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ORIGINAL ARTICLE

Identification of the first homozygous *POLG* mutation causing non-syndromic ovarian dysfunction

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ABSTRACT

Objective: To investigate the genetic cause of non-syndromic ovarian dysfunction in a patient from a consanguineous family.

Methods: This study examined a patient with irregular menstrual cycles and abnormal oocytes. The patient had undergone irregular hormone replacement therapy over 3 years to adjust the menstrual cycle and improve ovarian function. Prior to ovarian stimulation in our hospital, 3 months of androgen and regular hormone therapy were used as an intervention method. No follicular development was detected in the subsequent three cycles using letrozole treatment. The patient then received a constantly adjusted dose of menotropins, but produced only one oocyte.

Results: Whole-exome sequencing analysis identified the first homozygous *POLG* mutation (c.2890C > T; p.R964C) associated with ovarian dysfunction. Sanger sequencing was used to validate. *In silico* analysis suggested that the p.R964C mutation was pathogenic. Conservation analysis demonstrated that R964 was an important site for the DNA polymerase function of POLG.

Conclusions: Biallelic mutations in *POLG* may be associated with ovarian dysfunction. This study has improved our understanding of *POLG*-related genetic mutations in ovarian dysfunction, and the mode of inheritance of certain sequence variants. This information will assist genetic counseling and precision medicine in the future.

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Ovarian dysfunction; POLG; whole-exome sequencing; irregular menstrual cycle; abnormal oocyte

Introduction

Human ovarian dysfunction comprises a variety of different conditions that each result in irregular menstrual cycles, ovarian failure, and female infertility. Amenorrhea and irregular menstrual cycles are two main features of ovarian dysfunction. Genetic defects can cause ovarian dysfunction, including chromosomal abnormalities and single gene alterations[1–7]. Mutations in *STAG3*, *BMP15*, *FSHR*, *GDF9*, *NOBOX*, *MCM8*, *MCM9*, *NUP107*, *MSH4*, *CSB-PGBD3* and *MSH5* can cause recessive primary amenorrhea[8–17] or secondary amenorrhea[18–20]. Sequence variants in *POLG*, *NR5A1*, *KHDRBS1*, and *NOBOX* are reported to be associated with dominant primary[21] or secondary amenorrhea[22–24]. In our clinical practice, some patients exhibit irregular menstrual cycles and poor outcomes of *in vitro* fertilization, but there is a lack of knowledge of the potential genetic contribution to this ovarian dysfunction.

In this study, we examined a patient from a consanguineous family exhibiting irregular menstrual cycles and abnormal oocytes. Using whole-exome sequencing technology, we identified a novel homozygous *POLG* mutation in this patient. In contrast to previously reported heterozygous *POLG* mutations associated with premature ovarian failure (POF), this is the first recessive mode of inheritance of a *POLG* mutation in a patient with ovarian dysfunction.

Materials and methods

Patient

A Chinese patient with ovarian dysfunction was recruited from The First Affiliated Hospital of Anhui Medical University, and was from a consanguineous family (Figure 1A). The patient did not show any of the following: karyotypic abnormalities, autoimmune disorders, history of radiotherapy and chemotherapy, or pelvic surgery. This study was approved by the Ethics Committee of Anhui Medical University. Written informed consent was obtained from the patient and her parents, and 5 ml of peripheral blood was collected from the patient.

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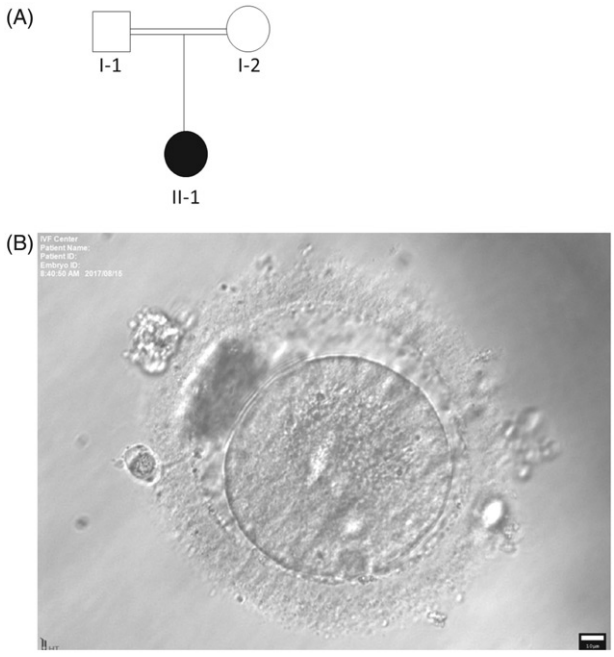

