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ARTICLE

Analysis of progesterone receptor membrane component 1 mutation in Han Chinese women with premature ovarian failure

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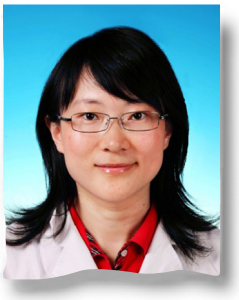

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Abstract The gene *PGRMC1* is highly expressed in the granulose and luteal cells of rodent and primate ovaries. Its role in anti-apoptosis and regulating cell-cycle progression suggests a role in regulating follicle growth. The hypothesis is supported by the study in mice and studies in Sweden. In this study, the coding exons of *PGRMC1* were sequenced among 196 Chinese women with premature ovarian failure (POF) and 200 controls, and one novel missense mutation was identified (C.556C>T, p. Pro186Ser) in the POF group and one novel SNP (C.533C>T, p. Trh177Ile) was identified in both groups. The mutation is not considered causative because protein prediction did not indicate a deleterious effect. It is concluded that coding mutations of *PGRMC1* do not seem to be a common cause of the disease in Han Chinese women. Future studies in larger cohorts from other ethnic groups are necessary to establish the role of *PGRMC1* in POF.

KEYWORDS: mutation screening, premature ovarian failure (POF), progesterone receptor membrane component 1 (PGRMC1)

Introduction

Premature ovarian failure (POF) is a syndrome characterized by elevated gonadotrophin level and low oestrogen level with amenorrhoea as a result of a cessation of ovarian function before the age of 40 years. The prevalence of POF is 1–2% of women in the general population (Coulam et al., 1986). To date, the pathogenesis of POF is unclear. The known possible causes include iatrogenic injury, autoimmune disease, infection factors and genetic factors, which will lead to defects

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Table 1 Primers for *PGRMC1* amplification.

Exon	*Primer sequence*	*Amplification length (bp)*	*Annealing temperature (°C)*
Exon1	F1 5′ GCTCAGAGGGAGGAGAAAGTGG 3′ R1 5′ TACCCGCCTACCCTAGCTCG 3′	534 bp	55
Exon2	F2 5′ AAATGGGGCTATGTTGATGAAT 3′ R2 5′ CACACCAAGCACCAAATCCTC 3′	549 bp	57
Exon3	F3 5′ GGTATAAACCACCTTTGTATCCTTC 3′ R3 5′ CACAAACACATCATGCATTCTAAG 3′	434 bp	55

in follicular development (Shelling, 2010; Simpson and Rajkovic, 1999).

Genetic factors are estimated to be present in over 30% of POF cases (Russell et al., 1982). Increasing numbers of genes involved in follicle development are considered causative by gene screening, such as *GDF9* (Dixit et al., 2005; Kovanci et al., 2007), *FOXO3* (Wang et al., 2010), *NOBOX* (Qin et al., 2007), *FIGLA* (Zhao et al., 2008), *FMR1* (Bodega et al., 2006; Bretherick et al., 2005), *FMR2* (Murray et al., 1999), *BMP15* (Di Pasquale et al., 2006), *NR5A1* (Harrison et al., 2013) and *POU5F1* (Wang et al., 2011). Although usually only 1–2% of a given ethnic cohort will show perturbation of a candidate gene, the cascade reaction of the perturbations may lead to impaired ovarian function (Eystathioy et al., 2001). Therefore, further screening of candidate genes is necessary for contributing to a final targeted array.

Progesterone receptor membrane component (PGRMC1) as a member of the heme-binding protein family is highly expressed in the granulose and luteal cells of rodent and primate ovaries (Peluso et al., 2009). It may serve as progestrone receptors in a non-classical progesterone signalling mechanism (Pru and Clark, 2013). It may regulate follicle growth by regulating expression of genes associated with apoptosis (Cahill, 2007; Crudden et al., 2006; Peluso et al., 2006, 2008, 2010) and modulating cell-cycle progression of granulosa cells (Lodde and Peluso, 2011).

PGRMC1's anti-apoptotic action in granulosa cells works in two ways: one suppresses the activity of the transcription factor, Tcf/Lef, which can influence the expression of genes that regulate cell survival (Peluso et al., 2012a, 2012b), and the other inhibits genes related to mitosis, consequently preventing a 'mitotic catastrophe' (Peluso et al., 2012a, 2012b).

A study using mice in which *PGRMC1* was conditionally depleted from granulosa cells demonstrates the role of *PGRMC1* in regulating follicle growth. The analysis shows that ovaries from immature (22–25 days old) *Pgrmc1* conditional knockout (−/−) mice have fewer antral follicles compared with either the controls (+/+) or the heterozygous (+/−) mice. Also, the heterozygous (+/−) mice have a higher percentage of atretic antral follicles than the controls (+/+) (Peluso and Pru, 2014). A study from Sweden showed that reduced level of *PGRMC1* in peripheral leukocytes is associated with impaired ovarian function (Schuster et al., 2010), and another study from Sweden suggested that mutant or reduced levels of PGMRC1 may cause POF by impairing activation of microsomal cytochrome P450 and increasing apoptosis of ovarian cells (Mansouri et al., 2008).

The present study was designed to investigate whether *PGRMC1* variants contribute to POF in Chinese origin.

Materials and methods

A total of 196 nulligravida experiencing POF, with no family history of POF or X chromosome abnormality, were recruited into the study. Diagnosis could be made if the following criteria were satisfied: amenorrhoea before the age of 40 years with repeated FSH concentrations exceeding 40 IU/L. Patients with associated endocrinopathies, autoimmune disorders or chromosomal abnormality were excluded. The control group consisted of 200 healthy women with regular menstrual cycles and no known history of infertility before the age of 40 years, each of whom provided written informed consent. In addition, the ethics approval was obtained from the Institutional Review Board of Reproductive Medicine of Shandong University (date of approval 03 June 2012, reference number 16).

Genomic DNA was extracted from peripheral leukocytes using QIAamp DNA Blood Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The three exons and exon–intron boundaries of *PGRMC1* (GeneBank Gene ID: 10857) were amplified by polymerase chain reaction with three pairs of primers. The primers and amplification conditions are shown in **Table 1**. After confirmation by agarose gel electrophoresis, the amplicons were sequenced with BigDye sequencing reagent V3.1 (Applied Biosystems, Forster City, CA) using a 3730 × 1 DNA Analyzer (Applied Biosystems, Forster City, CA). The exact location and novelty of the found mutation was determined via consulting the Ensemble database (www.ensembl.org). Once indentified, it was confirmed by repeating the whole procedure and sequencing both the forward and reverse strands.

Categorical variables were described with counts and percentages. The chi-squared test or Fisher's test was used to analyse nominal variables. $P < 0.05$ was considered statistically significant for all statistical tests.

Results

In the present study, a novel missense mutation (C.556C>T, p.Pro186Ser) was found in exon 3, which was not, however, found in the 200 controls (**Table 2**). The mutation detected was from a 40-year-old woman whose FSH was 52.88 IU/L. Transvaginal ultrasonography did not detect the existence of the follicles. This patient, with no genetic family history, experienced her menarche at age 15 years, and entered menopause at age 35 years. She presented with hot flashes, excessive sweating, lassitude, emotional lability and vaginal