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GDF9 Mutation Analyses in 100 Chinese Women with Premature Ovarian Failure (POF)

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Abstract

We screened growth differentiation factor 9 (GDF9) coding regions for mutations in a Chinese sample of 100 women with premature ovarian failure (POF) and discovered 4 novel SNPs: c.436C>T (p.Arg146Cys), c.588A>C (silent), c.712A>G (p.Thr238Ala) and c.1283G>C (p.Ser428Thr). Non-synonymous SNPs c.436C>T and c.1283G>C were also detected in the control population. The c.712A>G perturbation results in a missense mutation (p.Thr238Ala) and was not present in any of 96 controls. Substitution of the hydrophobic amino acid residue alanine for hydrophilic threonine may disrupt GDF9 function.

Keywords

Premature Ovarian Failure; Growth Differentiation Factor 9; Mutation; Single Nucleotide Polymorphism

Premature Ovarian Failure (POF) is defined as a primary ovarian defect characterized by premature depletion of ovarian follicles before the age of 40 years (secondary amenorrhea) (1,2), with elevated gonadotropins. POF affects approximately one in 10,000 women by age 20, one in 1,000 women by age 30, and one in 100 women by age 40 (3). POF is heritable in 4 to 31% of all cases of POF and sporadic mutations account for other cases (4–7). The molecular mechanisms underlying POF are still unclear, but the diverse etiologies include gonadotoxic chemotherapy, radiation treatment, chromosome abnormalities involving the X or autosomes and certain single gene mutation like Fragile X syndrome (8).

Women with POF have an increased incidence of ovarian follicle growth defects. Two members of the TGF β superfamily-growth differentiation factor 9 (GDF9) and bone

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Capsule We detected a novel missense mutation c.712A>G (p.Thr238Ala) in the growth differentiation factor 9 (GDF9) among 100 Chinese women with premature ovarian failure (POF). This substitution may disrupt GDF9 function.

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morphogenetic protein-15 (BMP15)-are germ cell specific factors secreted by oocytes and crucial for folliculogenesis. Previous studies (9–12) in non-Chinese subjects have shown heterozygous associations of single nucleotide variations in the GDF9 gene in women with ovarian failure. Because dimers form among TGF β gene products, the heterozygous perturbation is presumed to exert a dominant negative effect and, hence, may be clinically significant. Our goal was to determine if perturbations in the GDF9 gene occur in Chinese women with POF.

In this study, 100 Chinese women with POF, as well as 96 control women, were recruited at the Reproductive Medical Center, Shandong Provincial Hospital, Shandong University in Jinan, Shandong, China. An independent ethics committee at Shandong Provincial Hospital and Baylor College of Medicine Institutional Review Board for Human Subject Research approved the study. Written informed consent was obtained from all participants. Inclusion criteria were defined as two serum follicle stimulating hormone (FSH) concentrations greater than 40 IU/ml prior to 40 years of age, and the absence of a known chromosomal abnormality. Women with associated endocrinopathies or autoimmune disorders were also excluded. Pedigree analysis revealed that in 6 cases a mother or sister also showed amenorrhea before age of 40 years.

After genomic DNA was extracted from blood samples, the coding regions of GDF9 were amplified using polymerase chain reaction (PCR) with 3 pairs of GDF9 gene specific primers. The first exon was amplified using GDF9X1F (5'TTCCTCACTAGTTCTCCC AAGC3') and GDF9X1R (5'CATCTTCCCTCCACCCAGT3') primers. The first half of the second exon was amplified using GDF9X21F (5'TTCAAGCACTACTGGTAG3') and GDF9X21 (5'AGCCTGAGCACTTGTGTCATT3') primers; the second half was amplified using GDF9X22F (5'ATGAAAGACCAG CTGGAGCA3') and GDF9X22R (5'TTTGCCAAATAGGCTCAAGG3') primers. PCR products were denatured and re-annealed to form potential heteroduplexes (wild-type strand paired with mutant strand). Heteroduplexes were detected using denaturing high-performance liquid chromatography (DHPLC) on the WAVE System 3500 (Transgenomic Ltd, Omaha, NE). Samples which demonstrated heteroduplex formation on DHPLC were then sequenced directly after PCR amplification on an automated sequencer, ABI Prism Sequencer 3130XL (Applied Biosystems, Foster City, CA).

