Status of Estrogen Receptor Beta Gene Polymorphisms rs1256049 and rs4986938 in a cohort of South Indian women with Primary ovarian insufficiency

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ABSTRACT

The current study is aimed to investigate the role of estrogen receptor beta gene polymorphisms for causation of Primary Ovarian insufficiency (POI) in south Indian women. Primary Ovarian insufficiency (POI) is a menstrual disorder presenting with Primary and secondary amenorrhea, decreased estradiol levels and increased gonadotropin levels. Most cases of POI remain unresolved even after exhaustive investigations and is a significant cause of infertility in women in the reproductive age. In this study a total of 276 subjects, of which 138 POI patients and 138 controls were analysed for biochemical profiles of FSH, LH and Estradiol. Genotypic frequencies were calculated for different models of the SNP rs1256049 and rs4986938 in the Estrogen receptor Beta gene by PCR-RFLP (Restriction length polymorphism) analysis. Statistically, significant relation is found for FSH, LH and Estradiol profiles of a different group of Premature ovarian insufficiency and controls (p<0.0001). Genotypic frequencies between patients and controls were compared, and strength of the interdependence of genotypes and POI is confirmed by the odds ratio and 95% confidence intervals. Incidence of 1082 G>A (rs1256049) polymorphisms and 1730 G>A (rs4986938) were not significantly different in both patient and control group (P=0.21 and P=0.4). The presence of 1082 G>A (rs1256049) polymorphisms and 1730 G>A (rs4986938) is not associated with causation of Premature ovarian insufficiency in south Indian women.

INTRODUCTION

POI could be a common functional disorder of the ovaries within which accelerated loss of functional follicle occurs. The condition is characterised by amenorrhea and oligoamennorhoea with raised gonadotrophin levels (FSH >25 IU/L) and attenuated ovarian production of estrogen (estradiol <50 pmol/L) and progesterone. (Casson, 2000; Vujovic et al., 2010). POI is the leading cause of infertility in actively reproducing group as loss of function of the ovary occurs before the age of 40 years. (de Moraes-Ruehsen and Jones, 1967)

Depending on biochemical parameters and menstrual irregularities, three different forms of POI are categorised. Patients with regular menstrual cycles and normal biochemical profiles are considered occult. The second category that is biochemical POI is a disorder with a range of rising FSH
Estrogen is a gonadal steroid hormone which is produced by the developing follicle. Estrogen imparts a crucial role in the proper functioning of the reproductive tract, cardiovascular system, progesterone induction, endometrial proliferation and maintenance of bone mass, fetoplacental function and maturation. Estrogen appears to enact a pivotal role in the development of the primordial follicle and ovulation. Estrogen imparts both positive and negative feedback regulation of the production of FSH. During the follicular phase developing follicle produces more amounts of estrogen which inhibits FSH secretion. This inhibition is a negative feedback regulation which prevents the growth of resting pool of primordial follicles restricting the loss of follicular pool, contrarily during the ovulatory phase estrogen stimulates the anterior pituitary for an outsized surge of luteinising hormone and a lesser surge of FSH (Stocco, 2001; Sharma et al., 1999) which stimulates the growth of follicles for the next menstrual cycle.

Progression of various diseases is additionally associated with estrogen including breast, colorectal, endometrial, prostate, ovarian cancers, insulin resistance, cardiovascular diseases, lupus erythematosus, endometriosis, obesity and neurodegenerative disorders (Beck-Peccoz and Persani, 2006; Cramer, 1990; van der Schouw et al., 1996). The biological effect of estrogens is mediated through the activation of its receptors ER-α and ER-β, belonging to a nuclear receptor family and functioning as ligand-dependent transcription factors (Enmark and Gustafsson, 1999). In 1996 ESR2 was first identified in the rat prostate and ovary (Kuiper et al., 1996). In the ESR2, two typical SNPs were selected to be analysed, rs1256049 (G1082A) and (G1730A) a A1730G the ligand-binding domain in exon five and an untranslated 3′-area in exon eight respectively were selected.

MATERIALS AND METHODS

Patients and controls

The study group includes cases diagnosed with POI recruited from different hospitals in Hyderabad, and Institute of Genetics and hospital for Genetic disorders (n=138) and their age and sex-matched controls (n=138). All patients were selected based on at least four months of amenorrhea and two FSH serum measurements > 25 IU/L before 40 years of age. Blood samples were collected only after the informed consent proforma accepted by the institutes’ ethical committee was duly signed by the subjects.

