referent)	
A	0.324	0.22 (0.15–0.50)	0.0001	0.231	1.74 (0.82–2.34)	0.082	0.445	4.74 (2.37–7.31)	0.004
ER-β *Rsa*I									
GG	0.410	1.0 (Referent)		0.201	1.0 (Referent)		0.389	1.0 (Referent)	
AG	0.143	0.36 (0.22–0.66)	0.004	0.571	4.74 (2.63–6.65)	0.002	0.286	0.70 (0.49–0.81)	0.02
AA	0.500	0 (0.0)	NA	0.500	0 (0.0)	NA	0 (0.0)	NA	
G	0.406	1.0 (Referent)		0.206	1.0 (Referent)	0.388	1.0 (Referent)		
A	0.273	0.32 (0.24–0.53)	0.002	0.545	5.27 (3.74–9.21)	0.001	0.182	0.62 (0.34–0.71)	0.01
ER-β *Alu*I									
GG	0.556	1.0 (Referent)		0.083	1.0 (Referent)		0.361	1.0 (Referent)	
AG	0.177	0.25 (0.14–0.47)	0.0001	0.392	4.72 (2.41–6.72)	0.002	0.431	2.84 (1.58–3.76)	0.02
AA	0.571	1.14 (0.80–1.69)	0.22	0.286	3.53 (1.77–5.82)	0.006	0.143	0.38 (0.23–0.68)	0.003
G	0.456	1.0 (Referent)		0.164	1.0 (Referent)		0.380	1.0 (Referent)	
A	0.224	0.32 (0.21–0.57)	0.004	0.379	4.81 (2.62–7.31)	0.002	0.397	1.81 (0.84–2.76)	0.073
ER-α haplotypes									
*Pvu*II T–*Xba*I A	0.370	1.0 (Referent)		0.254	1.0 (Referent)		0.343	1.0 (Referent)	
*Pvu*II C–*Xba*I A	0.230	0.87 (0.72–1.76)	0.27	0.254	0.52 (0.31–0.74)	0.003	0.439	4.74 (2.64–7.38)	0.003
*Pvu*II C–*Xba*I G	0.536	7.47 (4.61–16.62)	0.0001	0.155	0.46 (0.32–0.75)	0.001	0.292	0.35 (0.24–0.67)	0.0006
*Pvu*II T–*Xba*I G	0 (0.00)	NA		0 (0.00)	NA			0 (0.00)	NA
ER-β haplotypes									
*Rsa*I G–*Alu*I A	0.505	1.0 (Referent)		0.385	1.0 (Referent)	0.110		1.0 (Referent)	
*Rsa*I G–*Alu*I G	0.165	0.27 (0.11–0.42)	0.0001	0.421	2.42 (0.72–3.71)	0.06	0.414	7.65 (4.29–14.61)	0.0002
*Rsa*I A–*Alu*I A	0.583	2.47 (1.64–4.66)	0.02	0.250	0.48 (0.34–0.72)	0.003	0.167	1.68 (0.84–2.68)	0.07
*Rsa*I A–*Alu*I G	0 (0.00)	NA		0 (0.00)	NA		NA		

AI: arterial insufficiency, VOD: venoocclusive disease, MVD: mixed vascular disease, OR: odds ratio, CI: confidence interval, ER: estrogen receptor, VED: vasculogenic erectile dysfunction, NA: not applicable. [a]Odds ratios were obtained from a logistic regression model with adjusting for age; body mass index; serum total cholesterol, triglyceride, LDL and HDL; smoking status, associated comorbidities, occupational status, and educational level. All p values are two-sided (χ^2 test).

p = 0.0002). Homozygotes for the ER-β *Alu*I A allele had significantly higher occurrences of VOD (OR = 3.53; 95% CI: 1.77–5.82, p = 0.006) and lower occurrences of mixed vasculogenic status (OR = 0.38; 95% CI: 0.23–0.68, p = 0.003) (for details see Table 6).

Compared between ER-α, and ER-β gene polymorphisms and reproductive hormones

Serum sex hormone-binding globulin, total testosterone, free testosterone, total oestradiol and free oestradiol levels were not significantly different in infertile patients compared with the controls (Table 1).

