

Human oocyte maturation arrest caused by a novel missense mutation in *TUBB8*

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Jingjing Xiang^{1,2,#}, Wei Wang^{1,2,#},
Chunfeng Qian^{1,2}, Jiangyang Xue^{1,2},
Ting Wang^{1,2}, Haibo Li^{1,2} and Hong Li^{1,2,#}

Abstract

Objective: To explore the etiology of human oocyte maturation arrest in two infertile Chinese sisters.

Methods: Clinical examination and genetic testing of all available family members were conducted, and the findings were used to create a pedigree. Mutation screening using PCR amplification and DNA Sanger sequencing of the entire tubulin beta 8 class VIII gene (*TUBB8*) including intron–exon boundaries was performed to identify mutations.

Results: A novel missense *TUBB8* mutation (c.1054G > T, p.A352S) in the patient and her elder sister was detected and shown to be associated with oocyte maturation arrest.

Conclusion: Our findings expand the known mutation spectrum of *TUBB8* and provide insights into the etiology of human oocyte maturation arrest.

Keywords

Oocyte maturation arrest, tubulin beta 8 class VIII, missense mutation, genetic pedigree, multiple sequence alignment, pathogenicity

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Introduction

In the fetal ovary, oocytes in the primordial follicle initiate meiosis, and pause at prophase I. Until puberty, the oocytes begin the maturation process and resume meiosis in response to a surge of luteinizing hormone (LH), with germinal vesicle

¹Center for Reproduction and Genetics, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou, Jiangsu, China

²Center for Reproduction and Genetics, Suzhou Municipal Hospital, Suzhou, Jiangsu, China

#These authors contributed equally to this work

Corresponding author:

Haibo Li, Center for Reproduction and Genetics, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou, Jiangsu, China.

Email: lihaibo-775@163.com



breakdown followed by spindle assembly, chromosome migration, asymmetric division, and extrusion of the first polar body. At this point, the oocytes are mature and arrest at metaphase II until fertilization.^{1,2}

Successful mammalian reproduction requires the fusion of a mature oocyte with a sperm cell. Although it is common for a small number of oocytes to be immature after ovary stimulation, human chorionic gonadotropin (hCG) administration, and *in vitro* culture in assisted reproduction treatments, it is rare for all retrieved oocytes to remain immature. Indeed, only a few cases of human oocyte maturation arrest have been reported in the literature.^{3–10}

Oocyte maturation arrest may occur at various stages of the cell cycle, such as the germinal vesicle, metaphase I, and metaphase II stage, but little is known about the underlying genetic pathology.

Recently, mutations in the primate-specific tubulin beta 8 class VIII gene (*TUBB8*) were identified as causative of human oocyte maturation arrest. *TUBB8* is expressed specifically in oocytes and early embryos. A total of 26 *TUBB8* mutations have been reported, including 21 heterozygous missense mutations, two homozygous missense mutations, and three homozygous deletion mutations. These mutations cause varying defects leading to arrest in human oocyte maturation, fertilization, or early embryonic development. Heterozygous missense mutations in *TUBB8* may have dominant-negative effects that affect the formation of the α/β -tubulin heterodimer, disrupt microtubule dynamics, and interfere with spindle assembly, leading to maturation arrest of mouse and human oocytes.^{11–14}

Here, we report a case of a patient and her sister with primary infertility caused by oocyte maturation arrest. Mutation screening of *TUBB8* revealed a novel heterozygous missense mutation that was causative of disease.

Patients and methods

Patient

This study was approved by the institutional ethics committee of the Affiliated Suzhou Hospital of Nanjing Medical University. Written informed consent was obtained from all participants. All available members of the patient's family were subjected to a full medical history evaluation and comprehensive physical examination. A pedigree of the family was constructed after clinical examination and genetic testing.

Mutational analysis

Peripheral blood was collected from the patient, her elder sister, and their mother. Genomic DNA was extracted from the blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. All four exons and intron–exon boundaries of *TUBB8* were amplified by PCR using Faststart Taq DNA polymerase (Roche, Basel, Switzerland). The PCR amplification program included an initial denaturation at 94°C for 5 minutes, followed by 16 cycles of denaturation at 94°C for 45 s, annealing for 45 s at 68°C then decreasing by 0.5°C each cycle, and extension at 72°C for 45 s, followed by 20 cycles of denaturation at 94°C for 45 s, annealing at 56°C for 45 s, extension at 72°C for 1 minute, a final extension at 72°C for 7 minutes, and holding at 4°C. The amplified DNA fragments were purified and sequenced in both directions using an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The resulting sequences were compared with the reference sequence of *TUBB8* (NM_177987.2) in the NCBI database.

