G769A Variation of Inhibin α-gene in Korean Women with Premature Ovarian Failure

Hye-Jin Jeong1, Sung-Won Cho1, Hyun-Ah Kim1, Sook-Hwan Lee1,2, Jung-Hyun Cho2, Dong-Hee Choi2, Hwang Kwon2, Won-Tae Cha3, Jee-Eun Han2, and Kwang-Yul Cha2

1Genome Research Center for Reproductive Medicine and Infertility of Korea Ministry of Health and Welfare;
2CHA General Hospital, College of Medicine, Pochon CHA University, Seoul, Korea;
3Duke University, Durham City P.O. Box 92330, North Carolina 27708, USA.

Premature ovarian failure (POF) is menopause before the age of 40 years. The frequency of POF is about 1% of all women. Recently inhibin alpha gene (INHα) has been indicated as candidate in POF pathogenesis. Inhibin, a glycoprotein, is a gonadal hormone, which can inhibit the synthesis and secretion of pituitary follicle-stimulating hormone (FSH), which has an important role in the recruitment and development of ovarian follicles during the folliculogenesis. G769A variation of INHα, alanine, is highly conserved across species, and has an important role of its receptor binding. We screened a G769A transition in the INHα from the total population of the patients of 84 women with POF and 100 normal fertile women. We found no variation between the normal subjects and the POF patients. G769A variation of INHα is rare in Korean women with POF.

Key Words: POF, inhibin, polymorphism, infertility

INTRODUCTION

Premature ovarian failure (POF) is defined as a syndrome characterized by menopause before the age of 40 years. The frequency of POF is about 1% in all women, and it may affect very young women in their late teens or twenties. Patients with this disorder suffer from the loss of fertility, and the clinical effects of hypo-oestrogenism in some women can be noted. In most cases, the etiology of POF remains unknown, but several factors, such as genetic factors, autoimmune diseases, iatrogenic agents, and environmental toxicants have been identified.

Inhibin, a glycoprotein, is a gonadal hormone, which can inhibits the synthesis and secretion of pituitary FSH. Inhibin is structurally related to the TGF-β (transforming growth factor-β superfamily, a group of multi-functional growth and differentiation factors. The mature inhibin is a 31-32kDa heterodimeric glycoprotein consisting of α-subunit (18kDa) and β-subunit (βA and βB, approximately 14kDa). There are two forms of inhibin: inhibin A (α-βA), and inhibin B (α-βB).

The serum inhibin is secreted from the granulose cells in females. A recent study suggests that the level of the serum inhibin is significantly raised in the infertile patients, as compared to the fertile controls. A defect in the inhibin secretion has been reported in women with POF.

According to Shelling et al., two variants of inhibin were found in patients with POF: C1032T transition in the INHβA gene in one patient, and the G769A transition in the INHα gene in three patients. The INHα variant is significantly associated with POF (3/43, 7%) compared with control samples (1/150, 0.7%) (Fisher’s exact test, p < 0.035). Marozzi et al. reported that the G769A transition was significantly more frequent in both the POF (7/157, 4.5%), Fisher’s exact test, p < 0.01) and primary amenorrhea (3/12, 25%, Fisher’s
exact test, p<0.05) patients. This loss would result in an increase in the concentrations of FSH by removing the negative feedback, leading to a premature depletion of follicles, and hence, result in POI. We have investigated whether the G769A variation of INHα gene exist in Korean women with POI.

MATERIALS AND METHODS

Patients and controls

From June 2000 to January 2002, 84 patient with POI and 100 healthy fertile women were recruited by CHA general hospital. All the women in this study were examined at the endocrinology or gynecology clinics and were diagnosed as having premature ovarian failure, based on the criteria of at least 6 months of amenorrhea and two serum FSH measurements above 40 mIU/ml in women <40 years. Women with a history of pelvic surgery, chemotherapy, autoimmune diseases and abnormal chromosome were excluded from the study.