ER-α polymorphism

There was a statistically significant difference in serum SHBG levels between subjects with ER-α *Pvu*II T allele and those with ER-α *Pvu*II C allele both in cases and controls (Tables 7 and 8). In healthy men, the mean values of SHBG (nmol/L) were 21.69 for the ER-α *Pvu*II TT genotype and 24.8 for the ER-α *Pvu*II CC genotype (p = 0.005). As shown in Tables 7 and 8, participants carrying the ER-α *Pvu*II CC and ER-α *Xba*I AA genotypes had highest mean serum total testosterone and total E2 levels and lowest mean serum fT and free E2 levels (for details see Table 8).

ER-β polymorphism

Participants with ER-β *Rsa*I GA variants had highest serum levels of SHBG, total testosterone and total E2, and lowest serum levels of fT and free E2. The same directions for the same above-mentioned hormones can be applied for ER-β *Alu*I AA genotype (for details see Tables 7 and 8).

DISCUSSION

This population-based study demonstrates evidence for the association of both ER-α and ER-β genes polymorphisms in developing VED. There were also significant correlation of the studied SNPs [rs2234693 (ER-α *Pvu*II), rs9340799 (ER-α *Xba*I), rs1256049 (ER-β *Rsa*I) and rs4986938 (ER-β *Alu*I)] and genotypes with plasma levels of T, E2, free T and free E2. In the multivariate logistic regression model, which adjusted for confounding factors, ER-α *Pvu*II C and ER-α *Xba*I A alleles had strong predisposing effects for developing VED (# fivefold) whereas ER-β *Rsa*I A and ER-β *Alu*I A alleles had strong protective effects against developing VED (OR = 0.37, and 0.29 respectively). To our knowledge, this is the first population-based study to demonstrate such an association. Two large prospective, population-based cohort studies, the Rotterdam study and the Framingham study, demonstrated association between ER-α *Pvu*II genotypes,

Table 7 Relationship between different ER-α, and ER-β genotypes and serum levels of SHBG, total and free testosterone, and estradiol in healthy men

	Sex hormone binding globulin[a]				Total testosterone (mean)			Free testosterone (mean)			Total estradiol (mean)			Free estradiol (mean)		
Variables	n	nmol/L	95% CI[b]	*p* value	ng/ml	95% CI[b]	*p* value*	pg/ml	95% CI[b]	*p* value*	pg/ml	95% CI[b]	*p* values*	pg/ml	95% CI[b]	*p* value*
ER-α *Pvu*II																
TT	106	21.69	1.0 (Ref.)		4.3	1.0 (Ref.)		147	1.0 (Ref.)		15.0	1.0 (Ref.)		0.40	1.0 (Ref.)	
TC	277	24.08	20.25–28.37	0.005	5.0	3.2–5.8	0.061	130	99–159	0.006	16.3	10.2–22.2	0.067	0.33	0.32–0.38	0.004
CC	149	24.32	20.02–28.67	0.003	6.1	4.0–7.7	0.003	123	91–156	0.005	17.8	11.1–24.3	0.004	0.35	0.29–0.37	0.005
ER-α *Xba*I																
GG	92	22.64	1.0 (Ref.)		4.1	1.0 (Ref.)		146	1.0 (Ref.)		13.3	1.0 (Ref.)		0.42	1.0 (Ref.)	
AG	307	22.28	18.72–26.74	0.074	4.6	2.8–5.8	0.071	126	87–161	0.006	15.6	9.8–22.3	0.003	0.34	0.30–0.38	0.004
AA	133	25.06	20.28–29.11	0.004	6.2	3.9–7.9	0.005	122	86–154	0.005	18.8	13.0–24.4	0.002	0.32	0.29–0.37	0.003
ER-β *Rsa*I																
GG	492	24.00	1.0 (Ref.)		5.4	1.0 (Ref.)		129	1.0 (Ref.)		16.6	1.0 (Ref.)		0.35	1.0 (Ref.)	
AG	33	24.36	20.24–28.43	0.070	6.3	3.8–8.0	0.062	128	87–170	0.91	21.2	17.6–25.4	0.002	0.31	0.29–0.34	0.004
AA	7	19.61	15.57–22.07	0.002	3.4	2.1–4.8	0.007	155	112–192	0.004	13.1	10.1–17.3	0.002	0.39	0.36–0.42	0.006
GA + AA	40	20.10	17.15–24.26	0.003	4.2	2.7–5.5	0.063	148	110–190	0.003	14.4	9.82–20.2	0.004	0.38	0.35–0.41	0.006
ER-β *Alu*I																
GG	261	25.80	1.0 (Ref.)		4.8	1.0 (Ref.)		130	1.0 (Ref.)		16.4	1.0 (Ref.)		0.31	1.0 (Ref.)	
AG	203	23.09	19.77–27.36	0.003	6.2	4.0–7.9	0.062	124–7.9	83–156	0.088	18.2	13.2–24.2	0.006	0.28	0.25–0.31	0.006
AA	68	21.38	17.26–25.84	0.01	4.4	2.9–5.9	0.061	143	96–179	0.076	14.7	9.4–21.1	0.005	0.35	0.32–0.38	0.005

