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GENETICS

A novel compound heterozygous mutation in *TUBB8* causing early embryonic developmental arrest

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Abstract

Purpose Mutations in the β-tubulin isotype, *TUBB8*, can cause female infertility. Although several mutations of *TUBB8* have been reported, the full spectrum for guiding genetics counseling still needs to be further explored. Here, we sought to identify novel variants in *TUBB8* and their phenotypic effects on microtubule network structure in vitro.

Methods Whole-exome sequence analysis was performed in two families with infertility to detect pathogenic variants, with validation by Sanger sequencing. All gene variants and protein structures were predicted in silico. Cells were transfected with wild-type and mutants, and immunofluorescence analysis was performed to visualize microtubule network changes.

Results We detected a novel compound heterozygous mutation, c.915_916delCC (p.Arg306Serfs*21) and c.82C > T (p.His28Tyr), and a benign heterozygous variant c.1286C > T (p.Thr429Met) in *TUBB8* in the two families. Female patients with p.Arg306Serfs*21 and p.His28Tyr were infertile with early embryonic developmental arrest. The female patient with p.Thr429Met gave birth to a healthy baby in the second in vitro fertilization frozen embryo transfer cycle. The p.Arg306Serfs*21 mutation was predicted to cause large structural alteration in the TUBB8 protein and was confirmed to produce a truncated and trace protein by western blot analysis. Immunofluorescence analysis of transfected HeLa cells showed that p.Arg306Serfs*21 significantly disrupted microtubule structure.

Conclusions Our findings expand the known mutational spectrum of *TUBB8* associated with early embryonic developmental arrest and female infertility.

Keywords *TUBB8* · Infertility · Compound heterozygous mutation · Microtubule

Introduction

Normal gamete maturation, sperm–oocyte fertilization, and embryonic development are essential to successful human reproduction [1, 2], and failure at any stage can lead to infertility. Two meiotic divisions are needed for oocytes to mature, and after fertilization, zygotes initiate mitosis to produce embryos for implantation. Although the oocyte meiotic spindle differs from the mitotic spindle in several respects [3, 4], microtubules are the main structural component in both [5]. Tubulins are basic microtubule proteins [6] that play an important role in spindle assembly and chromosome separation. Microtubules are dynamic polymers assembled from heterodimers of one α-tubulin and one β-tubulin polypeptide (α/β tubulin). The human genome contains seven α-tubulin and eight β-tubulin members, of which *TUBB8* encodes a special β-tubulin isotype, a major component of the oocyte and early embryo spindle, exists only in primates [7–9]. Mutations in *TUBB8* cause oocyte maturation defect 2 (OOMD2) and several consequent phenotypes including (i) metaphase I (MI) arrest [7, 10, 11], (ii) metaphase II (MII) arrest [12, 13], (iii) cleavage arrest [14, 15], (iv) early embryonic developmental arrest [16–18], (v) oocytes with large polar bodies [19, 20], and (vi) zygotes containing multiple pro-nuclei that subsequently arrest at an early stage [21, 22]. To date, a total of 139 *TUBB8* variants

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have been reported, including 113 heterozygous mutations, 15 homozygous mutations, and 11 compound heterozygous variants (Supplemental Table 1). These three types of mutations in *TUBB8* are all considered to primarily cause MI arrest, but some arrest during fertilization, cleavage, or early embryonic development also occurs.

Here, we report a novel compound heterozygous mutation that causes early embryonic developmental arrest as well as a benign heterozygous variant that leads to successful pregnancy. The new findings expand the known mutational spectrum in *TUBB8* to inform genetic counseling.

Materials and methods

Human subjects

Two patients diagnosed with female infertility were recruited from Chenzhou No.1 People's Hospital and the Second Xiangya Hospital, respectively. Peripheral blood samples of the probands ($n = 2$) and their family members ($n = 10$) were taken for DNA extraction. The studies were approved by the Ethics Committee of the Second Xiangya Hospital. All participants provided informed consent to participate in the research.