Both from patients and controls 5 ml of Peripheral blood was collected and stored in vacutainers coated with EDTA as an anticoagulant. Using the phenol-chloroform method according to changes in Blin & Stafford (1976) genomic DNA was extracted from peripheral blood lymphocytes. E2, FSH, luteinising hormone (LH), were quantitatively estimated in human serum samples using enzyme-linked immunosorbent assay (ELISA) kits. All values are presented as means ± standard deviation. Deviations were considered significant at p < 0.05

Genotyping of rs1256049 and rs4 986938

Using isolated DNA as a template, the nucleotide changes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. Primers for PCR amplification of each polymorphism were as follows. Using the following primers: A,5′-TCTTGCTTTCCCCAGGCTTT-3′; B,5′-ACCTGTCCAGAACAAGATCT-3′; C,5′-TCTTGCTTTCCCCATAGTAACA-3′; and D,5′-AATGAGGACCACACAG-3′. rs1256049 (G1082A) and (G1730A) rs4986938 of the ER β gene were amplified, respectively for 40 cycles at 55°C. PCR Amplification of exon 5 yielded 156bp, and exon 8 yielded the 307 bp amplicons. Exon5 amplicon when digested with RsaI produced one band of 156 bp in the normal ERb sequence (GG); three separate bands of 156, 125, and 31 for GA and two separate bands of 125 and 31 bp for AA genotype. Digestion of exon8 amplicon by AluI produces one band of 307 bp in the normal ERβ sequence (GG), three separate bands of 307, 240, and 67 bp, for GA genotype, and 240 and 67 bp, for (AA) genotype.

Statistical analysis

Allele and genotype frequencies between groups are compared with the chi-square test to estimate the Hardy-Weinberg equilibrium. Using SNP STAT software. Significance of statistical test and χ2 analysis were carried out. P-values were two-tailed, and 95% confidence intervals (CIs) were calculated. Statistical significance is considered with a p-value < 0.05. To identify the association of individual polymorphisms haplotype inference were performed with Gabriel’s rule applied in Haploviev software (version 4.1; Broad Institute of MIT and Harvard University, Boston, MA).
### Table 1: Mean of Control and Groups

|          | FSH       |            | LH        |            | Estradiol |            |
|----------|-----------|------------|-----------|------------|-----------|------------|
|          | Mean      | Std error  | Mean      | Std error  | Mean      | Std error  |
| Control  | 5.6       | 0.16       | 7.22      | 0.58       | 136.97    | 5.23       |
| Group 1  | 65.35     | 5.26       | 34.97     | 3.96       | 18.2      | 1.82       |
| Group 2  | 5.49      | 0.3        | 6.68      | 0.91       | 51.51     | 5.22       |
| Group 3  | 38.17     | 6.75       | 26.06     | 4.25       | 15.87     | 1.29       |

### Table 2: The paired t-test for FSH, LH and Estradiol

#### Follicle Stimulating Hormone (FSH)

|          | Group 1 FSH vs control | Group 2 FSH vs control | Group 3 FSH vs control |
|----------|------------------------|------------------------|------------------------|
| P value  | <0.0001                | 0.8593                 | <0.0001                |
| P value summary | ****       | ns                     | ****                   |
| Significantly different (P < 0.05) | Yes | No                     | Yes                    |
| One or two-tailed P value | Two-tailed | Two-tailed            | Two-tailed             |
| t, df    | t=10.48, df=41         | t=0.1783, df=41        | t=4.517, df=41         |
| Number of pairs | 42 | 42                     | 42                     |

#### Luteinizing Hormone (LH)

|          | Group 1 LH vs control LH | Group 2 LH vs control LH | Group 3 LH vs control LH |
|----------|--------------------------|--------------------------|--------------------------|
| P value  | <0.0001                  | 0.5834                   | <0.0001                  |
| P value summary | ****       | ns                     | ****                   |
| Significantly different (P < 0.05) | Yes | No                     | Yes                    |
| One or two-tailed P value | Two-tailed | Two-tailed            | Two-tailed             |
| t, df    | t=6.959, df=41           | t=0.5527, df=41         | t=4.351, df=41         |
| Number of pairs | 42 | 42                     | 42                     |

