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GENETICS

Novel mutations in *NLRP5* and *PATL2* cause female infertility characterized by primarily oocyte maturation abnormality and consequent early embryonic arrest

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Abstract

Purpose This study aims to identify the genetic causes of 12 women with primary infertility characterized by primarily oocyte maturation abnormality and consequent early embryonic arrest.

Methods Genomic DNA was isolated from peripheral blood samples. Whole-exome sequencing was performed on the probands, and the identified variants were confirmed by Sanger sequencing. The pathogenicity of the identified variants on the protein was accessed in silico. And we used qRT-PCR to detect the possible effects of the novel mutation on the mRNA level of *NLRP5*.

Results A novel homozygous frameshift variant (p.V429Efs*30) in *NLRP5* and compound heterozygous variants with a novel frameshift variant (p.A297Efs*20) and a recurrent variant (c. 223-14_223-2delCCCTCCTGTTCCA) in *PATL2* were identified in two unrelated affected individuals. qRT-PCR showed an obvious decrease of the mutant *NLRP5* mRNA. In addition, the truncated proteins of NLRP5 and PATL2 were predicted to be non-functional due to the deletion of the most or the whole region of the critical functional domain(s) respectively.

Conclusions This study identified novel mutations in *NLRP5* and *PATL2*, further expanding the mutational and phenotypic spectrum of both genes. This is the first report of the *NLRP5* mutations that associates with oocyte maturation abnormality in humans.

Keywords Female infertility · Oocyte maturation abnormality · Early embryonic arrest · *NLRP5* · *PATL2*

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Introduction

During in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI), it is common for a small proportion (8.6–15.2%) of oocytes to arrest at germinal vesicle (GV) stage or metaphase I (MI) stage [1]. When the proportion of immature oocytes was more than 25%, the rates of fertilization, blastocyst production, and clinical pregnancy were markedly reduced. When the proportion of immature oocytes exceeded 40% including at least one maturation-resistant oocyte, the pregnancy would be hardly achieved and the oocytes exhibited abnormal nuclear maturation [2]. In some severe but extremely rare cases, the collected oocytes were repeatedly all immature [3]; or a majority of the retrieved oocytes were immature, together with a very small proportion of oocytes showing an appearance of first polar body (PB1) extrusion but actually having abnormal nuclear maturation, consequently resulting in fertilization failure or early embryonic

arrest [4]; the underlying reason for these conditions is largely unknown.

Maternal components stored in oocyte are essential for oocyte maturation, and they are also the main factor that determines the embryo development and implantation potential [5]. These maternal components in oocytes were encoded by maternal-effect genes. If such a gene was impaired, it would bring bad effects on the maturation of oocyte, and subsequently the development of early-stage embryo [6]. Recently, numerous studies have identified that mutations in genes required for human oocyte maturation, including *TUBB8* (MIM: 616,768) [7] and *PATL2* (MIM:614,661) [8–11], *TRIP13* (MIM: 604,507) [12], are primarily responsible for oocyte maturation arrest. However, different mutations in these genes can result in variability in the clinical phenotypes, in terms of oocyte maturation arrest, fertilization failure, or early embryonic arrest [13]. For mutations in such genes encoding a protein as a member of subcortical maternal complex (SCMC), including *TLE6* (MIM: 612,399) [14], *PADI6* (MIM: 610,363) [15], *KHDC3L* (MIM: 610,363) [16], *NLRP2* (MIM:609,364), and *NLRP5* (MIM: 609,658) [17], they have been shown to mainly cause early embryonic arrest. *NLRP5*, as one of the maternal-effect genes, is highly expressed in oocytes. Initially, its biallelic mutations had been identified to cause multiple pregnancy loss or birth of offspring with multi-locus imprinting disturbances (MLID) or healthy offspring [18, 19]. Subsequently, biallelic *NLRP5* mutations had been also linked to human female infertility with embryonic arrest [17, 20, 21]. More recently, two studies had identified biallelic *NLRP5* mutations were causative for female infertility with recurrent fertilization failure [22, 23]. So far, no mutations in *NLRP5* have been reported in human in the context of oocyte maturation abnormality.

In this study, we used whole-exome sequencing (WES) to identify genetic causes of 12 primary infertile women with primarily oocyte maturation abnormality and consequent early embryonic arrest. We discovered novel mutations in *NLRP5* and *PATL2* in two affected women respectively, thereby expanding the mutational and phenotypic spectrum of both genes. This is the first study that associated biallelic *NLRP5* mutations with human oocyte maturation abnormality.