Exome and variant screening

Five-ml peripheral blood samples were obtained from two patients and their relatives. Genomic DNA was extracted from blood samples using DNA extraction kits (Tiangen Biotech, Beijing, China). Exome capture and sequencing were performed using Agilent SureSelect Whole-Exome capture (Agilent, Santa Clara, CA) and Illumina sequencing technology (Illumina, San Diego, CA). The Illumina bioinformatics analysis pipeline was used for data analysis. Basic bioinformatics analysis included mapping the raw FASTQ files to the human reference sequence (UCSC hg19). Single-nucleotide variants and short insertions and deletions were filtered according to the functional annotation using an in-house next-generation sequencing analysis platform and public databases. All suspected pathogenic gene variants were queried in the Human Gene Mutation Database (HGMD)(http://www.hgmd.cf.ac.uk) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar). SIFT, Polyphen2, Mutation Taster, FATHMM-MKL, PROVEAN, and ExAC (http://exac.broadinstitute.org/) were also applied to predict the functional effects of the variants. A *TUBB8* variant was considered a candidate mutation if it had a frequency below

Table 1 Clinical characteristics of patients and their retrieved oocytes and embryos

Age (years)	Duration of infertility (years)	Previous IVF/ICSI cycles	Total no. of oocyte retrieved	PB1 oocyte	Oocyte with abnormal morphology	Fertilized oocyte	3PN	No. of cleavage embryos	Embryos arrested at early stage	Usable embryos	Outcome of ET
38	12	1	7	7	0	6	1	5	4	1 (4cIII)	Failure
29	2	3	10	9	1	9	2	9	1(3PN)	5 (3 are high-quality)	Delivered at the 2nd ET

Abbreviation: *ICSI*, intracytoplasmic sperm injection; *IVF*, in vitro fertilization; and *ET*, embryo transfer

0.1% in three public databases: the 1000 Genomes variant database, the NHLBI exome sequencing project, and ExAC. Changes in protein structure and the conservation and tolerance of amino acid variants were predicted using Alphafold2, PyMOL 1.7.4 software, and MetaDome online.

Sanger sequencing

Candidate mutations in *TUBB8* were examined in patients, immediate family members, and 150 normal controls by Sanger sequencing. *TUBB8* exon's