#### Estradiol

|          | Group 1 Estradiol vs control Estradiol | Group 2 Estradiol vs control Estradiol | Group 3 Estradiol vs control Estradiol |
|----------|----------------------------------------|----------------------------------------|----------------------------------------|
| P-value  | <0.0001                                | <0.0001                                | <0.0001                                |
| P value summary | ****       | ****                                 | ****                   |
| Significantly different (P < 0.05) | Yes | Yes                                  | Yes                    |
| One or two-tailed P value | Two-tailed | Two-tailed            | Two-tailed             |
| t, df    | t=21.95, df=41                        | t=11.65, df=41                       | t=23.68, df=41           |
| Number of pairs | 42 | 42                     | 42                     |

*Difference between controls and the group with elevated FSH levels, and LH and estradiol was statistically significant (p > 0.001)
### Table 3: Un Paired t-test for FSH, LH and Estradiol

| Hormone                  | Group 1 vs control | Group 2 vs control | Group 3 vs control |
|--------------------------|--------------------|--------------------|--------------------|
| **Follicle Stimulating Hormone (FSH)** |                    |                    |                    |
| P-value                  | <0.0001            | 0.8557             | <0.0001            |
| P value summary          | ****               | ns                 | ****              |
| Significantly different (P, 0.05) | Yes                | No                 | Yes               |
| One or two-tailed P value | Two-tailed         | Two-tailed         | Two-tailed         |
| t, df                    | t=10.55, df=41.21  | t=0.1825, df=81.97 | t=4.497, df=41.12  |
| **Luteinizing Hormone (LH)** |                    |                    |                    |
| P-value                  | <0.0001            | 0.6183             | <0.0001            |
| P value summary          | ****               | ns                 | ****              |
| Significantly different (P, 0.05) | Yes                | No                 | Yes               |
| One or two-tailed P value | Two-tailed         | Two-tailed         | Two-tailed         |
| t, df                    | t=6.928, df=42.80  | t=0.5006, df=69.98 | t=4.389, df=42.56  |
| **Estradiol**            |                    |                    |                    |
| P-value                  | <0.0001            | <0.0001            | <0.0001            |
| P value summary          | ****               | ****              | ****              |
| Significantly different (P, 0.05) | Yes                | Yes               | Yes               |
| One or two-tailed P value | Two-tailed         | Two-tailed         | Two-tailed         |
| t, df                    | t=21.44, df=50.81  | t=11.56, df=82     | t=22.48, df=46    |

*Difference between controls and the group with elevated FSH levels, and LH and estradiol was statistically significant (p > 0.001)*

### Table 4: Correlation matrix of hormones with different groups of POI

| Hormone                  | Control | Group 1 | Group 2 | Group 3 |
|--------------------------|---------|---------|---------|---------|
| **Follicle Stimulating Hormone (FSH)** |         |         |         |         |
| Control                  | 1       | -       | -       | -       |
| Group 1                  | -0.6528 | 1       | -       | -       |
| Group 2                  | -0.827  | 0.2381  | 1       | -       |
| Group 3                  | -0.3293 | 0.1784  | 0.0724  | 1       |
| **Luteinizing Hormone (LH)** |         |         |         |         |
| Control                  | 1       | -       | -       | -       |
| Group 1                  | 0.6256  | 1       | -       | -       |
| Group 2                  | -0.5983 | 0.1559  | 1       | -       |
| Group 3                  | -0.3424 | -0.1337 | 0.0182  | 1       |
| **Estradiol**            |         |         |         |         |
| Control                  | 1       | -       | -       | -       |
| Group 1                  | 0.4667  | 1       | -       | -       |
| Group 2                  | -0.4918 | -0.06426| 1       | -       |
| Group 3                  | -0.632  | -0.02598| 0.0247  | 1       |