Materials and methods

Subjects and clinical investigation

A total of 12 women with primary infertility characterized by primarily oocyte maturation abnormality (> 60% of oocytes were immature) and consequent early embryonic arrest (embryos were arrested before 6-cell stage (grade III)) on day 3 were recruited during July 2014 and November 2021. All probands had normal karyotypes (46, XX). Peripheral blood samples were obtained from all probands and their available family members. Written informed consent was obtained from all participants.

Evaluation of oocyte and embryo phenotypes

The morphological evaluation of oocyte maturity, fertilization, and embryonic development was performed under a light microscope. Metaphase II (MII) oocytes were observed with the BP1 extruded (Fig. 1a). GV oocytes had an intact germinal vesicle (Fig. 1a). Metaphase I (MI) oocytes had no GV or polar body. GV oocytes and MI oocytes were considered to be immature. The fertilization assessment was performed 17 ± 1 h post-insemination. The embryos were cultured and incubated to day 5 or day 6.

Whole-exome sequencing

WES was performed using the DNA samples of the probands. Genomic DNA library was prepared using the Agilent Human SureSelect All Exon V6 kit and exome sequencing was performed on an Illumina NovaSeq 6000 platform. Clean sequencing reads were aligned to the human reference sequence (hg19). Sequence variants including single-nucleotide variants (SNVs) and small insertions or deletions (indels)

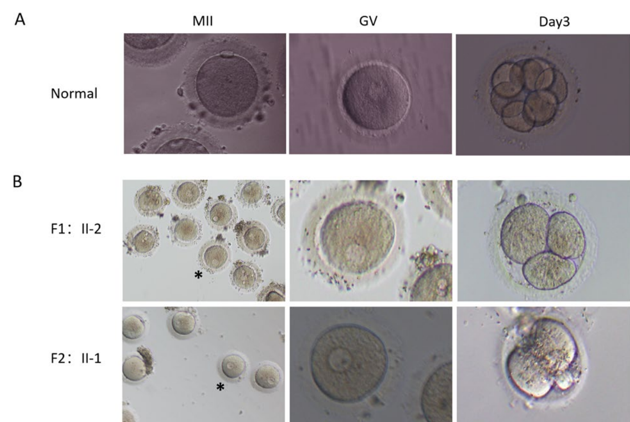

Fig. 1 Phenotypes of oocytes and embryos from a normal individual and two infertile women with primarily oocyte maturation abnormality and consequent early embryonic arrest. **a** Morphology of an MII oocyte, a GV oocyte, and an embryo of day 3 from a normal individual. *Left panel*, an MII oocyte. *Middle panel*, a GV oocyte. *Right panel*, an embryo of day 3. **b** Morphology of the oocytes and embryos from the two affected women with biallelic *NLRP5* and *PATL2* mutations respectively. *Left panels*, part of the oocytes retrieved. *Middle panels*, the oocyte marked with a star on the left panel was magnified. *Right panels*, embryos of day 3. *Upper panels*, the proband from family 1 (F1:II-2), the second cycle of an IVF attempt. *Lower panels*, the proband from family 2 (F2:II-1). Scale bars in all panels: 50 μm. MII = metaphase II; GV = germinal vesicle

were annotated by ANNOVAR pipeline. Common variants (defined as a minor allele frequency (MAF) above 1% in public databases: 1000 genome, dbSNP, ESP6500, gnomAD, or ExAC) were excluded. SNVs and indels were classified by position as intergenic, 5′ UTR, 3′UTR, intronic, splicing, or exonic. Exonic variants were then classified by predicted amino acid change as a stopgain, missense, synonymous, or frameshift variant, inframe insertion or deletion, or possible splicing mutation. For coding or potential splicing mutations, the conservation at the variant site and the predicted effect on protein function were evaluated with in silico tools: SIFT, PolyPhen-2, MutationTaster, and NNSplice.

Sanger sequencing

The coding regions flanking the variants in the *NLRP5* and *PATL2* gene were amplified by PCR using the specific primers respectively (Table S1). The PCR products were directly Sanger sequenced in both forward and reverse directions, using an ABI 3100 DNA analyzer (Applied Biosystem, Foster City, CA, USA). The variants were validated by Sanger sequencing in the probands and all their available family members.