Figure 1. Pedigree analysis of the patient in the family. (A) The patient with ovarian dysfunction in a Chinese consanguineous family. The black circle indicates the affected family member. (B) Abnormal oocyte from the patient. The oocyte was abnormal with the presence of two polar bodies and granular cytoplasm. The white bar =10 μm.

WES and sanger sequencing validation

Exome sequence capture was performed using the SureSelect Human All Exon V5 Kit (Agilent Technologies, Palo Alto, CA, USA) following the manufacturer's protocol. The captured libraries were sequenced using an Illumina HiSeq 2000 Sequencer. Sequence reads were mapped against a human reference genome (hg19) using the Burrows-Wheeler Aligner algorithm (http://bio-bwa.sourceforge.net/). The SNPs and Indels were detected by SAMtools (http://samtools.sourceforge.net/). Sanger sequencing was performed using gene-specific primers.

Results

Clinical characterization

The proband (II-1, Figure 1A) was diagnosed with ovarian dysfunction and infertility at 32 years of age in 2017. Her height was 160 cm and weight was 60 kg. She experienced menarche at 15 years of age. Her menstrual cycle was irregular from 24 years of age, when she had a cycle length of approximately 4 months. Both ovaries were not clearly detected by transvaginal color Doppler ultrasound at the first-time consultancy. Circulating hormone levels of the proband were: 10.48 IU/l follicle stimulating hormone (FSH); 2.77 IU/l luteinizing hormone (LH); 2.07 nmol/l testosterone; 54 pmol/l estradiol (E2); 10.03 ng/ml prolactin; and 0.05 ng/ml anti-Müllerian hormone (AMH). Magnetic resonance imaging showed the pituitary was normal. Examination revealed no progressive external ophthalmoplegia (PEO), sensory ataxic neuropathy dysarthria, ophthalmoparesis, mitochondrial DNA depletion syndrome 4A, Leigh syndrome, or spinocerebellar ataxia with epilepsy. The proband's mother (I-2) underwent menopause at the age of 54.

To adjust the menstrual cycle and improve ovarian function, this patient had undergone 3 years of irregular hormone replacement therapy. Prior to ovarian stimulation in our hospital, 3 months of androgen supplement (dehydroepiandrosterone (DHEA) 25 mg three times a day) and regular hormone therapy (oral contraceptive: ethinylestradiol and cyproterone acetate tablets (Diane-35); for 21 days of each month) were used as an intervention method. Hormone levels were measured in duplicate: 7.46 IU/l FSH; 1.12 IU/l LH; and 33.43 pmol/l E2. No follicular development was detected in the subsequent three cycles using letrozole tablets (Furui; Jiangsu Hengrui Pharmaceutical Co, Lianyungang, China). The patient then received a constantly adjusted injection dose of menotropins (hMG; Lizhu Pharmaceutical Co, Zhuhai, China) but produced only one oocyte. Details and endpoints of the treatment are shown in Table 1.

The pretreatment with DHEA and hormone replacement lowered the circulating FSH level, improved the ovarian sensitivity to FSH and increased the opportunity for oocyte development. The long stimulation lasted for about 1 month; one follicle about 20 × 17 mm could be detected on the left ovary. The only oocyte was retrieved from the left ovary by transvaginal ultrasound-guided puncture, approximately 36 h after induction of ovulation with 5000 IU human chorionic gonadotropin (hCG; Lizhu Pharmaceutical Co.). After denuding the oocyte from surrounding granular cells using hyaluronidase, the abnormal presence of two polar bodies and granular cytoplasm (Figure 1B) indicated poor oocyte quality. Despite the aberrant morphology, the oocyte underwent intracytoplasmic sperm injection *in vitro,* but displayed complete fertilization failure.