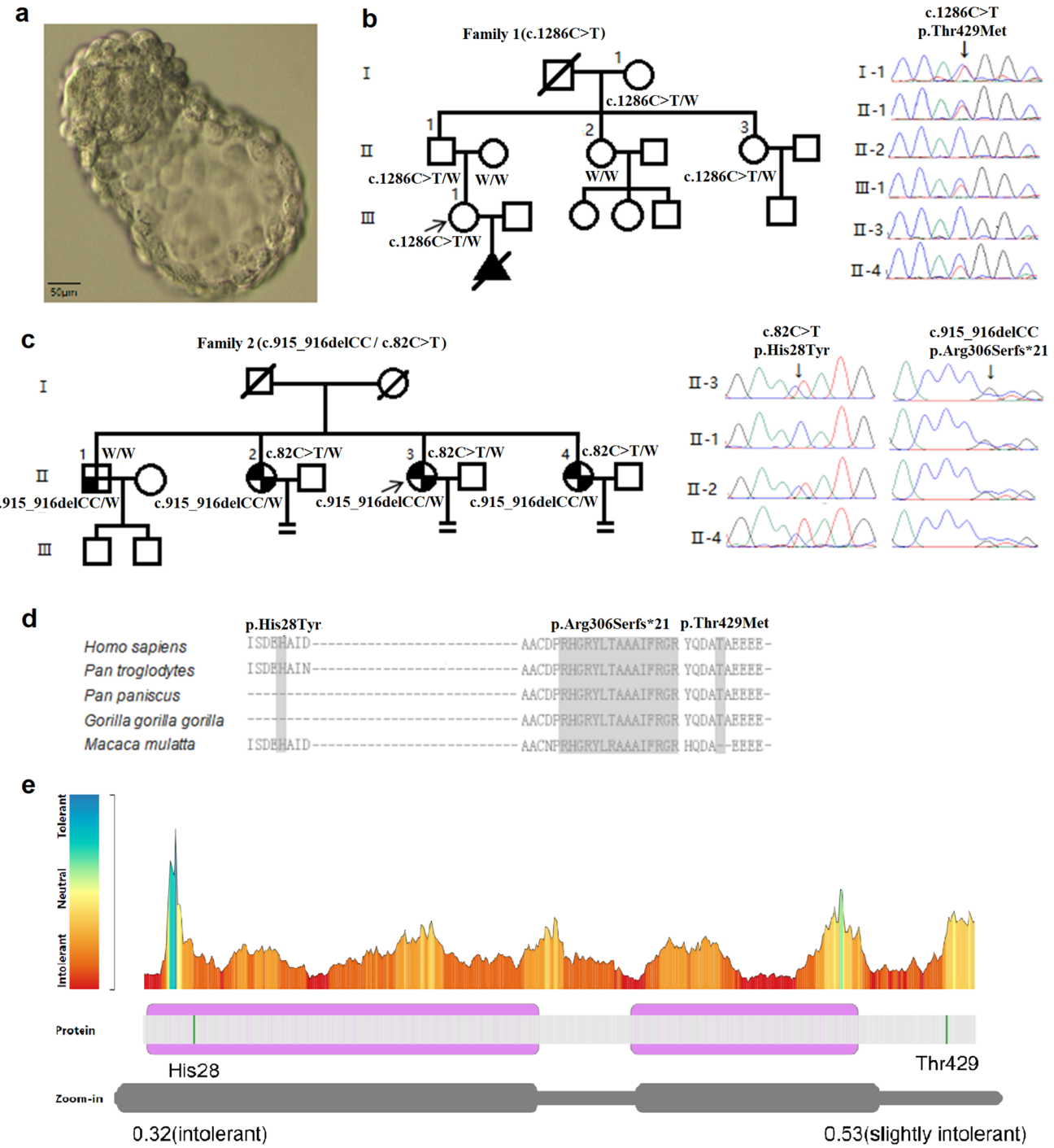


Fig. 1 Genetic analysis of *TUBB8* mutants. **a** Morphology of the blastocyst transferred in the second FET cycle from the patient in family 1. **b** Pedigree of the family 1 and Sanger sequencing of the family members. **c** Pedigree of the family 2 and Sanger sequencing of the family members. **d** Conservation analysis of altered amino acids in five primate species. **e** The graph represents the tolerance of TUBB8 to amino acid mutations. Each amino acid of TUBB8 is classified according to its tolerance score

Table 2 Effects of TUBB8 mutations predicted with in silico tools

Mutations	Amino acid change	Genotype	SIFT[a]	Polyphen2[b]	Mutation taster[c]	PROVEAN[d]	Fathmm_MKL[e]	1000 genomes[f]	gnomad_exon_total[g]	gnomad_exon_east Asian[h]
c.915_916del	p.Arg306Serfs*21	com-het	NA	NA	NA	NA	NA	NA	NA	NA
c.82C > T	p.His28Tyr	com-het	Damaging (0.0)	Damaging (0.999)	Disease causing (1)	Damaging (−2.63)	Damaging (0.900)	NA	2.596e-05	0.0004
c.1286C > T	p.Thr429Met	het	Damaging (0.0)	Probably damaging (0.989)	Disease causing (1)	Tolerable (−1.72)	Damaging (0.805)	0.000199681	2.97e-05	0.0002

[a]*SIFT*, sorting Intolerant from tolerant (http://sift.jcvi.org/)

[b]Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/)

[c]Mutation Taster (http://www.mutationtaster.org/)

[d]PROVEAN (http://provean.jcvi.org/protein_batch_submit.php?species=human)

[e]FATHMM-MKL (http://fathmm.biocompute.org.uk/fathmmMKL.htm)

[f]Frequency of variation in the 1000 Genomes database

[g]Frequency of variation in the total of exome gnomAD (genome Aggregation Database) (version 2.1)

[h]Frequency of variation in the East Asian population of exome gnomAD (version 2.1)

amplification primers and sequencing primers were quoted from the References 18 (Supplemental Table 2). PCR amplification was performed using the Q5 High-Fidelity Polymerase (NEB, MA, USA). PCR products were sequenced for the initial screening by Tsingke Biotechnology Co., Ltd (Beijing, China). All PCR products were sequenced using the ABI 3730XL automated sequencer according to the manufacturer's instructions (Applied Biosystems, Foster City, CA), and the sequencing results were analyzed using Chromas software (v2.6.5, Technelysium Pty Ltd, South Brisbane, Australia).