Quantitative reverse transcription PCR

Total RNA was extracted from the peripheral blood leukocytes of the proband from family 1 and from a normal fertile female by the Trizol Reagent (Thermo Fisher Scientific, Inc.). cDNA was synthesized using reverse transcriptase (TaKaRa PrimeScript reagent kit) and amplified by PCR with Taq polymerase using the specific primers spanned exons 13–15 of *NLRP5* shown in Table S2. The PCR products were analyzed by electrophoresis and further sequenced using the ABI 3100 DNA analyzer (Applied Biosystem, Foster City, CA, USA). Quantitative reverse transcription PCR (qRT-PCR) was performed using StepOnePlus Real-time PCR system (Applied Biosystems, Foster City, CA). qRT-PCR reactions contain iTaq Sybrgreen (Biorad) and specific primers. *GAPDH* was used as an internal control. The $2^{-\Delta\Delta CT}$ method [24] is used to analyze the relative expression of the *NLRP5* mRNA in the proband from family 1 (F1:II-2) with c.1286_1289del mutation and in a normal control. Three independent experiments were performed. *P* value was calculated using Student's unpaired, two-tailed *t*-test.

Results

Clinical phenotypes of the patients

Two out of twelve unrelated probands with primary infertility due to primarily oocyte maturation abnormality and consequent early embryonic arrest had been found to have a genetic cause for their clinical phenotypes. Their spouses had normal semen volume, sperm concentration, motility, and sperm morphology.

The 34-year-old proband in family 1 (F1:II-2) from a consanguineous family had a 1-year history of primary infertility. She had undergone two trials of failed intrauterine insemination (IUI) with her ex-husband. She remarried to a man who had a child with his ex-wife. She had regular menses. Hysterosalpingography indicated incomplete patency of the bilateral fallopian tubes. The proband had two failed cycles of IVF attempts (Table 1). In the first cycle, a gonadotropin-releasing hormone (GnRH) antagonist protocol was performed and 30 oocytes were retrieved but all arrested at GV stage, even after culture of in vitro maturation (IVM). In the second cycle, a progestin-primed ovarian stimulation (PPOS) protocol was utilized and 17 out of 24 oocytes remained immature even after IVM. A total of 4 of the other 7 oocytes with PB1 were fertilized by IVF but resulted in early embryonic arrest at the 2–3-cell stage (grade IV) on day 3 (Table 1) (Fig. 1b). Her sister (II-1) had a son and a daughter through natural pregnancy.

The 41-year-old proband in family 2 (F2:II-1) was from a non-consanguineous family with a 10-year history of primary infertility. She had regular menstrual cycles. Due to the venerable age of the patient and the long period of infertility, the couples accepted the IVF treatment. In the cycle of IVF attempt, a long protocol was performed and 14 oocytes were retrieved; among them, 11 oocytes were arrested at GV stage, one oocyte appeared degraded, one oocytes with PB1was abnormally fertilized resulting in a zygote with multiple pronuclei (multiple PN), and one oocyte was normally fertilized (2PN) but resulting in an unusable embryo arrested at the 2-cell stage (grade IV) on day 3 (Table 1) (Fig. 1b). Her sister (II-2) had two children through natural pregnancy.

Identification of novel mutations in *NLRP5* and *PATL2*

In family 1, the proband (F1:II-2) was identified with a novel homozygous frameshift mutation in *NLRP5* (NM_153447; c.1286_1289del; p.V429Efs*30). Her consanguineous parents were heterozygous for this mutation and her fertile sister was not found the mutation by Sanger sequencing (Fig. 2a). In family 2, the proband (F1:II-1) was identified with compound heterozygous mutations in *PATL2* (NM_001145112.1), including a recurrent splicing mutation (c. 223-14_223-2delCCCTCCTGTTCCA) (eventually causing a frameshift p.R75Vfs*21) and a novel frameshift mutation (c.890delC; p.A297Efs*20). Her mother and her fertile sister were heterozygous for the splicing mutation, but her father was found no mutations in *PATL2*. Five very rare homozygous SNPs (Table S3) in the proband were used for

Table 1 Oocyte and embryo characteristics of IVF and ICSI attempts of three probands

Individual	Age (years)	Duration of infertility (years)	IVF/ICSI attempts	Stages of oocytes				No. of fertilized oocytes			No. of usable D3 embryos	Outcomes
				GV	MI	PB1	Degenerated	2PN	1PN	Multiple PN		
F1:II-2	34	1	1st IVF	30	0	0	0	0	0	0	0	/
			2nd IVF	15	2	7	0	4	0	0	0	All embryos were arrested at the 2–3-cell stage on day 3
F2:II-1	41	10	1st IVF	11	0	2	1	1	0	1	0	The embryo was arrested at the 2-cell stage on day 3

