Reproductive genetics

Inhibin \( \alpha \) gene promoter polymorphisms in Korean women with idiopathic premature ovarian failure

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BACKGROUND: It has been suggested that variations in the inhibin \( \alpha \) gene (\( \text{INHA} \)) may affect the ovarian function of women. This study was performed to investigate whether the genetic polymorphisms of the \( \text{INHA} \) gene are associated with idiopathic premature ovarian failure (POF) in a Korean population.

METHODS: The subjects consisted of 159 idiopathic POF patients and 233 post-menopausal controls. Genotyping for the –16C>T polymorphism was performed by a minor groove binder (MGB) primer/probe Taqman assay, and the –124A>G polymorphism was identified using PCR restriction fragment length polymorphism analysis. Haplotypes were deduced by using the Haploview version 4.1.

RESULTS: There were no significant differences in the genotype distributions or allele frequencies of the \( \text{INHA} \) gene –16C>T and –124A>G polymorphisms between the POF and the control group. Haplotype analysis also showed no significant difference between groups.

CONCLUSIONS: The distribution of the \( \text{INHA} \) gene promoter polymorphisms in a Korean POF population was not significantly different from controls, implying that the \( \text{INHA} \) gene polymorphisms may not be associated with the risk of idiopathic POF.

Key words: inhibin \( \alpha \) / single-nucleotide polymorphisms / premature ovarian failure

Introduction

Premature ovarian failure (POF) is a complex disorder defined as a cessation of ovarian function before or at the age of 40, which causes hypergonadotropic amenorrhea and a hypo-estrogenic condition (Anasti, 1998; Goswami and Conway, 2005). POF occurs approximately in 1% of women of reproductive age (Coulam et al., 1986). Although the etiology of POF is obscure in a large proportion of cases, a number of studies have suggested that underlying genetic aberrations may play important roles in the etiology and pathogenesis of ovarian insufficiency (Therman et al., 1990; Davis et al., 2000; Yoon et al., 2010).

Considering that the rate of follicular depletion is associated with the age of menopause, genetic variants in FSH-regulating hormones such as inhibin could be potential candidates for POF pathogenesis (Shelling et al., 2000; Chand et al., 2010). The main function of inhibin is the negative regulation of pituitary secretion of FSH, which has a key role in the recruitment and development of ovarian follicles. It has been reported that an increase in FSH secretion coincides with an increased rate of follicular depletion during the menopausal transition (Richardson et al., 1987), and also with a defect of inhibin secretion in POF patients (Pampfer and Thomas, 1989; Halvorson and Decherney, 1996). Considering these reports, it could be postulated that variation in the inhibin gene could lead to an impaired bioactivity...
or decreased level of inhibin, which might also affect the action of FSH and the reproductive efficiency of women, namely premature depletion of ovarian follicles, and may serve as a susceptibility factor for POF.

Two forms of inhibin exist in human tissue: inhibin A (α-βA) and inhibin B (α-βB). The inhibin subtypes are encoded by inhibin α gene (INHA) on chromosome 2q33-34, INHβA on 2cen-q13 and INHβB on 7p15-p14, respectively (Barton et al., 1989). There were genetic studies that showed the INHα gene missense mutation (INHA G769A) was associated with the development of POF (Shelling et al., 2000; Marozzi et al., 2002; Dixit et al., 2004). However, this association was not replicated in Argentine women with POF (Sundblad et al., 2006) and, moreover, the G769A mutation was not found in Korean women with POF (Lee et al., 2006).

Recently, it has been reported that the two promoter polymorphisms of the INHA gene, which are located at the −16C>T (rs35118453) and −124A>G (rs11893842) restriction sites, are associated with POF in New Zealand and Slovenian population (Harris et al., 2005; Woad et al., 2009). They showed significant reductions in allele frequency for the −16T and −124G in POF patients. However, controversial results have also been reported that the distribution of the INHA gene promoter polymorphisms was not significantly different from controls in a cohort from Argentina (Sundblad et al., 2006) and, in Italian and German populations (Corre et al., 2009). We therefore investigated whether polymorphisms in the promoter region of the INHA gene are associated with idiopathic POF in our Korean population. For this, we analyzed the frequency of the −16C>T and −124A>G variants in patients with idiopathic POF in controls.

Materials and Methods

Subjects

We enrolled 159 idiopathic POF patients composed of 20 cases affected with primary amenorrhea (12.6%) and 139 cases with secondary amenorrhea (87.4%), who were recruited from several university hospitals in Korea between 1999 and 2010. Subjects underwent complete POF workup and gynecological examination in their centers as previously described (Yoon et al., 2010). All POF patients included in this study had normal karyotype as determined by conventional GTG banding. A total of 233 women who underwent menopause over age 45 served as controls. Recruiting controls from this age group has the merit of maximizing the probability that these subjects were not affected by POF. The review board for human research of Seoul National University Hospital approved this project, and informed consent was obtained from all subjects.