In silico analysis of mutations

Multiple sequence alignment of the *TUBB8* protein and its orthologs was performed

using MUSCLE alignment software (<http://www.drive5.com/muscle/>).¹⁵ The mutations were analyzed according to the Standards and Guidelines for the Interpretation of Sequence Variants released by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.¹⁶ The corresponding mutations were searched in the following databases: dbSNP (<http://www.ncbi.nlm.nih.gov/SNP/>), Exome Aggregation Consortium (ExAC) (<http://exac.broadinstitute.org/>), the Genome Aggregation Database (gnomAD) (<http://gnomad.broadinstitute.org/>), the 1000 Genomes Project (<http://www.1000genomes.org/>), and Chinese genomes in diseaseDX (<http://diseasedx.virgilbio.com/>). Mutation pathogenicity was predicted by Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>)¹⁷ and Mutation Taster (<http://www.mutationtaster.org/>) prediction algorithms.¹⁸

Results

Clinical data

A 30-year-old female with a 6-year history of primary unexplained infertility was referred to our center. Routine infertility investigation revealed that her ovulatory cycle was regular, and that her fallopian tubes were patent. The concentrations of endocrine hormones in her serum were normal: follicle stimulating hormone (FSH) 7.14 mIU/mL, LH 4.89 mIU/mL, estradiol 55 pg/mL, testosterone 0.24 ng/mL, and anti-Mullerian hormone 1.95 ng/mL. The sperm concentration, motility, and morphology of her spouse were normal, and the karyotypes of both individuals were normal. The patient underwent a failed *in vitro* fertilization (IVF) attempt at another IVF center, in which 18 oocytes were retrieved but none were fertilized. On her second IVF attempt in our center, her pituitary was downregulated with

gonadotropin-releasing hormone agonist, then her ovaries were stimulated for 10 days with a total dosage of 1725 IU gonadotropins, including 975 IU FSH and 750 IU human menopausal gonadotropin (HMG). Then, 6750 IU hCG was administered, at which point serum estradiol concentrations were 4335 pg/mL; 36 h later, 14 oocytes were aspirated by ultrasound-guided follicular puncture. The oocytes were divided into two equal groups; those in group I were stripped of their cumulus cells before intracytoplasmic sperm injection (ICSI), and were at the meiosis I stage. Twenty-four h after ICSI, no signs of fertilization were observed, and the seven oocytes were still at the meiosis I stage (Figure 1a). The remaining seven oocytes in group II were cultured overnight in culture medium supplemented with 75 mIU/mL HMG for *in vitro* maturation. However, 24 h later no polar body was extruded. After culture for a further 24 h, all seven oocytes were stripped of their cumulus cells and examined. Six oocytes were shown to have remained at the meiosis I stage, and one oocyte exhibited abnormal morphology (Figure 1b). Therefore ICSI was cancelled. The patient's elder sister also presented with primary unexplained infertility and experienced a failed IVF attempt at another IVF center.

Genetic analysis

A pedigree of this family is shown in Figure 2a. Sequencing analysis identified the heterozygous *TUBB8* mutation c.1054G > T in both sisters, which was not detected in their mother (Figure 2a). Because of a lack of genetic information regarding their father, the inheritance pattern of this mutation is unknown. c.1054G > T is a novel missense mutation found within exon 4 of *TUBB8*, and is responsible for converting a highly conserved alanine residue to a serine in the

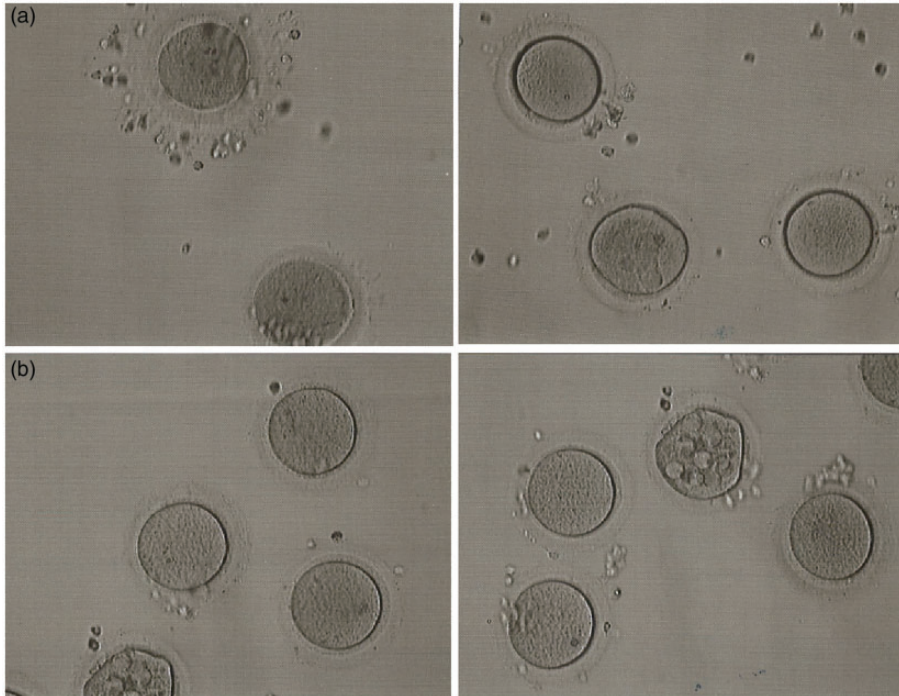


Figure 1. Phenotypes of oocytes from the patient with maturation arrest. (a) The retrieved oocytes examined by light microscopy after stripping of cumulus cells. (b) The oocytes observed by light microscopy 48 h after *in vitro* maturation treatment

TUBB8 protein (p.A352S). This mutation is not found in dbSNP, ExAC, gnomAD, 1000 Genomes Project, or diseaseDX databases. Additionally, the alanine residue is evolutionarily conserved among primate species (Figure 2b), and the p.A352S mutation is predicted to be probably damaging by Polyphen-2 and disease-causing by Mutation Taster.