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Table 5: Genotypic and allelic distribution of 1082 G>A (rs1256049) and 1730 G>A (rs4986938)

| Genotypes and alleles (rs1256049) | Control group | Patients group | OR (95% CI) | p-value |
|-----------------------------------|---------------|---------------|-------------|---------|
| **Co-dominant**                   |               |               |             |         |
| G/G                               | 131 (94.9%)   | 135 (97.1%)   | 1           | -       |
| G/A                               | 7 (5.1%)      | 3 (2.2%)      | 0.42 (0.11-1.64) | 0.21 |
| A/A                               | 0 (0%)        | 1 (0.7%)      | NA (0.00-NA) | -       |
| **Dominant**                      |               |               |             |         |
| G/G                               | 131 (94.9%)   | 135 (97.1%)   | 1           | 0.35    |
| G/A-A/A/A                        | 7 (5.1%)      | 4 (2.9%)      | 0.55 (0.16-1.94) | -     |
| **Recessive**                     |               |               |             |         |
| G/G-G/A/A                        | 138 (100%)    | 138 (99.3%)   | 1           | 0.24    |
| A/A                               | 0 (0%)        | 1 (0.7%)      | NA (0.00-NA) | -       |
| **Over Dominant**                |               |               |             |         |
| G/G-A/A/A                        | 131 (94.9%)   | 136 (97.8%)   | 1           | 0.19    |
| G/A                               | 7 (5.1%)      | 3 (2.2%)      | 0.41 (0.10-1.63) | -     |
| **Alleles**                       |               |               |             |         |
| G                                 | 0.97          | 0.98          | -           | -       |
| A                                 | 0.03          | 0.02          | -           | -       |

(rs4986938)

Table 6: Combined genotypic frequencies of 1082 G>A (rs1256049) and 1730 G>A (rs4986938)

| Genotype                  | Control | Case     | OR (95% CI)     | P values |
|---------------------------|---------|----------|-----------------|----------|
| GG-GG                     | 126     | 126      | 1 (Ref)         | -        |
| GG-GA                     | 5       | 8        | 1.60 (0.53-4.81)| 0.57     |
| GG-AA                     | 0       | 0        | -               | -        |
| GA-GG                     | 7       | 3        | 0.43 (0.12-1.58)| 0.33     |
| GA-GA                     | 0       | 0        | -               | -        |
| GA-AA                     | 0       | 0        | -               | -        |
| AA-GG                     | 0       | 1        | -               | -        |
| AA-GA                     | 0       | 0        | -               | -        |
| AA-AA                     | 0       | 0        | -               | -        |

Table 7: Haplotypic frequencies of 1082 G>A (rs1256049) and 1730 G>A (rs4986938)

| Haplotype association with response (n=277) |
|--------------------------------------------|
| (rs1256049)                                | (rs4986938) | Freq | OR (95% CI)     | P-value |
| 1                                         | G          | G    | 0.9549          | 1        | -     |
| 2                                         | G          | A    | 0.0235          | 1.60 (0.51 - 5.03) | 0.42 |
| 3                                         | A          | G    | 0.0217          | 0.75 (0.25 - 2.23) | 0.61 |

Global haplotype association p-value: 0.61
**RESULTS**

**Demographic analysis**

Total samples were categorised into three groups for biochemical analysis of FSH, LH and Estrogen hormones. Group 1 for premature ovarian failure cases, group 2 secondary amenorrhea, and group 3 for primary amenorrhea.

Group 1-POF, Group2-Secondary amenorrhea, Group3-Primary amenorrhea.

The calculated average FSH value for control is 5.6 ± 0.16 mIU/ml whereas for group 1, group 2 and group 3 were 65.35 ± 5.26 mIU/ml, 5.49 ± 0.30 mIU/ml and 38.17 ± 6.75 mIU/ml respectively.

**LH**

The calculated average LH value for control is 7.22 ± 0.58 mIU/ml whereas it is 34.97 ± 3.96 mIU/ml, 6.68 ± 0.91 mIU/ml and 26.06 ± 4.25 mIU/ml for group 1, group 2 and group 3 respectively.

**Estradiol**

The calculated average estradiol value for group 1, group 2 and group 3 are 18.20 ± 1.82 pg/mL, 51.51 ± 5.22 pg/mL and 15.87 ± 1.29 pg/mL respectively whereas for control is 136.97 ± 5.23 pg/mL.

**Analysing the Correlation matrix**

Correlation matrix of controls and different groups correlation coefficients between FSH, LH and Estradiol.

For 1082 G>A (rs1256049) polymorphism all the models including the codominant, G/A (OR, 0.42; 95% CI 0.11-1.64, p=0.21); GA vs AA (OR, 0.55; 95% CI 0.16-1.94, P=0.35; for 1730 G>A (rs4986938) G/A (OR 1.62; 95% CI (0.52-5.09), P=0.4) showed insignificant association with the pathogenesis of POI. 1082 G>A (rs1256049) polymorphism and 1730 G>A (rs4986938) combined genotypes does not show any significant association with the pathogenesis of POI.