Expression of wild-type and mutant TUBB8 in cultured cells

A full-length *TUBB8* cDNA cloned in a pCDNA3.1 (+) vector with a CMV promoter and an in-frame C-terminal FLAG tag was purchased from Chubo BioTech, Inc. Point mutations were generated by quick-change polymerase chain reaction for the expression of the wild-type (WT). For transient expression of wild-type or mutant *TUBB8*, approximately 20,000 HeLa cells were seeded into 24-well plate 16 h before transfection. 0.1 μg (low density) or 0.5 μg (high density) plasmids were transfected into HeLa cells using 0.5ul or 2.5 μl Lipofectamine 2000 per well according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA). Forty-eight hours after transfection, the cells were fixed, permeabilized, and labeled with antibodies targeting to the FLAG epitope (1:200, #14,793) (Cell Signaling Technology, Beverly, MA, USA) and α-tubulin (1:200, #3873) (Cell Signaling Technology, Beverly, MA, USA). To quantify microtubule phenotypes, over 200 cells expressing either wild-type or mutant *TUBB8* were examined in each of the three independent experiments. All FLAG-positive cells were classified according to the level of expression of the transgene as judged by the fluorescence intensity and microtubule structure, where abnormal microtubule structures were visualized as diffuse, mottled FLAG labeling as previously described.

In order to detect whether the expression level of wild-type and mutant proteins is different, approximately 350,000 HeLa cells were seeded into 6-well plate 16 h before transfection. 2.5 μg plasmids (1.25 μg c.82C > T plasmids and 1.25 μg c.915_916delCC plasmids in p.H28Y & p.R306S*21 group) were transfected into HeLa cells using 15 μl Lipofectamine 2000 per well according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA). Forty-eight hours after transfection, cultured Hela cells were dissolved in RIPA lysis buffer (Beyotime, China)

and proteinase inhibitor. The protein expression was detected by Western blot.

Results

Clinical characteristics and phenotypes of oocytes/embryos harboring TUBB8 mutations

Two patients with female infertility were identified. In family 1, the patient was diagnosed with secondary infertility at 29 years of age after spontaneous abortion during the first trimester 2 years previously. Ten oocytes were retrieved from three IVF cycles; of nine matured oocytes, seven fertilized normally, and the other two were three pro-nuclei. Five embryos were usable and vitrified (Table 1), three of which were high-quality. At the first frozen embryo transfer (FET), two blastocysts (3BB and 3BC) were thawed and transferred but failed. At the second FET, one blastocyst (6AA, Fig. 1a) was transferred, and the patient conceived and gave birth to a healthy baby.

In family 2, the proband had been diagnosed with primary infertility 12 years previously at 38 years of age and underwent one intracytoplasmic sperm injection (ICSI) cycle, from which seven oocytes were obtained. All seven oocytes matured and six were fertilized, five of which arrested at an early stage. One four-cell embryo was transferred on D3 but failed to produce a pregnancy (Table 1). The patient's two sisters also suffered from primary infertility: the elder sister underwent three IVF/ICSI cycles but also failed to become pregnant due to early embryonic developmental arrest. Eventually, the patient conceived through oocyte donation.

Novel TUBB8 variants

Whole-exome sequencing analysis of the two probands with validation by Sanger sequencing implicated a heterozygous missense mutation c.1286C > T (p.Thr429Met) in family 1 and a novel compound heterozygous mutations c.915_916delCC (p.Arg306Serfs*21) and c.82C > T (p.His28Tyr) in family 2. In family 1, the *TUBB8* p.Thr429Met mutation was detected in four family members (I-1,II-1,II-3,and III-1, family 1), two of whom were female with offspring (I-1, II-3, family 1, Fig. 1b). All three sisters in family 2 (II-2, II-3, and II-4, family 2) harbored the same compound heterozygous mutations and suffered primary infertility (Fig. 1c).