IVF in vitro fertilization; *ICSI* intracytoplasmic sperm injection; *GV* germinal vesicle; *MI* metaphase I; *PB1* the first polar body; *PN* pronucleus/pronuclei

paternity test. Sanger sequencing showed that all these SNPs were heterozygous in her mother and father (Fig. S1), which demonstrated a very high possibility of paternity. Since the heterozygous frameshift mutation (p.A297Efs*20) was not inherited from her mother nor her father; it might be a de novo mutation in the patient (Fig. 2b).

The c.1286_1289del (p.V429Efs*30) mutation is recorded as rs769276313 in dbSNP (v153) and has a low allele frequency in the gnomAD browser (14/280438). The mutation c.890delC (p.A297Efs*20) was not recorded in dbSNP (v153) or the gnomAD browser (Table 2).

Prediction of the effects of the novel mutations on the protein

The frameshift mutations c.1286_1289del (p.V429Efs*30) and c.890delC (p.A297Efs*20) were predicted to produce a premature termination code (PTC) in the mRNA of *NLRP5* and *PATL2* respectively. If the aberrant mRNAs were translated, the truncated protein of NLRP5 would lack large part of NACHT (NAIP, CIITA, HET-E, and TP1) domain and the whole leucine-rich repeats (LRR) domain; and the truncated protein of PATL2 would lack nearly the whole PAT1 domain (Fig. 2c).

Effect of a novel mutation on the expression of *NLRP5* mRNA

qRT-PCR was performed to explore the effect of the novel homozygous frameshift mutation on the expression of *NLRP5* mRNA. The results indicated that the relative expression of *NLRP5* mRNA was significantly reduced in the proband (F1:II-2) with c.1286_1289del mutation compared with a wild-type normal control (Fig. 2d).

Discussion

In this study, we identified a novel homozygous frameshift *NLRP5* mutation and compound heterozygous mutations of *PATL2* including a recurrent splicing mutation and a novel frameshift mutation in two out of twelve affected women with primary infertility due to primarily oocyte maturation abnormality and consequent early embryonic arrest. Their typical clinical phenotypes indicated by IVF/ICSI procedures were that the whole or most oocytes were immature and only a small part of oocytes were fertilized but embryos were arrested at an early stage of development.

The novel frameshift mutations c.1286_1289del (p.V429Efs*30) and c.890delC (p.A297Efs*20) producing a PTC in the mRNA of *NLRP5* and *PATL2* respectively were predicted to trigger the rapid degradation by the pathway of nonsense-mediated decay (NMD) [25], resulting in no