Haplotype analysis was performed by combining two polymorphisms rs1256049 and rs4986938. No significant association is observed with the combination of haplotypes GA (P=0.42, OR 1.60, CI (0.51-5.03) and AG haplotype (P=0.61, OR 0.75, CI (0.25-2.23) Table 7.

The genotypic distributions of estrogen receptor beta1082 G>A (rs1256049) and 1730 G>A rs4986938 polymorphisms were in agreement with Hardy-Weinberg equilibrium. The genotypic frequencies of GG, GA and AA in women with premature ovarian insufficiency of the estrogen receptor beta gene 1082 G>A (rs1256049) polymorphisms were 135(97.1%), 3 (2.2%) and 1 (0.7%) and in controls 131(94.9%), 7(5.1%) and 0(0.0%), showing no significant difference between the patient and the control group (P=0.21) illustrated in Table 5. Significant association is not observed with combination genotype models of dominant P=0.35(OR 0.55, CI (0.16-1.94)), recessive P=0.24 and Over Dominant P=0.19(OR 0.41, CI (0.10-1.63)), as illustrated in Table 6. The incidence of 1082 G>A (rs1256049) polymorphisms and rs4986938 (G1730A) are not significantly different in both patient and control group (P=0.21 and P=0.4) respectively. The Alu A A genotype for rs4986938 (G1730A) polymorphisms was not found in either normal women or women with premature ovarian insufficiency. The GG genotypic frequency 133 (96.4%), 131 (94.2%) in controls and cases and GA genotypic frequency 5(3.6%) and 8(5.8%) in cases and controls respectively without any significant association (p=0.4).

**DISCUSSION**

Estrogens put their biological effects into action by activating their receptors ER-α and ER-β. ERβ gene (ESR2) spans of eight exons localised to chromosome 14q23–24.1. (Enmark et al., 1997) and is the main receptor in ovarian tissue. ERα and ERβ mRNA expression patterns with RT-PCR of Human fetal ovaries revealed the importance of estrogen and its receptors in ovaries (Revelli et al., 1996; Chiang et al., 2000). Many animal studies in adult hamster (Yang et al., 2002), rat (Palter et al., 2001; Bao et al., 2000), monkey (Pau et al., 1998), reported low levels of ERα and high levels of ERβ expression in the granulosa cells attributing its important role in the development and arrest of the resting primordial follicle pool.

Patients in the POI community were predicted to have substantially higher levels of serum FSH and LH and lower levels of E2 relative to healthy controls. High FSH in females indicates low ovarian reserve, and women with high FSH have significantly lower pregnancy chances than women with normal FSH levels. Exogenous estrogens were hypothesised to function by decreasing serum FSH, restoring follicles sensitivity or directly enhancing granulose cell sensitive to the impact of FSH.

For confirmed diagnosis of premature ovarian failure, FSH levels > 40 IU/l, with an interval of 4-6 weeks and recorded twice, is considered (Rebar et al., 1982). The average mean FSH levels in the patient’s groups 1 (65.35) and group 3 (38.17) are 11 and 7 times higher that of control mean (5.60) whereas the average mean FSH value of group 2 (5.49) is comparable with that of control mean.
value. The LH mean of group 1 and group 2 are determined to be 34.97 and 26.06, which are 4.8 and 3.6 times higher than the normal range in comparison to control mean (7.22). The mean LH value of group 2 is almost equal to that of control mean. Normal development of oocytes is critically dependent upon the pituitary hormones, luteinising hormone (LH) and follicle-stimulating hormone (FSH). Many of ovarian follicles fail to function normally because they become luteinised prematurely due to the associated chronically elevated serum LH levels (Nelson, 1994). The usual range of LH determined in women at reproductive age with the regular menstrual cycle is LH 1–5 IU/l. In POF, defects in LH receptor are typically associated with a serum LH elevation (> 10 IU/L) more pronounced than that of serum FSH (Seeman et al., 1988). The mean estradiol levels in group 1, group 2 and group 3 are 18.20, 51.51 and 15.87 respectively. These levels are 7.5, 2.6 and 8.6 times less than that of control mean value (136.97) (Table 1). Examination of FSH levels in women with hypogonadotropic hypogonadism in the absence of follicle development, during follicle development, and ovulatory cycles, evidenced that estradiol, inhibin A, and inhibin B play a role in the dynamic negative feedback control of FSH secretion across the normal menstrual cycle (Welt et al., 2003). The absence of estradiol suggests aromatase defects (Bulun, 1996) or LH receptor abnormalities (Latronico and Segaloff, 1999); however, these congenital defects are associated with primary amenorrhea and are not acquired defects as in the hypogonadotropic woman studied. Plasma levels of E2, inhibin B, and AMH are of limited value in predicting the presence of an ovarian reserve in patients with POF (Bachelot et al., 2009).