In silico analysis of TUBB8 variants

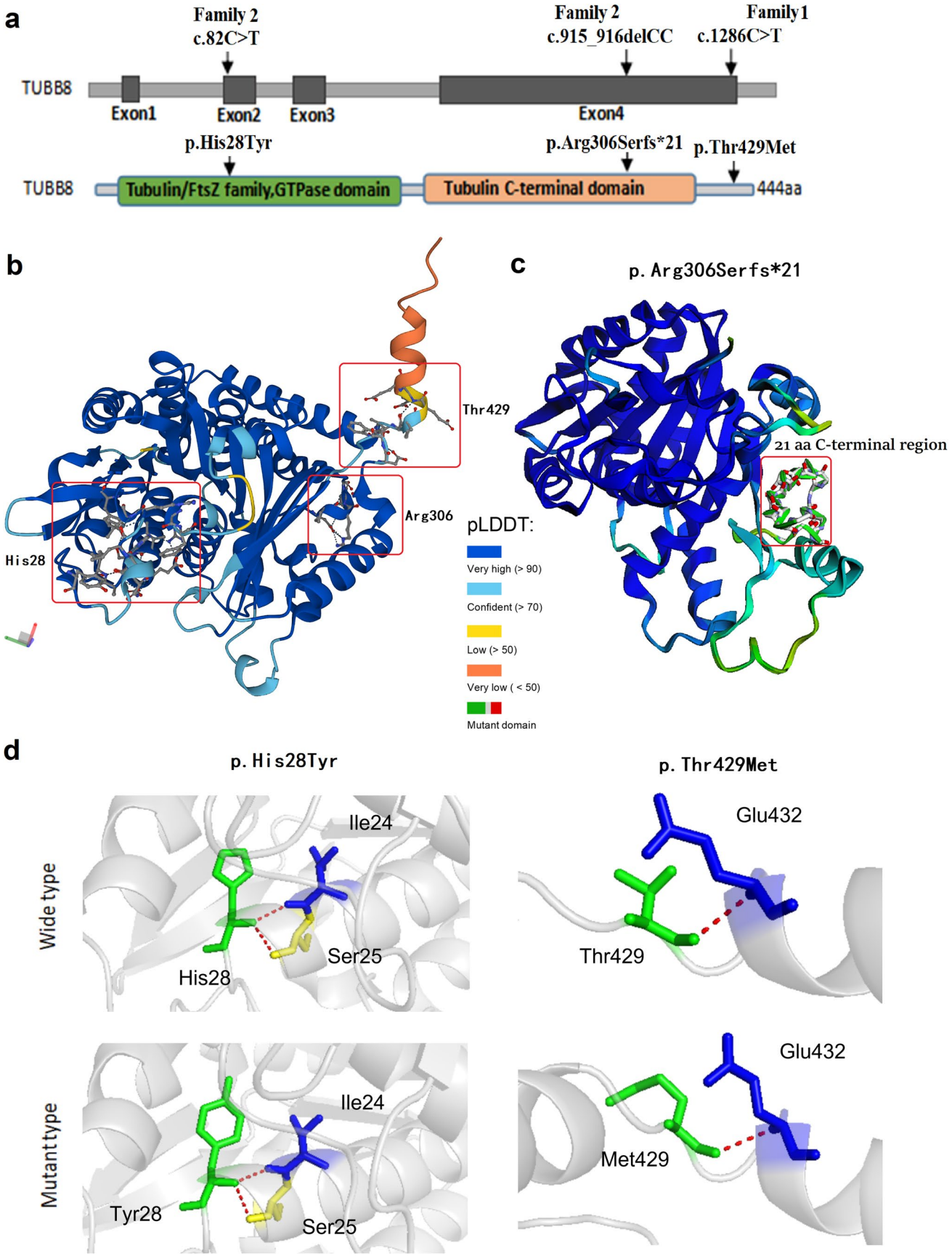
According to SIFT, Polyphen2, MutationTaster, PROVEAN, and FATHMM-MKL, the p.His28Tyr mutation was predicted to be damaging, while the p.Thr429Met mutation was predicted to be damaging or possibly damaging (Table 2). No prediction tool derived scores for the p.Arg306Serfs*21 mutation (Table 2), which is a frameshift mutation resulting in a premature stop codon that predicts a truncated protein from 444 to 326 amino acids and a terminal 21 amino acid sequence bearing no homology to the corresponding amino acid sequence of TUBB8. The residues altered by p.Arg306Serfs*21 in TUBB8 are highly evolutionarily conserved in primates, but the residues altered by p.His28Tyr and p.Thr429Met are not (Fig. 1d). p.28 (c.82–84) corresponds to a histidine residue with a tolerance score of 0.32, indicating intolerance to mutations at this site. Similarly, p.429 (c.1285–1287) corresponds to a threonine with a tolerance score of 0.53, indicating slight intolerance to the mutation at this locus (Fig. 1e).

