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ANGIOTENSIN CONVERTING ENZYME GENE AND RECURRENT PREGNANCY LOSS

Polymorphisms of the angiotensin converting enzyme gene in Iranian Azeri Turkish women with unexplained recurrent pregnancy loss

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Abstract
Several factors which influence the balance between fibrinolysis and coagulation pathways play role in the outcome of conception. A large body of studies demonstrate that gene variations are associated with recurrent pregnancy loss (RPL) by different mechanisms. We aimed to determine the allele and genotype frequencies of the angiotensin converting enzyme (ACE) gene in Iranian Azeri Turkish women with unexplained RPL. Fifty patients with RPL and 63 fertile healthy women as controls were entered in the study. A standard method was used for DNA isolation. All genotypes were determined using PCR. Our analysis showed that patient ($\chi^2 = 0.347$, $p = 0.84$) and control ($\chi^2 = 0.77$, $p = 0.68$) groups fitted the Hardy–Weinberg equilibrium strongly. No significant differences were found regarding the frequencies of ACE genotypes [deletion/deletion (D/D), insertion/deletion (I/D) and insertion/insertion (I/I)] and alleles between cases and controls. Based on these findings, we could not find any association between ACE (D/D, I/D and I/I) gene polymorphisms and RPL.

Keywords: *ACE, unexplained RPL, Azeri Turkish women*

Introduction

Several factors play a role in recurrent pregnancy loss (RPL) including thrombophilic conditions which can be influenced by gene polymorphisms (Coulam et al., 2006; Goodman et al., 2006). Recent studies have focused on plasminogen activator inhibitor-1 (PAI-1), angiotensin converting enzyme (ACE) and some other gene variations which affect fibrinolysis activities (Buchholz & Thaler, 2003; Buchholz et al., 2003; Coulam et al., 2006; Goodman et al., 2006; Goodman et al., 2009). ACE zinc-containing peptidylpeptide hydrolase plays a critical role in the renin–angiotensin system and is involved in the conversion of decapeptide angiotensin I to active octapeptide angiotensin II. ACE is expressed in different tissues such as lung, vascular endothelium, kidney epithelium and Leydig cells in the testis. ACE not only degrades vasoactive peptides but also contributes to the metabolism of neurotransmitters (Erdos & Skidgel, 1987; Villard & Soubrier, 1996). ACE isozyme production is regulated by different hormones including glucocorticoids in endothelium and androgens in the testis (Krulewitz et al., 1984; Velletri et al., 1985). The human ACE gene is mapped to chromosome 17q23.3 with 26 exons and 25 introns and contains approximately 21 kb of genomic DNA. The ACE gene polymorphism was initially reported by Rigat et al (1990). This polymorphism is defined by the presence (insertion, I) or absence (deletion, D) of a 287 bp fragment in intron 16 that leads to three I/I, I/D, and D/D genotypes (Rigat et al., 1990). The Presence of the D allele or D/D genotype is correlated with elevated plasma and tissue specific ACE activity (Rigat et al., 1990). The D/D genotype also enhances production of angiotensin II from angiotensin I, and is associated with high levels of circulating PAI-1 which result in reduced levels of fibrinolysis (Ueda et al., 1995; Kim et al., 1997). These thrombophilic polymorphisms may play a role in susceptibility to human disorders and drug responses in different

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ethnic populations (Johanning et al., 1995). Several studies have been carried out to evaluate possible associations of ACE with human disorders such as Alzheimer's disease (Farrer et al., 2000), diabetes mellitus and nephropathy (Kvetny et al., 2001), polycystic kidney disease (Cambien et al., 1992; Schunkert et al., 1994), hyper-homocysteinemia (Heby et al., 1995), hypertension (Girard et al., 2005), coronary artery disease (Zee et al., 2002) and complications in pregnancy such as RPL (Griendling et al., 1993; Preston et al., 1996; Stephenson, 1996; Vaughan, 1998; Fatini et al., 2000; Buchholz & Thaler, 2003; Buchholz et al., 2003; Fatini et al., 2003; Mello et al., 2003; Suzuki et al., 2003). We aimed to determine the allele and genotype frequencies of the ACE (I/D) gene in Iranian Azeri Turkish women with unexplained RPL.

Materials and methods

The study was approved by the Ethics Committee of Urmia University of Medical Sciences. One hundred and thirteen women (50 women with unexplained RPL and 63 women as healthy controls) were included in the study. Fifty patients with RPL were referred from the Obstetric and Gynaecology Department to the Genetics Department at Motahari Educational Hospital (Urmia, Iran); all of the cases had a history of at least three (mean $\pm$ SD: 3.15 ± 1.07, median: 3) pregnancy losses with unknown aetiology. Patients and controls were enrolled according to the criteria described by Vettriselvi et al. (2008). RPL refers to any pregnancy losses (three or more consecutive) that occur before 20 weeks gestational age, with the same partner. Women excluded from the investigation were those with chromosomal, anatomical, endocrine, infectious or immune disorders. Diagnostic tests such as karyotyping of cases and related partners, maternal infections, antiphospholipid and cardiolipin antibody, protein C and S and antitrombin III were carried out to determine the aetiology of RPL. Controls were fertile healthy females with at least two successful live-births, randomly selected from the same geographical region and ethnic group. Cases and controls with abnormal findings and problems in their medical history such as chromosomal aberrations, cardiovascular diseases, diabetes mellitus, obesity, insulin resistance and other confounding factors known to cause RPL were excluded from the study. Patients and controls all gave written consent and their histories were assessed in the departments of Genetics and Obstetrics and Gynaecology. Three to five millitres whole blood were obtained from the patients and controls and added to EDTA-containing tubes. Genomic DNA was extracted by a standard salting out method (Miller et al., 1988). ACE genotyping was performed by polymerase chain reaction (PCR) using primers 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′ and 5′-GAT GTG GCC ATC ACA TTC GTC AGA T-3′ and the cycling programme consisted of 35 cycles (denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min) (Rigat et al., 1992). PCR products were separated by electrophoresis on 2% agarose gel containing ethidium bromide. The presence or absence of amplified fragments (490 and 190 bp) was visualised by UV transilluminator. Presence of a single 490 bp fragment corresponding to the I/I genotype and a single 190 bp band was indicative of the D/D genotype. The presence of both 490 and 190 bp fragments indicated the I/D genotype.