Paired t-test for FSH and LH between control & group 1 and control & group 3 shows that the difference between the control mean and group mean significant whereas in case of control and group 2 there is no significant difference between the control mean and group mean. The paired test for estradiol exhibits a significant difference between the means of control and the three groups, p=0.0001(Table 2).

Welch’s test when performed for FSH and LH, the calculated p-value is much higher between control & group 1 and control & group 3, indicating the difference between the control mean, and group means are significant. Whereas in case of control and group 2 for FSH and LH, there is an insignificant difference between the means of control and groups. In the case of estradiol, Welch’s Test showed higher p-value indicating a significant difference between the means of control and groups (Table 3).

Correlation matrix of FSH indicates a moderate negative degree of correlation between the Control and Group 1, negative reasonably high degree of correlation between control and group 2, and low negative degree of correlation between control and group 3. Further, a positive, meagre degree of correlation between group 1 and group 3. In the case of LH, there is a low negative degree to moderate negative degree of correlation between control and group 1, 2 and 3. In the case of estradiol, a meagre degree of negative correlation between control and groups (1, 2, 3). It indicates that the secretion of estradiol has been decreased in group 1, group 2 and group 3 in comparison to control Table 4.

In (Krege et al., 1998) developed female ERβ knockout mice to study the role of estrogen beta in follicular development. The follicular growth retardation of these mice confirmed its essential role in normal ovulation, but not in lactation, sexual differentiation and fertility. Tsukamoto et al. first identified a silent G1082A transition in exon 5 of the ERβ gene and association were reported by Arko et al. in a small cohort of postmenopausal Slovenian women with Rsol polymorphism (Arko et al., 2002). The same kind of association was reported in Italian postmenopausal women with the G1082A single nucleotide polymorphism on BMD and vertebral fractures (Becherini et al., 2001). (Sundarajan, 2001) reported a positive association with ovulatory dysfunction including primary amenorrhea, secondary amenorrhea, POF and PCOS for the G1730A polymorphism in exon 8 of the ERβ gene.

Increased risk of breast cancer in postmenopausal women not in premenopausal women has been associated with polymorphisms of ESR2 (Zheng et al., 2003). Bianco and cols (Bianco et al., 2009) the effect of +1730 G/A polymorphism in ERβ gene (rs4986938) was investigated in infertile women with endometriosis and control and the risk of endometriosis, regardless the stage of the disease was increased. A positive relationship was established first by Aschim et al. (2005) between genetic variants of the ERβ gene and male infertility, showing that the frequency of Rs11 AG genotype in infertile men has been increased compared with the reference group. In the present study, genotype distributions of single SNPs1082 G-A (rs1256049) polymorphisms and rs4986938 (G1730A) are not significantly differing in the patients and control group. Even though Rs11 and AluI polymorphisms in the ERβ gene are silent mutations, they might confer susceptibility to a diseased condition when they are in linkage disequilibrium with other gene polymorphisms. (Noble et al., 1996)
CONCLUSIONS

Estrogen exerts beneficial effects on the development and regulation of follicular development. An early loss of ovarian function puts a woman at higher risk for cardiovascular diseases, osteoporosis, and ovarian cancer, increases the risk of mortality as estrogen receptor-mediated signalling pathway plays an essential role. In the present study, it was observed that polymorphisms of estrogen receptors ER-β were not significantly associated with primary ovarian insufficiency. Our finding is in discordance with previous studies that were conducted to explore the association between SNPs of ER-β gene and POI in Singapore population, where it was found that these SNP’s were significantly associated with POI. While the individual contribution of these polymorphisms to POI pathogenesis need to be carried out in a large number of association studies, remains to be verified and confirmed.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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REFERENCES

Arko, B., Preželj, J., Komel, R., Kocijančič, A., Marc, J. 2002. No major effect of estrogen receptor beta gene Rsal polymorphism on bone mineral density and response to alendronate therapy in postmenopausal osteoporosis. The Journal of Steroid Biochemistry and Molecular Biology, 81(2):147–152.