Molecular genetic analysis

Whole-exome sequencing with aligned sequence reads variant identification were performed, as previously described[23]. Pedigree analysis suggested an autosomal recessive mode of inheritance associated with this family (Figure 1A). We filtered out polymorphisms with allele frequencies >1% in The Short Genetic Variations database (dbSNP), 1000 Genomes (1000G), Exome Aggregation Consortium (ExAC), and Genome Aggregation Database (gnomAD) databases. A list of genes harboring coding/splicing homozygous variants were further filtered by the functional impact of the mutation (e.g. conservation and functional prediction by *in silico* prediction modules PolyPhen2, SIFT, and Mutationtaster).

One homozygous *POLG* mutation (NM_002693:exon18: c.2890C > T: p.R964C) passed the filtering steps. This mutation was segregated within the family (Figure 2A). The *POLG* gene encodes a DNA polymerase-γ involved in the replication of human mitochondrial DNA[25]. Mutations in *POLG* can cause POF or premature menopause, and all cases to date showed dominant inheritance[22,26–28]. Our study found the first *POLG* homozygous mutation in a patient with ovarian dysfunction. *In silico* analysis by five online prediction tools suggested that the p.R964C mutation was pathogenic

Table 1. Ovarian stimulation and follicular ultrasound monitoring of patient.

hMG (start from day 1)	*Date of TVS*	*Right ovary (mm)*	*Left ovary (mm)*	*Mean diameter of uterus (mm)*	*Endometrial thickness (mm)*
150 U*6 day (days 1–6)	Day 1	Blurred image	15 × 12	32	Line
225 U*6 days (days 7–12)	Day 7	Blurred image	15 × 13	35	5.4
300 U*5 days (days 13–17)	Day 13	Blurred image	15 × 13 F: 4 × 4	35	5.5
225 U*7 days (days 18–24)	Day 18	Blurred image	21 × 21 F: 11 × 10	44	8.9
225 U*3 days (days 25–27)	Day 25	20 × 19	29 × 24 F: 15 × 16	38	10.9
	Day 28	17 × 16	28 × 27 F: 20 × 17	34	11

hMG, human menopausal gonadotropin (menotropin); TVS, transvaginal ultrasound; F, follicle.

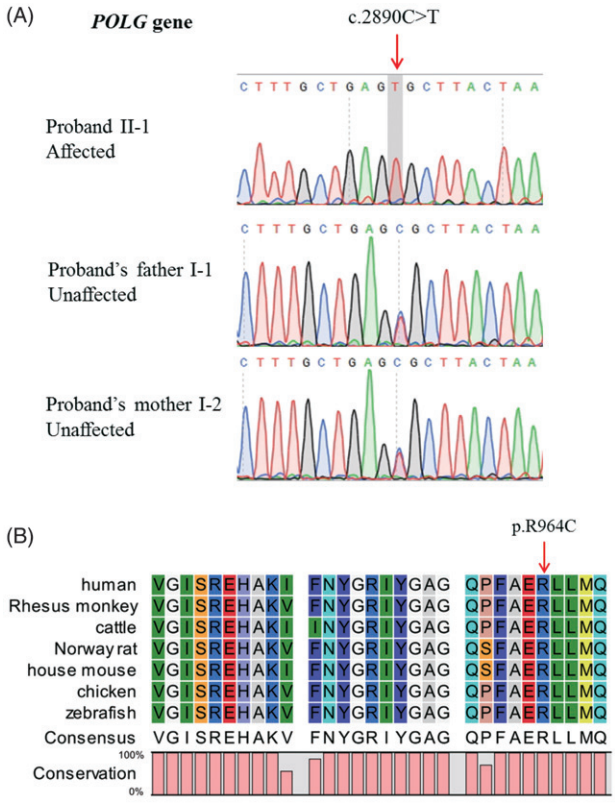

Figure 2. Genetic analysis of the patient. (A) Sanger sequencing validation of the mutation in family members. Red arrow points to the mutation site. (B) Sequence alignment of POLG in different species. Red arrow points to the R964 site in human POLG.

(Table 2). The mutation was also predicted a high risk for pathogenicity using the POLG Pathogenicity Prediction Server (http://polg.bmb.msu.edu/query.php). Amino acid R964 is located in the polymerase domain of POLG, and is highly conserved among different species ranging from human to zebrafish (Figure 2B), suggesting the functional importance of the R964 site.