Genotyping of −16C>T polymorphism

Genomic DNA was isolated and extracted from peripheral blood leukocytes by the Wizard DNA purification kit (Promega, Madison, WI, USA). The promoter −16C>T polymorphism in the INHA gene was genotyped by an MGB primer/probe Taqman assay on the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The following primers and probes were used: −16C>T forward primer, 5′-GG CAGACCTGCGCAAGA-3′, −16C>T reverse primer, 5′-GCAGCA AGAAAGACGACTGTT-3′, −16C>T reporter ‘C′ 5′-VIC-CTGCCC CTGCTAGTG-NFQ-3′, −16C>T reporter ‘T′ 5′-(FAM)-CTGCC CTACTAGTG-NFQ-3′. The PCR mixture consisted of 10 µl of TaqMan Universal PCR Master Mix 2× (Applied Biosystems) and 25 ng DNA. The PCR cycling conditions consisted of one 2-min cycle at 50°C, and one 10-min cycle at 95°C, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. We used distilled water as a negative PCR control in each amplification.

Genotyping of −124A>G polymorphism

Analysis of the −124A>G polymorphism was carried out using a restriction fragment length polymorphism assay. The PCR mixture had a total volume of 25 µl and contained 0.025 µg of genomic DNA, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 200 µM dNTPs, 1 U Taq polymerase (Takara, Shiga, Japan) and 0.4 µM of each upstream and downstream primer. The oligonucleotide primers used in PCR were as follows: forward 5′-AGGTGCGTTAGGCGCAAATCCTTCC-3′ and reverse 5′-TCCACACCCACCCCTTCACCTACCTTGTA-3′. The PCR cycling conditions were as follows: an initial denaturation step at 94°C for 5 min, amplification for 35 rounds of PCR at 94°C for 30 s, 55°C for 30 s and 72°C for 30 s followed by a final extension time of 5 min at 72°C. PCR products were digested with 2 U of restriction enzyme MboI (New England Biolabs, USA) at 37°C for 3 h and separated by 3% agarose gel electrophoresis, and identified by using ethidium bromide staining. The product remained uncut for the −124G allele (196 bp) and cut into two fragments of 164 and 32 bp for the −124A allele.

Statistical analysis

The differences in the genotype distributions, frequencies of alleles and haplotypes were assessed by using the χ² test. All data analyses were performed by using the Statistical Package for the Social Sciences (SPSS) software (version 15.0, SPSS Inc., Chicago, IL, USA). Haplotype frequencies were estimated by utilizing the Haploview version 4.1 (available at http://www.broad.mit.edu/mpg/haploview). To decrease the probability for type I error caused by multiple testing (two single-nucleotide polymorphisms and haplotype), the statistical significance was specified by means of Bonferroni correction and therefore the P < 0.017 was considered significant.

Results

We have investigated the INHA gene polymorphisms from 159 idiopathic POF patients and 233 post-menopausal women. The mean age of onset of ovarian failure was 28.1 ± 8.2 (SD) years and the mean values of FSH, LH and estradiol in the POF group were 73.8 ± 35.9 mIU/ml, 32.9 ± 19.2 mIU/ml and 25.5 ± 24.7 pg/ml, respectively. Genotypes of the two promoter polymorphic loci in the INHA gene were successfully determined in all subjects, and the genotype distributions in both groups were compatible with Hardy–Weinberg equilibrium.

There was no significant difference in distribution of the INHA gene −16C>T polymorphism between the POF and control group (CC/CT/TT rates; 69.2/27.7/3.1% in the POF versus 65.7/29.2/5.1% in the control group, P = 0.569) (Table I). For the −124A>G polymorphism, the genotype distribution in POF patients was not significantly different from that of controls (AA/AG/GG rates; 40.9/47.8/11.3% in the POF versus 33.5/48.5/18.0% in the control group, P = 0.122) (Table II). For further analysis, when the POF patients were divided into the primary and the secondary POF group, neither group was significantly different compared with controls.

Haplotype analysis for the INHA gene −16C>T/−124A>G polymorphisms showed that four haplotypes were estimated to be
Table I The distribution of the INHA gene –16C>T polymorphism in the POF patients and controls.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Genotypes</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CC (%)</td>
<td>CT (%)</td>
</tr>
<tr>
<td>Total POF</td>
<td>159</td>
<td>110 (69.2)</td>
<td>44 (27.7)</td>
</tr>
<tr>
<td>Primary</td>
<td>20</td>
<td>13 (65.0)</td>
<td>6 (30.0)</td>
</tr>
<tr>
<td>Secondary</td>
<td>139</td>
<td>97 (69.8)</td>
<td>38 (27.3)</td>
</tr>
<tr>
<td>Control</td>
<td>233</td>
<td>153 (65.7)</td>
<td>68 (29.2)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Evaluated by the χ<sup>2</sup> test in comparison with the control group.