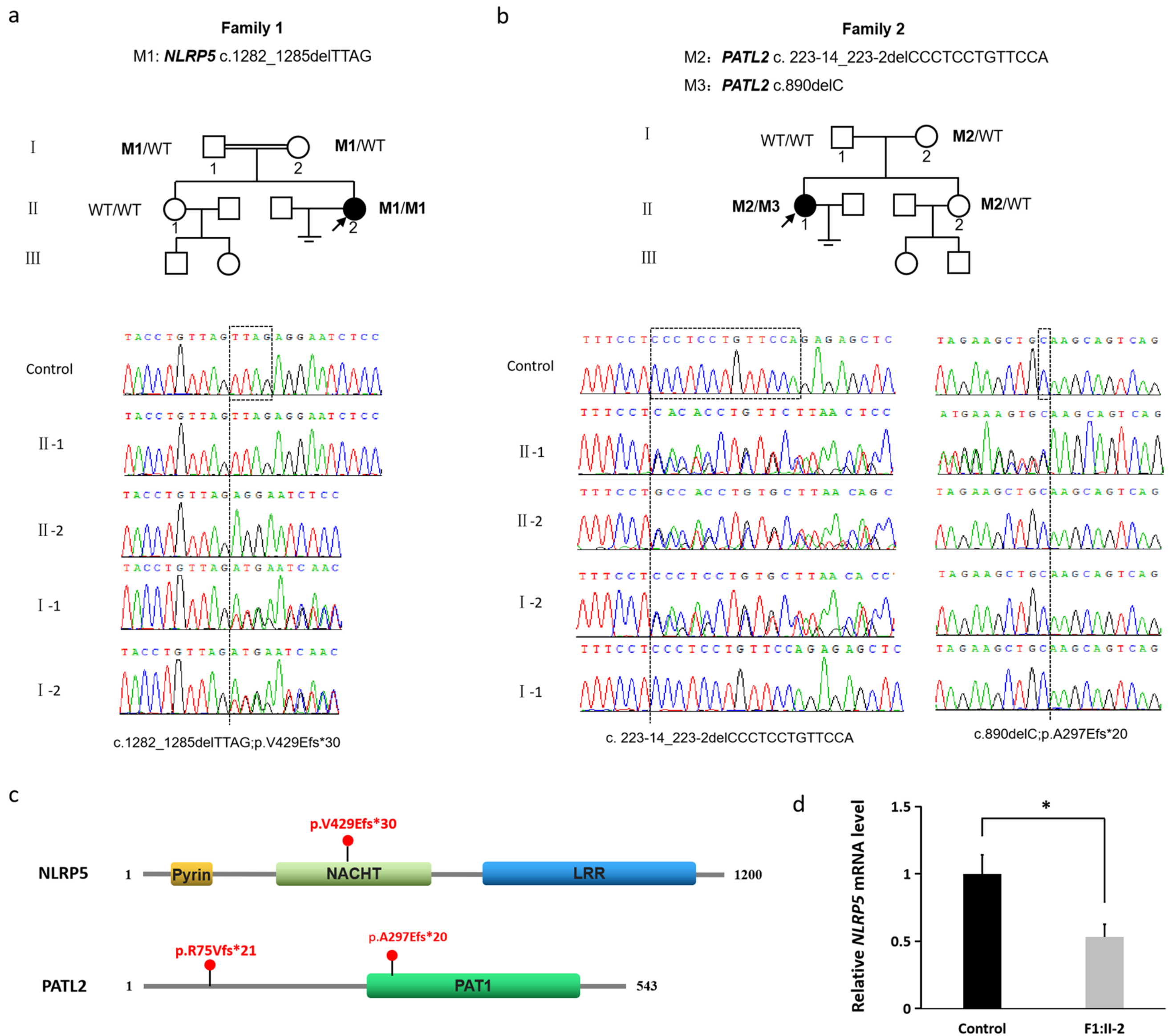

Fig. 2 Identification of novel mutations in *NLRP5* and *PATL2* in two unrelated primary infertile patients with primarily oocyte maturation abnormality and consequent early embryonic arrest. **a** Pedigree analysis of the family 1 with *NLRP5* mutations and chromatograms of partial *NLRP5* sequences in family members. **b** Pedigree analysis of the family 1 with *PATL2* mutations and chromatograms of partial *PATL2* sequences in family members. For **a** and **b**, a black circle with an arrow presents the proband. The individual with wild type is marked as a clear square or square. The double line is consanguinity. The chromatograms were the results of Sanger sequencing. The dashed lines indicate the positions of variants. **c** The positions of the mutations in the structure of NLRP5 and PATL2 protein. **d** Relative expression of *NLRP5* mRNA in the peripheral blood leukocytes of the proband in family 1 (F1:II-2) and a normal control. The relative expression was measured by qRT-PCR and normalized to *GAPDH*. The bars show the mean of three independent experiments and error bars denote standard deviations. *, $p < 0.001$

translation of protein. qRT-PCR results showed an obvious decrease of the mutant *NLRP5* mRNA (Fig. 2d), supporting that the mutant *NLRP5* mRNA expression was under the regulation of NMD. Even if the mutant *NLRP5* mRNAs were not all degraded, a significantly reduced amount of the truncated protein, lacking a large part of NACHT domain and the entire LRR domain in the C-terminus (Fig. 2c), could be translated but would be non-functional. Similarly, if the aberrant *PALT2* mRNAs were not all degraded by NMD, the truncated protein lacking nearly the whole part of PAT1 domain in the C-terminus (Fig. 2c) would lose its main function.