Aschim, E. L., Giwercman, A., Ståhl, O., Eberhard, J., Cwikiel, M., Nordenskjöld, A., Giwercman 2005. The Rsa I Polymorphism in the Estrogen Receptor-β Gene Is Associated with Male Infertility. The Journal of Clinical Endocrinology & Metabolism, 90(9):5343–5348.

Bachelot, A., et al. 2009. Phenotyping and genetic studies of 357 consecutive patients presenting with premature ovarian failure. European Journal of Endocrinology, 161(1):179–187.

Bao, B., Kumar, N., Karp, R. M., Garverick, H. A., Sundaram, K. 2000. Estrogen Receptor-β Expression in Relation to the Expression of Luteinizing Hormone Receptor and Cytochrome P450 Enzymes in Rat Ovarian Follicles1. Biology of Reproduction, 63(6):1747–1755.

Becherini, L., Semprini, S., Mango, R. 2001. Estrogen receptor beta gene polymorphism and osteoporotic risk in aged males and females from Italy. Calcif. Tissue Int, 67:496–496.

Beck-Peccoz, P., Persani, L. 2006. Premature ovarian failure. Orphanet Journal of Rare Diseases, 1(1):9.

Bianco, B., Christofolini, D. M., Mafra, F. A., Brandes, A., Zulli, K., Barbosa, C. P. 2009. +1730 G/A polymorphism of the estrogen receptor β gene (ERβ) may be an important genetic factor predisposing to endometriosis. Acta Obstetrica et Gynaecologica Scandinavica, 88(12):1397–1401.

Bulun, S. E. 1996. Clinical review 78: Aromatase deficiency in women and men: would you have predicted the phenotypes. Journal of Clinical Endocrinology & Metabolism, 81(3):867–871.

Casson, P. R. 2000. Dehydroepiandrosterone supplementation augments ovarian stimulation in poor responders: a case series. Human Reproduction, 15(10):2129–2132.

Chiang, C. H., Cheng, K. W., Igarashi, S., Nathwani, P. S., Leung, P. C. K. 2000. Hormonal Regulation of Estrogen Receptor α and β Gene Expression in Human Granulosa-Luteal Cells in Vitro1. The Journal of Clinical Endocrinology & Metabolism, 85(10):3828–3839.

Cramer, D. W. 1990. Epidemiologic Aspects of Early Menopause and Ovarian Cancer. Annals of the New York Academy of Sciences, 592(1):363–375.

de Moraes-Ruehsen, M., Jones, G. S. 1967. Premature Ovarian Failure. Fertility and Sterility, 18(4):440–461.

Enmark, E., Gustafsson, J. A. 1999. Oestrogen receptors - an overview. Journal of Internal Medicine, 246(2):133–138.

Enmark, E., Pelto-Huikko, M., Grandien, K., Lagercrantz, S., Lagercrantz, J., Fried, G., Nordenskjöld, M., Åke Gustafsson, J. 1997. Human Estrogen Receptor β-Gene Structure, Chromosomal Localization, and Expression Pattern1. The Journal of Clinical Endocrinology & Metabolism, 82(12):4258–4265.

Krege, J. H., Hodgin, J. B., Couse, J. F., Enmark, E., Warner, M., Mahler, J. F., Sar, M., Korach, K. S., Gustafsson, J. A., Smithies, O. 1998. Generation and reproductive phenotypes of mice lacking estrogen receptor. Proceedings of the National Academy of Sciences, 95(26):15677–15682.

Kuiper, G. G., Enmark, E., Pelto-Huikko, M., Nilsson, S., Gustafsson, J. A. 1996. Cloning of a novel receptor expressed in rat prostate and ovary. Proceedings of the National Academy of Sciences, 93(12):5925–5930.

Latronico, A. C., Segallo, D. L. 1999. Naturally Occur-
ring Mutations of the Luteinizing-Hormone Receptor: Lessons Learned about Reproductive Physiology and G Protein–Coupled Receptors. The American Journal of Human Genetics, 65(4):949–958.

Nelson, L. M. 1994. Development of luteinized graafian follicles in patients with karyotypically normal spontaneous premature ovarian failure. Journal of Clinical Endocrinology & Metabolism, 79(5):1470–1475.

