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Association of oestrogen receptor alpha gene SNPs Arg157Ter C>T and Val364Glu T>A with female infertility

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Globally, around 15% of couples suffer from infertility [1]. Factors linked to female infertility including uterine abnormalities, endometriosis, chromosomal abnormalities, endocrine disorders, and immune defects. As many aspects of these are influenced by genetic factors, many studies have attempted to identify susceptible genes for female fertility [2]. Oestrogen is synthesized by ovarian granulosa cells under the control of Hypothalamus-Pituitary-Ovarian (HPO) axis. The biological functions of oestrogen are mainly mediated by binding to the oestrogen receptors (ERs), of which there are two isoforms, ERα and ERβ. ERα is encoded by *ESR1* at 6q25.1 and is formed from 8 exons with >2200 SNPs [3,4]. Specific polymorphisms in *ESR1* may directly or indirectly lead to variations in its activity and have significant impacts on different diseases. Some have considered ESR_1 variants to be one of the important causative factors in female infertility [5]. One of the important *ESR1* SNPs is rs104893956 (Arg157Ter: C > T) found in exon 2 leading to a stop codon [6]. A further SNP is rs121913044 (Val364Glu T > A) located at exon 4 which results in the substitution of Valine (Val) to Glutamic acid (Glu) at codon 364 [6]. It is located within the binding site of ERα protein.

Several investigations in different populations suggest significant association of ERα polymorphisms with reproductive disorders among women. The association of single SNPs with oestrogen receptor activity in relation to different diseases including oestrogen resistance disease, endometriosis, and infertility has been studied in different populations. The identification of genetic variations associated with altered oestrogen response is of potential public health importance. Gaining more insights into the pathogenesis of different oestrogen sensitive diseases including infertility would significantly help the development and application of newer therapies for these disorders. Furthermore, genetic variants that alter sensitivity to oestrogen may affect both therapeutic and harmful responses to exogenous oestrogen administered in the form of the oral contraceptive pill or hormone replacement therapy. We hypothesised an association of *ESR1* missense mutations Arg157Ter and Val364Glu with female infertility.

We recruited 165 infertile women and 190 healthy controls with at least one successful pregnancy from Alzahra Hospital, Rasht, Iran. Infertile women had been unable to conceive for at least 2 years. Among them, 72% had disorders including polycystic ovary syndrome (36%), endometriosis (32%), and uterine fibroids (28%), and 25% had a history of recurrent miscarriage, whilst 22% of patients also reported a history of infertility in a first-degree relative. Clinical data on infertile women were collected from clinical notes. Informed consent documents were obtained from all the participants prior to sampling, and the study was conducted in accordance with the declaration of Helsinki. Genomic DNA was extracted from whole blood samples by a Gpp solution kit (Gen Pajoohan, Iran) following standard procedures. DNA integrity was quantified by electrophoresis on 1.8% agarose gel stained by ethidium bromide (0.5 mg/ml). The purified genomic DNA was maintained at a temperature of −20°C until the molecular analysis. Arg157Ter and Val364Glu were genotyped by ARMS-PCR technique. PCR amplification was carried out by utilizing primers designed by Oligo7 Software (version 7.54, Molecular Biology Insights Inc., Cascade, CO, USA), these being Forward (F): 5′- CCCCAGGCCAAATTCAGA TAGTC-3′ and Reverse (R): 5′-CTACCAAAGATACTAGTG GACC-3′ for C allele (Dominant) of Arg157Ter site (PCR product = 338 bp), F: 5′-CCCCAGGCCAAATTCAGATAGTT -3′ and R: 5′-TTCCTTCCTCAGTCGCTTT-3′ for T allele of Arg157Ter site (PCR product = 274 bp), F: 5′-GATCA ACTGGGCGAAGAGTGT-3′ and R: 5′-TACCATGTAGTGTC CACCC-3′ for T allele (Dominant) of Ala419Thr site (PCR product = 313 bp), and F: 5′-GATCAACTGGGCG AAGAGAGA-3′ and R: 5′-ATTTCTCCCATGACATCACA-3′ for A allele of Ala419Thr site (PCR product: 98 bp). PCR