ER: estrogen receptor, LH: luteinizing hormone, FSH: follicle stimulating hormone. [a]Geometric least-squares means adjusted for, age, body mass index, smoking status, occupational status, and educational level. [b]95% CI for mean. **p* values are for the ORs.

Table 8 Relationship between different ER-α, and ER–β genotypes and serum levels of SHBG, total and free testosterone, and estradiol in men with VED

	Sex hormone binding globulin[a]				Total testosterone (mean)			Free testosterone (mean)			Total estradiol (mean)			Free estradiol (mean)		
Variables	n	nmol/L	95% CI[b]	*p* value	ng/ml	95% CI[b]	*p* value*	pg/ml	95% CI[b]	*p* value*	pg/ml	95% CI[b]	*p* value*	pg/ml	95% CI[b]	*p* value*
ER–α *Pvu*II																
TT	19	22.77	1.0 (Ref.)		4.2	1.0 (Ref.)		148	1.0 (Ref.)		14.7	1.0 (Ref.)		0.41	1.0 (Ref.)	
TC	143	24.64	20.29–28.20	0.004	4.7	2.6–6.5	0.061	136	93–180	0.004	16.0	10.2–22.2	0.067	0.35	0.32–0.38	0.005
CC	104	25.36	20.77–29.66	0.005	5.9	3.8–7.2	0.004	137	101–156	0.003	17.4	11.3–24.1	0.004	0.34	0.31–0.36	0.004
ER–α *Xba*I																
GG	32	22.65	1.0 (Ref.)		4.7	1.0 (Ref.)		133	1.0 (Ref.)		15.7	1.0 (Ref.)		0.35	1.0 (Ref.)	
AG	155	22.29	18.37–26.30	0.074	4.0	2.0–6.1	0.067	151	136–194	0.004	13.3	7.5–19.5	0.005	0.43	0.41–0.45	0.004
AA	79	24.5	20.77–29.36	0.004	5.9	3.8–7.9	0.005	127	84–174	0.003	18.6	12.4–24.1	0.002	0.31	0.29–0.33	0.003
ER–β *Rsa*I																
GG	256	23.80	1.0 (Ref.)		5.3	1.0 (Ref.)		133	1.0 (Ref.)		16.7	1.0 (Ref.)		0.35	1.0 (Ref.)	
GA	8	24.50	19.5–28.27	0.068	6.3	4.2–8.4	0.064	132	83–174	0.94	21.4	17.7–25.2	0.003	0.30	0.26–0.33	0.003
AA	2	20.47	16.71–24.22	0.004	3.6	1.9–5.5	0.005	161	116–192	0.004	13.1	9.2–16.6	0.002	0.39	0.36–0.42	0.006
GA + AA	10	20.46	17.5–24.83	0.003	3.8	2.7–5.4	0.062	155	1118–194	0.006	15.4	10.0–18.2	0.004	0.37	0.34–0.40	0.006
ER–β *Alu*I																
GG	152	25.80	1.0 (Ref.)		6.2	1.0 (Ref.)		122	1.0 (Ref.)		18.4	1.0 (Ref.)		0.23	1.0 (Ref.)	
AG	107	23.09	19.16–27.27	0.003	4.8	3.3–6.2	0.073	132	82–176	0.074	15.2	11.3–21.2	0.004	0.31	0.28–0.33	0.007
AA	7	21.26	17.48–24.77	0.01	4.2	2.9–5.7	0.072	145	83–201	0.068	14.4	9.4–19.5	0.005	0.3435	0.31–0.38	0.006