Among the 100 POF subjects, we found 3 novel non-synonymous SNPs: c.436C>T (p.Arg146Cys), c.712A>G (p.Thr238Ala) and c.1283G>C (p.Ser428Thr); two of these (c.436C>T and c.1283G>C) were also found in the controls (Table 1). The c.712A>G variant detected only in the POF group was present in a 30-year-old woman with secondary amenorrhea. She has a healthy 4-year-old child but upon presentation had elevated FSH and luteinizing hormone (LH): 84.6IU/L and 39.2IU/L, respectively. Transvaginal ultrasonography showed a small uterus (41×33mm), an atrophic endometrium and small ovaries devoid of follicles (17 × 12mm and 18×14mm for the right and left ovaries, respectively). She had no evident somatic anomalies. Her parents and two sisters are healthy and no other family members had POF.

We also found 3 synonymous (silent) SNPs in the POF group, two of which were also present in controls (Table 1).

Heterozygous mutations of GDF9 have been described in Indian, French and American women with POF. Non-synonymous SNPs in the Indian POF subjects included c.199A>C, and c.646G>A; c.557C>A was detected among 203 French women with POF, whereas among 61 subjects of American Caucasian POF women, c.307C>T was identified. None of these SNPs were detected in our Chinese subjects. The c.712A>G SNP detected in one Chinese subject is

a novel SNP that has not previously been reported to SNP databases. The result is a substitution of the hydrophobic amino acid residue alanine for hydrophilic threonine at position 238 in the GDF9 protein.

GDF9 is synthesized as a preproprotein. After cleavage of the signal peptide, the GDF9 proprotein forms homodimers and heterodimers with BMP15 (13). Female mice lacking GDF9 arrest folliculogenesis at the primary follicle stage (14). Primary follicles lacking GDF9 can not recruit a thecal cell layer. Molecular markers such as Inhibin α and Kit ligand are also misregulated in the Gdf9 knockout mouse ovaries (15).

Naturally occurring variants in BMP15 and GDF9 have a profound effect on fertility in sheep (16–18), impairing normal processing of the proprotein dimers and leading to targeted degradation of the misfolded proteins (19). Amino acid substitutions are more likely to affect protein function when differences in the biochemical properties are large and the amino acids located at residues highly conserved among species. Predicting the likely effects of the mutations (20) suggests that the variant p. Thr238Ala would be an important amino acid substitution, given its occurrence at a position conserved in all mammalian and zebra fish GDF9 proteins sequences. Consistent with the manner in which perturbations of GDF9 are considered to exert untoward effects, the Thr238Ala substitution molecule could cause abnormal homodimers or heterodimers with BMP15, thereby adversely affecting intercellular follicular signaling in the ovary.

In summary, we have discovered 3 novel non-synonymous SNPs in the GDF9 gene among 100 Chinese women with POF. Two of these three SNPs were also present in the control population, whereas the third SNP, c.712A>G, caused the missense mutation p.Thr238Ala. Future functional studies are necessary to determine the functional significance of this missense mutation.

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Table 1

GDF9 mutations in 100 Chinese women with POF

Mutation	Exon	Sequence Variation	Amino Acid Variation	Protein Domain	Number of Cases (N=100)	Number of Controls (N=96)
Novel	2	c.436C>T	p.Arg146Cys	Propeptide	1/100	1/96
Rs254286	2	c.447C>T	Silent	Propeptide	56/100	56/96
Rs10491279	2	c.546G>A	Silent	Propeptide	26/100	28/96
Novel	2	c.588A>C	Silent	Propeptide	2/100	0/96
Novel	2	c.712A>G	p.Thr238Ala	Propeptide	1/100	0/96
Novel	2	c.1283G>C	p.Ser428Thr	Maure peptide	2/100	2/96

Note: POF=Premature Ovarian Failure; GDF9 = growth differentiation factor 9