Noble, L. S., Simpson, E. R., Johns, A., Bulun, S. E. 1996. Aromatase expression in endometriosis. The Journal of Clinical Endocrinology & Metabolism, 81(1):174–179.

Palter, S. F., Tavares, A. B., Hourvitz, A., Veldhuis, J. D., Adashi, E. Y. 2001. Are Estrogens of Import to Primate/Human Ovarian Folliculogenesis. Endocrine Reviews, 22(3):389–424.

Pau, C. Y., Pau, K. Y. F., Spies, H. G. 1998. Putative estrogen receptor \( \beta \) and \( \alpha \) mRNA expression in male and female rhesus macaques. Molecular and Cellular Endocrinology, 146(1-2):59–68.

Rebar, R. W., Erickson, G. F., Yen, S. S. C. 1982. Idiopathic premature ovarian failure: clinical and endocrine characteristics Supported by NIH Center grant HD-12303 and NIH grant HD-15162. The University of California, San Diego, General Clinical Research Center is supported by NIH grant RR-00827. Fertility and Sterility, 37(1):35–41.

Revelli, A., Paahioni, D., Cassoni, P., Bussolati, G., Massobrio, M. 1996. In situhybridization study of messenger RNA for estrogen receptor and immunohistochemical detection of estrogen and progesterone receptors in the human ovary. Gynecological Endocrinology, 10(3):177–186.

Seeman, E., Cooper, M. E., Hopper, J. L., Parkinson, E., McKay, J., Jerums, G. 1988. Effect of early menopause on bone mass in normal women and patients with osteoporosis. The American Journal of Medicine, 85(2):213–216.

Sharma, S. C., et al. 1999. Expression and Function of Estrogen Receptor Subtypes in Granulosa Cells: Regulation by Estradiol and Forskolin1. Endocrinology, 140(9):4320–4334.

Stocco, D. M. 2001. Star Protein and the Regulation of Steroid Hormone Biosynthesis. Annual Review of Physiology, 63(1):193–213.

Sundarajanan, C. 2001. Association between Estrogen Receptor- Gene Polymorphisms and Ovulatory Dysfunctions in Patients with Menstrual Disorders. Journal of Clinical Endocrinology & Metabolism, 86(1):135–139.

Tartagni, M., Cicinelli, E., Pergola, G. D., Salvia, M. A. D., Lavopa, C., Loverro, G. 2007. Effects of pre-treatment with estrogens on ovarian stimulation with gonadotropins in women with premature ovarian failure: a randomized, placebo-controlled trial. Fertility and Sterility, 87(4):858–861.

van der Schouw, Y. T., van der Graaf, Y., Steyerberg, E. W., Eijkemans, M. J. C., Banga, J. D. 1996. Age at menopause as a risk factor for cardiovascular mortality. The Lancet, 347(9003):714–718.

Vujovic, S., Brincat, M., Erel, T., Gambacciani, M., Lambrinoudaki, I., Moen, M. H., Schenck-Gustafsson, K., Tremollieres, F., Rozenberg, S., Rees, M. 2010. EMAS position statement: Managing women with premature ovarian failure. Maturitas, 67(1):91–93.

Welt, C. K. 2008. Primary ovarian insufficiency: a more accurate term for premature ovarian failure. Clinical Endocrinology, 68(4):499–509.

Welt, C. K., Pagan, Y. L., Smith, P. C., Rado, K. B., Hall, J. E. 2003. Control of Follicle-Stimulating Hormone by Estradiol and the Inhibins: Critical Role of Estradiol at the Hypothalamus during the Luteal-Follicular Transition. The Journal of Clinical Endocrinology & Metabolism, 88(4):1766–1771.

Yang, P., Kriatchko, A., Roy, S. K. 2002. Expression of ER-\( \alpha \) and ER-\( \beta \) in the Hamster Ovary: Differential Regulation by Gonadotropins and Ovarian Steroid Hormones. Endocrinology, 143(6):2385–2398.

Zheng, S. L., Zheng, W., Chang, B. L., Shu, X. O., Cai, Q., Yu, H., Gao 2003. The joint effect of estrogen receptor \( \beta \) sequence variants and endogenous estrogen exposure on breast cancer risk in Chinese women. Cancer Research, 63(22):7624–7629.