Statistical analysis

The ACE allelic/genotypic frequencies were found via direct counting. Allelic/genotypic frequencies were compared with controls using the χ^2 test or Fisher's exact test. For each group, the expected genotype frequencies were compared with determined and compared with those of observed genotype frequencies. The cases and control frequencies were tested for their fit to the Hardy–Weinberg equilibrium. SPSS version 16.0 and Microsoft Excel 2003 were used for statistical analysis to calculate the χ^2 and p-value, the odds ratio (OR), and 95% confidence interval (CI). Two-sided tests with power (1-β): 90% were performed and a p-value < 0.05 was considered statistically significant.

Results

Participants comprised 113 women including 50 with unexplained RPL aged 28.27 ± 5.29 (mean $\pm$ SD) with a body mass index of 24.767 ± 3.402 (BMI $\pm$ SD) kg/m^2 and 63 healthy controls aged 29.58 ± 4.95 (mean $\pm$ SD) with a body mass index of 23.327 ± 3.193 (BMI $\pm$ SD) kg/m^2. Cases *versus* controls showed no significant differences regarding BMI ($\chi^2 = 0.972$, df $= 4$, p-value $= 0.337$). Findings are summarised in Table I. Figure 1 shows ACE D/I genotypes using electrophoresis. Statistical analysis showed that patients ($\chi^2 = 0.347 < 3.84$, df $= 2$, p-value$=0.84 > 0.05$) and controls ($\chi^2 = 0.77 < 3.84$, df $= 2$, p-value $= 0.68 > 0.05$) strongly fitted the Hardy–Weinberg equilibrium. There were no significant differences regarding the alleles/genotypes frequencies of ACE [deletion/deletion (D/D), insertion/deletion (I/D) and insertion/insertion (I/I)] between cases and controls; χ^2 and p-values and OR (95% CI) are reported in Table I.

Table I. The frequencies of ACE alleles/genotypes in control patients and those with unexplained RPL.

	Patients			Controls					
ACE	*F* (%*F*)	Frequency	Expected	*F* (%*F*)	Frequency	Expected	OR (95% CI)	χ^2	*p*-value
D	60 (60)	0.6	–	75 (59.52)	0.59	–	1.02 (0.6–1.74)	0.01	0.94
I	40 (40)	0.4		51 (40.48	0.40		0.98 (0.57–1.67)	0.01	0.94
DD	17 (34)	0.34	18	24 (38.1)	0.38	22.32	0.8 (0.4–1.8)	0.2	0.65
ID	26 (52)	0.52	24	27 (42.9)	0.43	30.36	1.4 (0.7–3)	0.94	0.33
II	7 (14)	0.14	8	12 (19)	0.19	10.32	0.7 (0.3–1.9)	0.51	0.48

F, Frequency of observed alleles/genotypes.

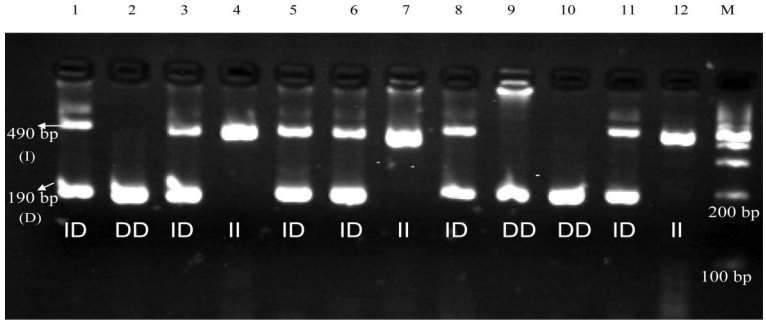

Figure 1. ACE D/D, I/D and I/I genotypes on 2% agarose gel in 12 samples.

Discussion

Unexplained RPL is defined as the occurrence of at least three or more sequential pregnancy losses commonly occurring before 12 weeks gestational age. The aetiology of RPL in most cases is not understood (Finan et al., 2002). In this study, we analysed three possible genotypes of the ACE gene (D/D, I/D, and I/I) in Iranian Azeri Turkish women with unexplained RPL. The findings failed to show any association between ACE polymorphisms and unexplained RPL in these women. Our results are supported by Goodman et al. (2009) and Vettriselvi et al. (2008), but not by others (Fatini et al., 2000; Buchholz & Thaler, 2003; Buchholz et al., 2003). The D allele of ACE results in a reduced level of fibrinolysis and a balance between fibrinolysis and coagulation is necessary for successful pregnancy. Several factors which influence the balance between fibrinolysis and coagulation pathways play a role in the outcome of conception. A large body of studies recently demonstrated that several gene variations, for example methylenetetrahydrofolate reductase, Factor V Leiden, prothrombin G20210A mutations are associated with RPL by different mechanisms (Nelen et al., 1997; Foka et al., 2000; Lachmeijer et al., 2001; Unfried et al., 2002; Kosmas et al., 2004). Reports within and between different ethnic groups are complicated by issues such as study design and family linkages. Based on the findings of this study, we could not detect any association between ACE (D/D, I/D and I/I) gene polymorphisms and RPL.

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