ER: estrogen receptor, LH: luteinizing hormone, FSH: follicle stimulating hormone. [a]Geometric least–squares means adjusted for, age, body mass index, smoking status, occupational status, and educational level. [b]95% CI for mean. **p* values are for the ORs.

and the risk of cardiovascular disease (Shearman *et al.*, 2003; Schuit *et al.*, 2004). In the Framingham study, male carriers of the ER-α *Pvu*II CC genotype had a threefold increased myocardial infarction (MI) risk (Shearman *et al.*, 2003). Study of more than 7 000 whites in five cohorts from four countries provided strong evidence that ER-α *Pvu*II CC genotype is a risk factor for acute myocardial infarction (MI) (Shearman *et al.*, 2006). Roumeguère *et al.* (2003) demonstrated that ED is associated with a high prevalence of coronary heart disease risk. In this study, male carriers of the ER-α *Pvu*II CC genotype had a 4.6-fold increased VED risk. In haplotype analysis, carriers of the ER-α *Pvu*II C- ER-α *Xba*I A alleles had more than eightfold increased risk for developing VED. These results are in agreement with the data from the Framingham Heart study in which carriers of the ER-α *Pvu*II CC genotype had a threefold increased risk of MI, and a twofold greater risk of major atherosclerotic cardiovascular disease (Shearman *et al.*, 2003). In a study by Rokach *et al.* (2005), carriers of one copy of the ER-α *Pvu*II C- ER-α *Xba*I G haplotype had the lowest number of obstructed coronary artery vessels when compared with carriers of no or two copies of this haplotype. This finding is also in consistent with our results, in which men who carriers ER-α *Pvu*II C- ER-α *Xba*I G haplotype had more than 50% decreased risk against developing VED. Observational epidemiological studies in human strongly support an atheroprotective role for endogenous E2 (Villablanca *et al.*, 2009). ER-α is a major mediator of E2 protection in advanced atherosclerotic lesions (The Writing Group for the PEPI Trial, 1995). In our study, ER-α polymorphisms with lowest serum levels of free E2 (ER-α *Pvu*II CC and ER-α *Xba*I AA genotypes) had highest risk for developing VED. The atheroprotective effects of oestrogens are caused by direct effects on the vessel wall and indirect effects on lipoprotein metabolism (Geraldes *et al.*, 2002). One large, randomized, controlled trial has confirmed that oestrogens reduce LDL cholesterol levels and increase HDL cholesterol levels (Geraldes *et al.*, 2002). In addition, there are receptor-mediated antiproliferative effects of E2 on vascular smooth muscle cells, and the trophic effects on vascular endothelial cells (Geraldes *et al.*, 2002). Therefore, there may be a true effect of ER-α gene polymorphisms on penile arteries, partially through an indirect effect via HDL metabolism and through a direct effect on penile arteries. The impact of ER-β gene polymorphism can also be explained by same mechanisms. Subjects with ER-β *Rsa*I AA and ER-β *Alu*I AA genotypes had highest serum free E2. These subjects had 68 and 46% decreased risk against developing VED respectively. In fact, ER-β gene deficiency worsens cardiac dysfunction following myocardial ischaemic injury (Pelzer *et al.*, 2005). In addition, subjects with lowest serum free E2 levels and highest risk for developing VED had also highest level of mixed vascular type VED. This demonstrates that serum oestrogen levels affect all types of penile vasculature. In addition, patients with lower serum E2 levels had more sever VED. Therefore, oestrogen receptors seem to play an important role in the prevention or in the occurrence of penile vascular disorders. ER-α gene polymorphisms can also influence healing response to vascular injury (Ferrero *et al.*, 2003). Ferrero *et al.* (2003) investigated the role of ER-α gene polymorphisms in the occurrence of restenosis after coronary stent implantation.