Discussion

In this study, the patient's ovaries showed a normal response to gonadotropins, and the follicular growth rate was normal, suggesting that the oocyte maturation arrest could not be attributed to the ovarian stimulation protocol. Additionally, the regular menstrual cycle, patent fallopian tubes, and primary infertility of this patient whose spouse

has normal semen suggested that the oocyte maturation arrest occurred in natural cycles. It has been reported that the supplementation of gonadotropins in culture medium augments the maturation and fertilization of human immature oocytes *in vitro*.¹⁹ However, *in vitro* maturation treatment by HMG supplementation did not promote maturation of the patient's oocytes after a 48-h culture.

TUBB8 is composed of four exons and three introns, and most of the previously identified mutations in *TUBB8* are located in exon 4. In this study, a novel heterozygous mutation, c.1054G > T, in exon 4 of *TUBB8* was identified in the patient with oocyte maturation arrest and her elder sister. c.1054G > T caused an amino acid change from a hydrophobic alanine residue to a hydrophilic serine residue in the TUBB8

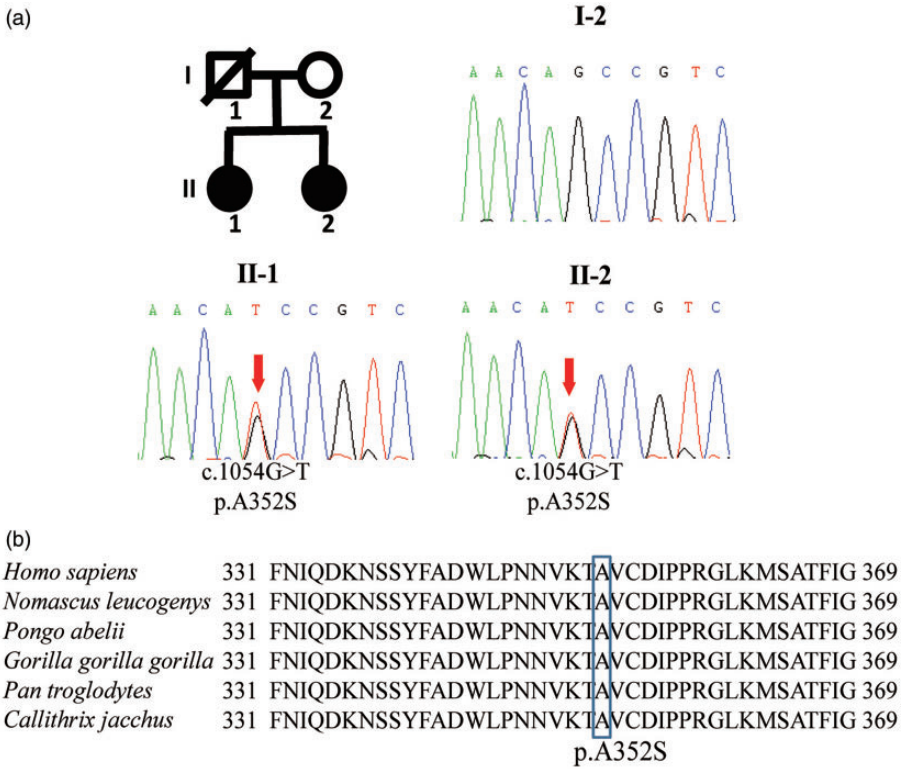


Figure 2. Genetic analysis of the family. (a) The pedigree of the family. Slash indicates a deceased individual. Sanger sequencing chromatographs of *TUBB8* in available family members revealing a heterozygous mutation in the patient and her elder sister, as indicated by red arrows. (b) Sequence alignment of the *TUBB8* protein and its homologs from five primate species revealing conservation of the affected amino acid, as indicated by the blue frame

protein (p.A352S), which is predicted to be damaging. Furthermore, the alanine residue is highly conserved among primate species, while p.A352S is located next to the previously identified heterozygous missense mutation p.V353I which was responsible for arresting four out of eight oocytes at meiosis I.¹⁴ A352 and V353 are buried within the β -tubulin structure, and mutations at these amino acids could affect the folding or stability of β -tubulin, thus impairing microtubule behavior and spindle assembly by dominant negative effects. Therefore, we propose that the A352S mutation affects microtubule dynamics and spindle assembly in a similar way to the V353I mutation.

In conclusion, we identified a novel missense mutation of *TUBB8* in a patient with oocyte maturation arrest. This finding not only substantiates the key role of *TUBB8* in human oocyte maturation, but also extends the spectrum of *TUBB8* mutations.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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