Table II The distribution of the INHA gene –124A>G polymorphism in the POF patients and controls.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Genotypes</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AA (%)</td>
<td>AG (%)</td>
</tr>
<tr>
<td>Total POF</td>
<td>159</td>
<td>65 (40.9)</td>
<td>76 (47.8)</td>
</tr>
<tr>
<td>Primary</td>
<td>20</td>
<td>8 (40.0)</td>
<td>11 (55.0)</td>
</tr>
<tr>
<td>Secondary</td>
<td>139</td>
<td>57 (41.0)</td>
<td>65 (46.8)</td>
</tr>
<tr>
<td>Control</td>
<td>233</td>
<td>78 (33.5)</td>
<td>113 (48.5)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Evaluated by the χ<sup>2</sup> test in comparison with the control group.

<sup>b</sup>p < 0.017 are considered significant after Bonferroni correction.

Discussion

No significant differences were found in the genotype distributions of the INHA gene polymorphisms in our Korean cohort. These findings are not consistent with the previous report in which significantly reduced allele frequencies of the –16T and –124G were observed in the POF group (Woad et al., 2009), but are consistent with other reports that there were no significant differences in the risk of POF development for the INHA promoter variants (Dixit et al., 2006; Sundblad et al., 2006; Corre et al., 2009).

A discrepancy of allele distribution in the POF group between studies could be explained as follows. First, ethnic variations might influence the pattern of the INHA gene polymorphisms in POF patients. There was a significantly reduced frequency of the –16T allele in a New Zealand POF and a reduced frequency of the –124G allele in a combined population of New Zealand and Slovenia (Woad et al., 2009). However, the –16T allele frequency was not significantly reduced in combined POF of New Zealand and Slovenia, and no significant association of the –124G allele was also found when considering each population in isolation. Moreover, significant associations were not replicated in other studies, i.e. in Argentine POF (Sundblad et al., 2006), in Indian POF (Dixit et al., 2006), in Italian/German POF (Corre et al., 2009) and, finally, in our Korean POF. Taken together, it is reasonable to assume that different ethnicities may bring conflicting results on the INHA genotype distributions in POF patients. More studies from different populations are needed to confirm these ethnic variations. Secondly, in most cases, the numbers of POF patients were small in studies that showed positive results compared with studies that showed negative results including our study; e.g. 70 in New Zealand/Slovenia POF, and 56 in New Zealand POF (Harris et al., 2005; Woad et al., 2009), versus 133 in Indian POF, 138 in Slovenian POF, 169 in Central Italian POF/299 in Northern Italian POF/143 in German POF, and 159 in our Korean POF (Dixit et al., 2006; Corre et al., 2009; Woad et al., 2009). A significant difference was only found in a small New Zealand cohort and, moreover, there was a great difference in the frequency of the –16T allele between same New Zealand controls, e.g. 44.3 versus 21.5% (Harris et al., 2005;
Conflict of interest
None declared.

References


Authors’ roles

S.H.Y. contributed to the conception and design, and participated in the analysis and interpretation of data and in the drafting of the paper. Y.M.C. contributed to the conception and design, and participated in the final approval of the version of the paper to be published. M.A.H. performed genomic DNA analysis. S.Y.M. and J.J.K. contributed to the revision of the article. H.J.I. participated in the analysis and interpretation of data. G.H.L. participated in the review of the manuscript. B.M.K. contributed to the acquisition of data.

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Woad et al., 2009), which seriously impairs the reliability of their positive results. The value of 19.7% in our data almost coincides with other reports. In addition, the previous significant result from Italian POF by Marozzi et al., was not replicated later in a larger Italian POF cohort (Corre et al., 2009). Small numbers of patients in studies may have resulted in different findings. A sample size large enough to achieve a satisfactory power is important in genetic association studies.

It was suggested that the –16T allele in the INHA gene is protective against POF (Harris et al., 2005). In theory, the promoter polymorphisms of the INHA gene could regulate the function of the INHA gene and also affect the biological action of inhibin on reproductive ageing. However, they might not be susceptible allelic variants or be insufficient to cause POF since –16C>T and –124 A>G INHA promoter variants are not located within regulatory elements for modulating INHA gene expression, and its significance was not demonstrated simultaneously at both loci which are thought to exist in linkage disequilibrium. Moreover, there was a report that serum inhibin levels were found to be similar in women with different –16C>T polymorphism genotypes, implying that ovarian production of inhibins may not be affected by –16C>T allelic variants (Sundblad et al., 2006). It is still unclear whether promoter polymorphic variants could affect the expression of the INHA gene or not, and functional studies on the influence of this polymorphism are needed.

In the present study, we postulated that primary amenorrhea patients would have more significant variations than secondary amenorrhea group. In subgroup analyses, there was no significant difference between groups (data not shown). We also think that the present study has significance in itself in spite of negative results because it is the first report on the association between the INHA polymorphisms and POF in East-Asian populations.

In summary, the promoter polymorphisms in the INHA gene did not show any correlation with idiopathic POF in Korean women, implying that it might not have clinical consequences or be unlikely to cause POF. Further studies from diverse ethnicities are needed to elucidate the biological mechanism of the INHA gene and to better understand the etiology of POF.