The findings of this study can have clinical implications. If there is a causal association between a genetic marker and VED then we could plan a strategy of individual treatment scheme. Nonetheless, our interpretation remains suppositional, as the functional relevance of the ER-α and ER-β polymorphisms cannot be investigated in this type of association studies but rather needs a specific identification of the quantitative trait.

Calculation of sample size in our investigation was a priori, considering an α error <0.05 and a β error <0.1. This demonstrates that our sample size was statistically adequate to minimize type I error.

However, as with any new genetic association study, the results of this study should be considered investigative and await confrontation with further researches, particularly with larger sample size and in different ethnic groups. In addition, although the subjects of this study were unrelated, hidden population structure can cause bias in association studies and/or result in overestimation of the effect of tested SNPs.

CONCLUSION

The findings of our study demonstrate that ER-α and ER-β polymorphisms are strongly associated with the presence, severity and type of VED in Iranian population. Whether this scenario can apply to other population is worth further investigation.

AUTHORS' CONTRIBUTIONS

M.R.S. and N.S. were involved in conception and design. M.R.S., S.S. and A.T. were involved in acquisition of data. M.R.S., N.S. and A.T. were involved in analysis and interpretation of data. M.R.S., S.S. and N.S. drafted the article and revised it for intellectual content. M.R.S. and A.T. provided final approval of the completed article.

REFERENCES

Aléssio AM, Höehr NF, Siqueira LH, Ozelo MC, de Pádua Mansur A & Annichino-Bizzacchi JM. (2007) Association between estrogen receptor alpha and beta gene polymorphisms and deep vein thrombosis. *Thromb Res* 120, 639–645.

American Psychological Association (2000) *Diagnostic and Statistical Manual of Mental Disorders. DSM-IV-TR*. American Psychological Association, Washington, DC.

Bing RJ & Conforto A. (1992) Reversal of acetylcholine effect on atherosclerotic coronary arteries by estrogen: pharmacologic phenomenon of clinical importance? *J Am Coll Cardiol* 20, 458–459.

Christian RC, Liu PY, Harrington S, Ruan M, Miller VM & Fitzpatrick LA. (2006) Intimal estrogen receptor (ER) beta, but not ER alpha expression, is correlated with coronary calcification and atherosclerosis in pre- and postmenopausal women. *J Clin Endocrinol Metab* 91, 2713–2720.

Deroo BJ & Korach KS. (2006) Estrogen receptors and human disease. *J Clin Invest* 116, 561–570.

Doherty TM, Asotra K, Fitzpatrick LA, Qiao JH, Wilkin DJ, Detrano RC *et al.* (2003) Calcification in atherosclerosis: bone biology and chronic inflammation at the arterial crossroads. *Proc Natl Acad Sci U S A* 100, 11201–11206.

Evangelopoulos D, Alevizaki M, Lekakis J, Cimponeriu A, Papamichael C, Kominakis A *et al.* (2003) Molecular analysis of the estrogen receptor alpha gene in men with coronary artery disease: association with disease status. *Clin Chim Acta* 331, 37–44.

Favre J, Gao J, Henry JP, Remy-Jouet I, Fourquaux I, Billon-Gales A *et al.* (2010) Endothelial estrogen receptor {alpha} plays an essential role in the coronary and myocardial protective effects of estradiol in ischemia/reperfusion. *Arterioscler Thromb Vasc Biol.* 30, 2562–2567.