So far, only five studies have linked biallelic *NLRP5* mutations to female infertility [17, 20–23]. The first study identified two pairs of compound heterozygous *NLRP5* mutations (p.Gln98* and p.Thr694Ile, p.Gly289Glu and

Table 2 Effects of novel *NLRP5* and *PATL2* mutations predicted using in silico tools

	Position on chromosome	cDNA alteration	Amino acid alteration	Exon	Mutation	gnomAD allele frequency	gnomAD homozygote frequency	dbSNP (v153)	SIFT	Polyphen-2	MutationTaster
F1:II-2	NLRP5 Chr19: 56538881_56538884delTTAG	c.1286_1289del	p.V429Efs*30	7	Frameshift	14/280438	0/280438	rs769276313	-	-	1 (D)
F2:II-1	PATL2 Chr15:44961748delG	c.890delC	p.A297Efs*20	10	Frameshift	Not found	Not found	-	-	-	1 (D)

a. Mutation assessment by SIFT, PolyPhen-2, and MutationTaster

D damaging; *P* probably damaging

p.Thr1107Ile) in two unrelated primary infertile females with early embryonic arrest [17]. The second study found a homozygous missense *NLRP5* mutation (p.P354L) in one primary infertile woman from a consanguineous family with early embryonic arrest [20]. The third study reported three affected individuals with primary fertility characterized by early embryonic arrest with biallelic *NLRP5* mutations, including a homozygous missense mutation (p.Arg635Cys), compound heterozygous missense mutations (p.Ser893Thr and p.Leu1116Trp) in two unrelated affected individuals respectively and two homozygous missense mutations (p.Arg143Pro and p.Arg462Cys) in another affected individual [21]. More recently, one study has identified a homozygous frameshift *NLRP5* mutation c.2274_2275del (Trp759Aspfs*4) in an affected woman [22] and another study has identified compound heterozygous missense mutations c.1598G > C (p.Arg533Pro) and c.1919 T > G (p.Leu640Arg) in another affected woman with primary infertility and total fertilization failure [23]. These two studies at least suggested that NLRP5 may exert its function before fertilization. To date, no mutations in the gene of *NLRP5*, which is highly expressed in oocytes, have been reported to be associated with human oocyte maturation abnormality.

Here, we identified the second homozygous frameshift *NLRP5* mutation c.1286_1289del (p.V429Efs*30) in a patient from a consanguineous family with primary infertility. This patient experienced two failed IVF attempts that all retrieved 30 oocytes were arrested at GV in the first cycle even after IVM, and in the second cycle, the majority oocyte (17/24) were immature (oocyte maturity was only 29.17%) and a small part of oocytes (4/24) were fertilized but embryos were all arrested at an early stage. As far as we know, this is the first study that linked the biallelic *NLRP5* mutations to oocyte maturation abnormality. In five previous studies, phenotypes of seriously decreased oocyte maturity were not demonstrated in those affected individuals with biallelic *NLRP5* mutations [15, 18–21]. We supposed that the novel homozygous frameshift mutation p.V429Efs*30, if not degraded by NMD, lacking a larger fragment in the C-terminus of protein, may cause more serious effect on the protein function of NLRP5, compared with the homozygous frameshift mutation p.Trp759Aspfs*4 and other currently identified homozygous or compound heterozygous missense mutations. It requires further studies to illustrate whether the protein of NLRP5 plays a role in regulating or modifying the maturation of oocyte.

In addition, we also identified a novel mutation of *PATL2* in a primary infertile patient with a large proportion of oocyte maturation abnormality and a small number of oocytes fertilized resulting in early embryonic arrest. So far, numerous studies have shown that biallelic mutations in *PATL2* are mainly responsible for human oocyte germinal

vesicle-stage arrest [10, 11] and that different mutations may result in a wide range of phenotypes in oocyte maturation arrest, fertilization failure, or embryonic developmental arrest [13, 26]. At present, one additional novel *PATL2* mutation was identified, which further supported the evidence that PATL2 plays a critical role in oocyte maturation and early embryonic development.

In conclusion, this study identified novel mutations in the maternal-effect genes *NLRP5* and *PATL2*, expanding the spectrum of genetic causes of female infertility with primarily oocyte maturation abnormality and consequent early embryonic arrest. As far as we know, this is the first study that associated biallelic *NLRP5* mutations with the phenotype of oocyte maturation abnormality. The genetic analysis of larger cohorts of infertile patients due to oocyte maturation abnormality and/or consequent early embryonic arrest is needed to formulate more accurate genotype and phenotype correlations.

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Author contribution L.H., R.J., and X.T. conceived and designed the experiments. L.H., F.L., Q.J, J.J., L.L., R.J., and X.T. collected the samples. L.H. and Y.W. performed the experiments. L.H., Y.W., and G.S. analyzed the data. L.H. wrote the paper. All authors approved the final manuscript.

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Declarations

Ethics approval The study was approved by the biomedical research ethics committee of the University of Science and Technology of China.

Conflict of interest The authors declare no competing interests.

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