Discussion

In this study, we report that a novel homozygous *POLG* mutation is associated with ovarian dysfunction in a patient with consanguineous pedigree. The *POLG* gene encodes the catalytic subunit of DNA polymerase-γ involved in the replication of mitochondrial DNA[25]. *Polg* knock-out mice develop an mtDNA mutator phenotype associated with reduced lifespan, premature onset of aging-related phenotypes and reduced fertility[29]. Dominant or recessive mutations in human *POLG* can cause a spectrum of disorders associated with mitochondria dysfunction, including PEO, sensory ataxic neuropathy, familial Parkinsonism and progressive sclerosing poliodystrophy[30–32]. Most women with PEO exhibit early menopause[26]. The heterozygous p.Y955C, p.R943H, p.Y831C and p.S511N mutations of *POLG* can segregate with POF and PEO[22,26,28,33,34]. In addition, another heterozygous p.Y951N mutation in *POLG* was found in a patient with cataracts, early-onset distal muscle weakness and atrophy, ovarian dysgenesis (a severe form of POF) and 3-methylglutaconic aciduria[27]. Another study screened *POLG* mutations in 201 patients with spontaneous primary ovarian insufficiency and found one heterozygous p.R953C variant in a patient[35]. Therefore, *POLG* heterozygous mutations, including p.S511N, p.Y831C, p.R943H, p.Y951N, p.R953C, and p.Y955C, may be associated with syndromic or non-syndromic ovarian failure.

Previous studies have identified dominant *POLG* mutations in patients with ovarian failure. However, using pedigree and WES analysis, we have found the first homozygous *POLG* mutation in a patient with non-syndromic ovarian dysfunction, suggesting a recessive mode of mutation inheritance. The functional difference between the p.R964C mutation and previously reported heterozygous *POLG* mutations remains to be determined. However, as our reported case manifested as a non-syndromic ovarian dysfunction phenotype (a mild form compared with POF), we speculate that p.R964C produces a milder functional defect in POLG activity than the six above-mentioned mutations. Further experiments are required to directly examine this proposal.

Our study demonstrates, for the first time, that a novel biallelic *POLG* mutation (p.R964C) may cause ovarian dysfunction. This study expands our understanding of *POLG* mutations related to ovarian dysfunction, and the mode of inheritance of certain sequence variants. The information provided by this study may also assist in future genetic counseling and precision medicine approaches.

Authors' contributions

C.B.L. wrote the paper and contributed reagents, materials, analysis tools and data. L.L. analyzed and interpreted the data and wrote the paper. W.J. performed the experiments and analyzed the data. Z.Y.R. and Z.J. contributed reagents, materials, analysis tools and data. L.T.Y., P.H. and L.B.H.

Table 2. *In silico* analysis of the *POLG* mutation.

Gene	*Mutation*	*Amino acid change*	*Zygosity*	*Polyphen-2*[a]	*SIFT*[b]	*Mutation taster*[c]	*SNPs & GO*[d]	*FATHMM-MKL*[e]	*ExAC (total)*[f]	*ExAC (East Asian)*[g]	*gnomAD (total)*[h]
POLG	c.2890C > T	p.R964C	Homozygous	Probably damaging (1.000)	Damaging (0.002)	Disease causing (1.000)	Disease (0.720)	Damaging (0.942)	0.0006708	0.008988	0.0006459

[a]Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/). Prediction scores range from 0 to 1, with high scores indicating probably or possibly damaging; [b]SIFT, i.e. sorting intolerant from tolerant (http://sift.jcvi.org/). Scores vary between 0 and 1. Variants with scores close or equal to 0 are predicted to be damaging; [c]Mutation taster (http://www.mutationtaster.org/). The probability value is the probability of the prediction, i.e. a value close to 1 indicates a high 'security' of the prediction; [d]SNPs & GO (http://snps.biofold.org/snps-and-go/). Disease probability (if >0.5 mutation is predicted disease); [e]FATHMM-MKL (http://fathmm.biocompute.org.uk/fathmmMKL.htm). Values above 0.5 are predicted to be deleterious, while those below 0.5 are predicted to be neutral or benign; [f]Frequency of variations in total of ExAC database; [g]Frequency of variations in East Asian population of ExAC database; [h]Frequency of variation in total of gnomAD (genome Aggregation Database).

performed the experiments. C.Y.X. and W.B.B. conceived and designed the experiments.

Conflict of interest The authors declare that they have no conflict of interest.

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