Ferrero V, Ribichini F, Matullo G, Guarrera S, Carturan S, Vado A *et al.* (2003) Oestrogen receptor-alpha polymorphisms and angiographic

outcome after coronary artery stenting. *Arterioscler Thromb Vasc Biol* 23, 2223–2228.
Figtree GA, Grieve SM, Speller B, Geiger MJ, Robinson BG, Channon KM *et al.* (2008) A commonly occurring polymorphism upstream of the estrogen receptor alpha alters transcription and is associated with increased HDL. *Atherosclerosis* 199, 354–361.
Geraldes P, Sirois MG, Bernatchez PN & Tanguay JF. (2002) Estrogen regulation of endothelial and smooth muscle cell migration and proliferation: role of p38 and p42/44 mitogen-activated protein kinase. *Arterioscler Thromb Vasc Biol* 12, 1585–1590.
Gerhard M & Ganz P. (1995) How do we explain the clinical benefits of estrogen? From bedside to bench. *Circulation* 92, 5–8.
Godsland IF. (2001) Effects of postmenopausal hormone replacement therapy on lipid, lipoprotein, and apolipoprotein (a) concentrations: analysis of studies published from 1974-2000. *Fertil Steril* 75, 898–915.
Hodgin JB, Krege JH, Reddick RL, Korach KS, Smithies O & Maeda N. (2001) Estrogen receptor alpha is a major mediator of 17beta-estradiol's atheroprotective effects on lesion size in Apoe-/-mice. *J Clin Invest* 107, 333–340.
Karas RH, Patterson BL & Mendelsohn ME. (1994) Human vascular smooth muscle cells contain functional estrogen receptor. *Circulation* 89, 1943–1950.
Kendirci M, Nowfar S & Hellstrom WJ. (2005) The impact of vascular risk factors on erectile function. *Timely Top Med Cardiovasc Dis* 9, E11.
Laumann EO, Paik A & Rosen Raymond C. (1999) Sexual dysfunction in the United States: prevalence and predictors. *JAMA* 281, 537–544.
Lehrer S, Rabin J, Kalir T & Schachter BS. (1993) Estrogen receptor variant and hypertension in women. *Hypertension* 21, 439–441.
Lussana F, Faioni EM, Mavilia C, Bucciarelli P, Brandi ML & Cattaneo M. (2006) Association of estrogen receptor-alpha genepolymorphisms with venous thrombosis. *Haematologica* 91, 279–280.
Mansur AP, Nogueira CCM, Strunz CMC, Aldrighi JM & Ramires AF. (2005) Genetic polymorphisms of estrogen receptors in patients with premature coronary artery disease. *Arch Med Res* 36, 511–517.
Melman A & Gingell JC. (1999) The epidemiology and pathophysiology of erectile dysfunction. *J Urol* 161, 5–11.
Miller H, Poon S, Hibbert B, Rayner K, Chen YX & O'Brien ER. (2005) Modulation of estrogen signaling by the novel interaction of heat shock protein 27, a biomarker for atherosclerosis, and estrogen receptor beta: mechanistic insight into the vascular effects of estrogens. *Arterioscler Thromb Vasc Biol* 25, e10–e14.
Morey AK, Pedram A, Razandi M, Prins BA, Hu RM, Biesiada E *et al.* (1997) Estrogen and progesterone inhibit vascular smooth muscle proliferation. *Endocrinology* 138, 3330–3339.
Mosselman S, Polman J & Dijkema R. (1996) ER beta: identification and characterization of a novel human estrogen receptor. *FEBS Lett* 392, 49–53.
NIH Consensus Conference (1993) NIH Consensus Development Panel on Impotence. *JAMA* 270, 83–90.
Pelzer T, Loza PA, Hu K, Bayer B, Dienesch C, Calvillo L *et al.* (2005) Increased mortality and aggravation of heart failure in estrogen receptor-beta knockout mice after myocardial infarction. *Circulation* 111, 1492–1498.
Pollak A, Rokach A, Blumenfeld A, Rosen LJ, Resnik L & Dresner-Pollak R. (2004) Association of oestrogen receptor alpha gene polymorphism with the angiographic extent of coronary artery disease. *Eur Heart J* 25, 240–245.
Ponglikitmongkol M, Green S & Chambon P. (1988) Genomic organization of the human oestrogen receptor gene. *EMBO J* 7, 3385–3388.
Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G, Walts B *et al.* (2006) The human obesity gene map: the 2005 update. *Obesity (Silver Spring)* 14, 529–644.
Rokach A, Pollak A, Rosen L, Friedlander Y, Blumenfeld A, Reznik L *et al.* (2005) Estrogen receptor alpha gene polymorphisms are associated with the angiographic extent of coronary artery disease. *J Clin Endocrinol Metab* 90, 6556–6560.
Rosen RC, Cappelleri JC, Smith MD, Lipsky J & Pena MB. (1999) Development and evaluation of an abridged, 5-item version of the International Index of Erectile Function as a diagnostic tool for erectile dysfunction. *Int J Impot Res* 11, 319–326.
Rosenkranz K, Hinney A, Ziegler A, Hermann H, Fichter M, Mayer H *et al.* (1998) Systematic mutation screening of the estrogen receptor beta gene in probands of different weight extremes: identification of several genetic variants. *J Clin Endocrinol Metab* 83, 4524–4527.
Ross R. (1993) The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature,* 362, 801–809.
Roumeguère T, Wespes E, Carpentier Y, Hoffmann P & Schulman CC. (2003) Erectile dysfunction is associated with a high prevalence of hyperlipidemia and coronary heart disease risk. *Eur Urol* 44, 355–359.
Safarinejad MR, Safarinejad S, Shafiei N & Safarinejad S. (2012) Estrogen receptors alpha (rs2234693 and rs9340799), and beta (rs4986938 and rs1256049) genes polymorphism in prostate cancer: evidence for association with risk and histopathological tumor characteristics in Iranian men. *Mol Carcinog* 51(Suppl 1), E104–E117.
Schuit SC, Oei HH, Witteman JC, Geurts van Kessel CH, van Meurs JB, Nijhuis RL *et al.* (2004) Estrogen receptor α gene polymorphisms and risk of myocardial infarction. *JAMA* 291, 2969–2977.
Serock MR, Wells AK & Khalil RA. (2008) Modulators of vascular sex hormone receptors and their effects in estrogen deficiency states associated with menopause. *Recent Pat Cardiovasc Drug Discov* 3, 165–186.
Shearman AM, Cupples LA, Demissie S, Peter I, Schmid CH, Karas RH *et al.* (2003) Association between estrogen receptor alpha gene variation and cardiovascular disease. *JAMA* 290, 2263–2270.
Shearman AM, Cooper JA, Kotwinski PJ, Miller GJ, Humphries SE, Ardlie KG *et al.* (2006) Estrogen receptor alpha gene variation is associated with risk of myocardial infarction in more than seven thousand men from five cohorts. *Circ Res* 98, 590–592.
Sudhir K, Chou TM, Chatterjee K, Smith EP, Williams TC, Kane JP *et al.* (1997) Premature coronary artery disease associated with a disruptive mutation in the estrogen receptor gene in a man. *Circulation* 111, 1492–1498.
The Writing Group for the PEPI Trial (1995) Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women: the Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. *JAMA* 273, 199–208.
Villablanca A, Lubahn D, Shelby L, Lloyd K & Barthold S. (2004) Susceptibility to early atherosclerosis in male mice is mediated by estrogen receptor alpha. *Arterioscler Thromb Vasc Biol* 24, 1055–1061.
Villablanca AC, Tenwolde A, Lee M, Huck M, Mumenthaler S & Rutledge JC. (2009) 17 beta-estradiol prevents early-stage atherosclerosis in estrogen receptor-alpha deficient female mice. *J Cardiovasc Transl Res* 2, 289–299.
Walter P, Green S, Greene G, Krust A, Bornert JM, Jeltsch JM *et al.* (1985) Cloning of the human estrogen receptor cDNA. *Proc Natl Acad Sci U S A* 82, 7889–7893.
Watanabe T, Akishita M, Nakaoka T, Kozaki K, Miyahara Y, He H *et al.* (2003) Estrogen receptor beta mediates the inhibitory effect of estradiol on vascular smooth muscle cell proliferation. *Cardiovasc Res* 59, 734–744.
Wolinsky H. (1972) Effects of estrogen and progestogen treatment on the response of the aorta of male rats to hypertension. Morphological and chemical studies. *Circ Res* 30, 341–349.