Polymerase chain reaction (PCR) and RFLP

Genomic DNA was extracted from 5 ml samples of blood. DNA was stored in 200 μl Tris-EDTA buffer at -20°C. PCR primers were designed to amplify INHα. (Forward: 5’-GGCCCACACTCG GACACAAGC-3’ and Reverse: 5’-AGCCACAAC CACCATGACTAG-3’).11 Genomic DNA was amplified in 50 μl volume reaction containing 5 μl of PCR buffer, 50 nmol of each dNTP, 5 nmol of forward and reverse primers, and 1 U Taq DNA polymerase. Standard PCR conditions comprised 94°C initial denaturation for 5 min, 94°C denaturation for 45 sec, 63°C annealing for 45 sec, and 72°C extension for 45 sec for 35 cycles. The final extension was carried out at 72°C for 7 min. The PCR product was separated on 2% agarose gel and visualized under UV light.

The PCR product was treated with 2 U BbvI, 1X restriction buffer and sterile water. The reaction mixtures were incubated at 37°C for 2-3 hr, and then heat inactivation was undertaken at 65°C for 20 min, and the mixtures were electrophoresed in 3% agarose gel.

RESULTS

Patients (n=84) with POI and normal fertile women (n=100) were investigated whether they displayed the mutations in INHα G769A. RFLP was used to identify the presence of the INHα G769A variant. This variant was G→A missense substitution at nucleotide 769 that alters codon 257 from GCT to ACT, resulting in alanine by threonine amino acid substitution in the INHα subunit. This variant abolished the BbvI restriction enzyme site. The wild-type INHα PCR product yields three fragments of 85 bp, 25 bp, and 133 bp when digested with BbvI. In the presence of the G769A variant, the enzyme recognition site is abolished, and hence, yields only two fragments of 85 bp and 158 bp. The RFLP analysis from the DNA samples of the 84 POI patients and 100 normal fertile individuals showed no variation (Table 1). All the samples had three fragments length: 133 bp, 85 bp, and 25 bp (Fig.1) in length.

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<th>Table 1. The INHα G769A Variation of POI Patients and Normal Fertile Women</th>
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<td>G769A variant</td>
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<td>POF patients</td>
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<td>Normal fertile women</td>
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<td>*number of G769A variant.</td>
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Fig. 1. RFLP analysis of INHα G769A variant using BbvI Lane 1-6: Enzyme-digested PCR product of POI patient. Lane 7-11: Enzyme-digested PCR product of normal fertile women. Lane 12: Undigested PCR product of POI patient. M: 100bp marker.
DISCUSSION

POF is a condition characterized by cessation of the ovarian function before the age of 40 years. Significant consequences of POF include the loss of fertility and the clinical effects of hypo-oestrogenism. The best known causes of ovarian failure are sex chromosome abnormalities, such as Turner's syndrome. However, the cause of most POF cases is not clear.

McCullagh used the term 'inhibin' to describe the inhibitory activity. With the advent of the specific FSH immunoassays in the 1970s, the gonadal fractions were identified, which specifically inhibited (contained inhibin and follistatin) or stimulated (activin) the FSH synthesis and secretion.

The INHα G769A variant was first reported in New Zealand patients (3/43 POF patients, 7%) compared with 150 control samples from New Zealand and Slovenia (1/150 normal control, 0.7%) (Fisher's exact test, p < 0.035). Recently, this variant was reported to have been found in 7 cases in patients with POF and in 3 cases in primary amenorrhea patients in Italy. These studies suggest the existence of a correlation between the mutation and the early onset of premature menopause. The human INHα gene has 80% homology compared with equine, bovine, porcine, ovine, rat and mouse sequences. The site of the mutation of the alanine at codon 257 was conserved in horse, porcine, ovine, mouse, bovine, primate and chicken species except the rat sequences. So far, the functional significance of the amino acid variant at codon 257 is unknown. Analysis of the protein structure suggests that it could interfere with the receptor binding. The inhibin molecule belongs to the TGF-β superfamily, a group of multi-functional growth and differentiation factors. INHα has the seven conserved cysteins, like the other members of the TGF-β superfamily. This region is thought to be involved in the receptor binding, because the amino-terminal region, upstream in respect of the first cystein, is distinguishable from other members of the TGF-β superfamily. The alanine to threonine change belongs to this putative receptor binding region of the final mature peptide. Thus, the alanine at codon 257 may have an important role in the function of the protein. It would be interesting to find out whether patients with POF display this mutation.

We investigated whether a G769A transition in the INHα gene exist in the Korean women with POF. The results obtained in this study have shown that patients with POF (n=84) and normal fertile populations (n=100) have no the INHα G769A variant. Therefore the INHα gene mutations may be rare in Korean patients with POF.

REFERENCES