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reaction mixture (25 µl) contained approximately 30 ng of genomic DNA, 1x PCR buffer, 0.2 mM dNTP, 1.5 mM of $MgCl_2$, 0.5 µM of primers and 2 units of Taq DNA Polymerase (Bio flux, Japan). DNA amplification by PCR was performed by the following steps: initial denaturation at 95°C for 5 min, 35 cycles of denaturation of 95°C for 45 s, annealing at 54°C for 30 s, and 53°C for 30 s, extension at 72°C for 45 s, and final elongation at 72°C for 5 min. PCR products were separated on 2% agarose gel and visualized by ethidium bromide staining. All statistical analyses were assessed by SPSS ver. 20, SNPAlyze (ver. 8.1.1, Dynacom, Japan), and Web-Assotest program (available at http://www.ekstroem.com/). Genotype and allele frequencies of Arg157Ter and Val364Glu between case and control groups were compared and measured through Pearson χ2 – statistics. The analyses were also performed considering hereditary models including dominant, co-dominant, and recessive models and odds ratios (ORs), their 95% CI ranges and corresponding P-values were also computed by both SNPAlyze and the Web-Assotest online software. The analysis of variance (ANOVA) or the unpaired two-tailed Student's test was performed to compare the clinical data in allelic and genotypic levels of studied variants. The significance level of the statistical tests considered <0.05.

Results are shown in Table 1. The frequency of the CC Arg157Ter (C > T) genotype SNP was >2-fold higher in controls than in cases, whereas the TT genotype was 6-fold higher in the cases compared to the controls (overall chi-squared 26.8, p < 0.001). The C allele was more frequent in the controls and the T allele in the cases (chi-squared 8.71, p = 0.003, odds ratio/95% CI) for T allele and infertility 1.56 (1.16–2.10). The dominant model (CC v CT+TT) gave an odds ratio/95% CI of 2.39 (1.07–5.53)(p = 0.02), the co-dominant model (CT v CC +TT) was 3.84 (2.13–6.94)(p < 0.001) and the recessive model (TT v CC+CT) was 8.22 (3.11–21.74)(p < 0.001). Similarly, the frequency of the TT Val364Glu (T > A) genotype SNP was over 3-fold higher in the cases (overall chi-squared 5.39) but due to the small number of women controls with a TT genotype and the small number of cases with an AA genotype, this was not significant (p = 0.067). The difference in the allele frequencies was not significant (chi-squared 0.58, p = 0.45). However, the dominant model (TT v TA+AA) gave a significant odds ratio/95% CI of 0.25 (0.07–0.92) (p = 0.02), the co-dominant model (TA v TT+AA) was 0.4 (0.17–0.98) (p = 0.03) and the recessive model (AA v TT+TA) was 0.65 (0.19–2.26) (p = 0.49).

Table 1. Genotype and allele frequencies in cases and controls.

Genotype/Allele	Controls (n = 190)	Cases (n = 165)
Arg157Ter (C > T)		
CC	23 (12.1)	9 (5.4)
CT	162 (85.3)	126 (76.4)
TT	5 (2.6)	30 (18.2)
C	208 (54.7)	144 (43.6)
T	172 (45.3)	186 (56.3)
Val364Glu (T > A)		
TT	3 (1.6)	10 (6.1)
TA	180 (97.7)	151 (91.5)
AA	7 (3.7)	4 (2.4)
T	186 (51.0)	171 (51.8)
A	194 (49.0)	159 (48.2)

Data n (%)

These results indicate that both Arg157Ter and Val364Glu are associated with female infertility, although a link with the former is much stronger and more consistent. Oestrogen receptor alpha controls a diverse set of essential functions such as development, proliferation and reproduction. These findings resulted in the concept that the functional SNPs of *ESR1* might be linked to infertility. Arg157Ter has a functional SNP of *ESR1* within exon 2 which is located in DNA binding domain results in a premature stop codon [7]. It has been suggested that this functional SNP may confer a high level of risk in some diseases as it can truncate and even inactivate ERα [6].

Others have also investigated *ESR1* and its genetic variations in the pathogenesis of female infertility. These include the finding of a significant association between *ESR1* SNP rs9340779 and endometriosis-related infertile women who had no blastocyst implantation [8]. The fertility status association with ERa polymorphisms has also been investigated in women with endometriosis, results suggesting that variants in *ESR1* led to a significant modification in their susceptibility to endometriosis and influenced their fertility [9]. Also, a significant association between rs9322331 and osteopenia has been reported [10]. Anousha et al., in a study on foetal tissue samples from spontaneous abortions and normal term placental tissues, demonstrated a strong association between intronic SNPs rs2234693 and rs9340799 and the decreased risk of spontaneous abortion [11]. Idiopathic female infertility has been linked to *ESR1* SNPs rs9340799 and rs2234693 [12]. Other polymorphisms of *ESR1* have been found to impact the pregnancy rate, IVF outcome, and fertility rate in different populations [5,13–15]. Our data support and extend these findings, and we speculate that the links are causal in that the SNPs lead directly to loss-of-function of the oestrogen receptor and so infertility.

This work represents an advance in biomedical science because it reports the significant association of the *ESR1* SNPs Arg157Ter and Val364Glu with female infertility.

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