Alterations in TUBB8 protein structure

The TUBB8 protein contains a tubulin domain (aa 47–244), a tubulin C-terminal domain (aa 246–383), a coiled-coil domain (aa 405–444), and other domains. The p.His28Tyr variant is located in the tubulin domain, p.Arg306Serfs*21 in the tubulin C-terminal domain, and p.Thr429Met in the coiled-coil domain (Fig. 2a). We predicted the three-dimensional structure of wild-type TUBB8 using Alphafold2 based on the Q76FS2 PDB template (Fig. 2b). The entire protein model of the p.Arg306Serfs*21 mutation was also predicted, especially the 21 aa C-terminal region (Fig. 2c). In p.Arg306Serfs*21, the original α-helix and β-fold regions near the mutation site were lost and resulted in the loose structure of the entire protein. For the two missense variants, p.His28Tyr and p.Thr429Met, the protein 3D structure and atomic model were rendered by PyMOL 1.7.4 software. It showed that there were no obvious effects on the protein structure and did not display changes in hydrogen bonding. Both the original His28 residue and replacement Tyr28 residue formed hydrogen bonds with Ile24 and Ser25, while the original Thr429 residue and the replacement Met429 residue formed hydrogen bonds with Glu432 (Fig. 2d).

TUBB8 variants disrupt microtubule structure in HeLa cells

To determine the influence of the *TUBB8* mutations on microtubule behavior in vivo, we transfected FLAG-tagged constructs into HeLa cells (Fig. 3a). When exogenous


a
Family 2
c.82C>T
Family 2
c.915_916delCC
Family1
c.1286C>T
TUBB8
Exon1
Exon2
Exon3
Exon4
p.His28Tyr
p.Arg306Serfs*21
p.Thr429Met
TUBB8
Tubulin/FtsZ family,GTPase domain
Tubulin C-terminal domain
444aa
b
Thr429
Arg306
His28
c
p. Arg306Serfs*21
21 aa C-terminal region
pLDDT:
Very high (> 90)
Confident (> 70)
Low (> 50)
Very low (< 50)
Mutant domain
d
p. His28Tyr
p. Thr429Met
Wide type
Ile24
Ser25
His28
Glu432
Thr429
Mutant type
Ile24
Tyr28
Ser25
Glu432
Met429

◂**Fig. 2** Protein conformation predictions caused by variants in TUBB8 using Alphafold2 and PyMol. **a** The positions of altered alleles are shown on the *TUBB8* gene, and the corresponding amino acids are indicated on the TUBB8 protein. **b** The 3D protein structure of wild-type TUBB8 predicted by Alphafold2. **c** The predicted protein conformation of p.Arg306Serfs*21 by Alphafold2 shows a large structural alteration. **d** TUBB8 variants were mapped to the atomic model using PyMol. Red dashed lines represent hydrogen bonds

TUBB8 proteins were expressed at a relatively low level, the wild-type and mutant proteins had little effect on the organization of the cytoplasmic microtubule network. High expression of p.His28Tyr or p.Thr429Met mutant TUBB8 was also typically associated with normal microtubule phenotypes, but p.Arg306Serfs*21 or p.Arg306Serfs*21 and p.His28Tyr mutations were significantly more frequently abnormal than wild-type (Fig. 3b) ($P < 0.01$). Moreover, the p.Arg306Serfs*21 (85.0%) mutation was significantly more frequently abnormal (Fig. 3b) ($P < 0.05$) than p.Arg306Serfs*21 and p.His28Tyr co-transfection (58.7%). It is worth noting that p.Arg306Serfs*21 shows a diffuse mottled stain throughout the cytoplasm, with no evidence of co-assembly into microtubules. Western blot analysis confirmed that p.Arg306Serfs*21 mutation resulted in a truncated protein (326 N-terminal amino acids rather than the full-length 444 amino acid polypeptide) (Fig. 3c) and a dramatic lowered expression (2.8%, $P < 0.001$) than that of wild-type (Fig. 3d), resulting in a severely impaired protein function following a loss-of-function mechanism. The TUBB8 expression of p.His28Tyr mutation also showed significantly decreased, while p.Thr429Met mutation showed no significant influence. The expression of mutant proteins showed the same trend as the proportion of normal cells detected by immunofluorescence.

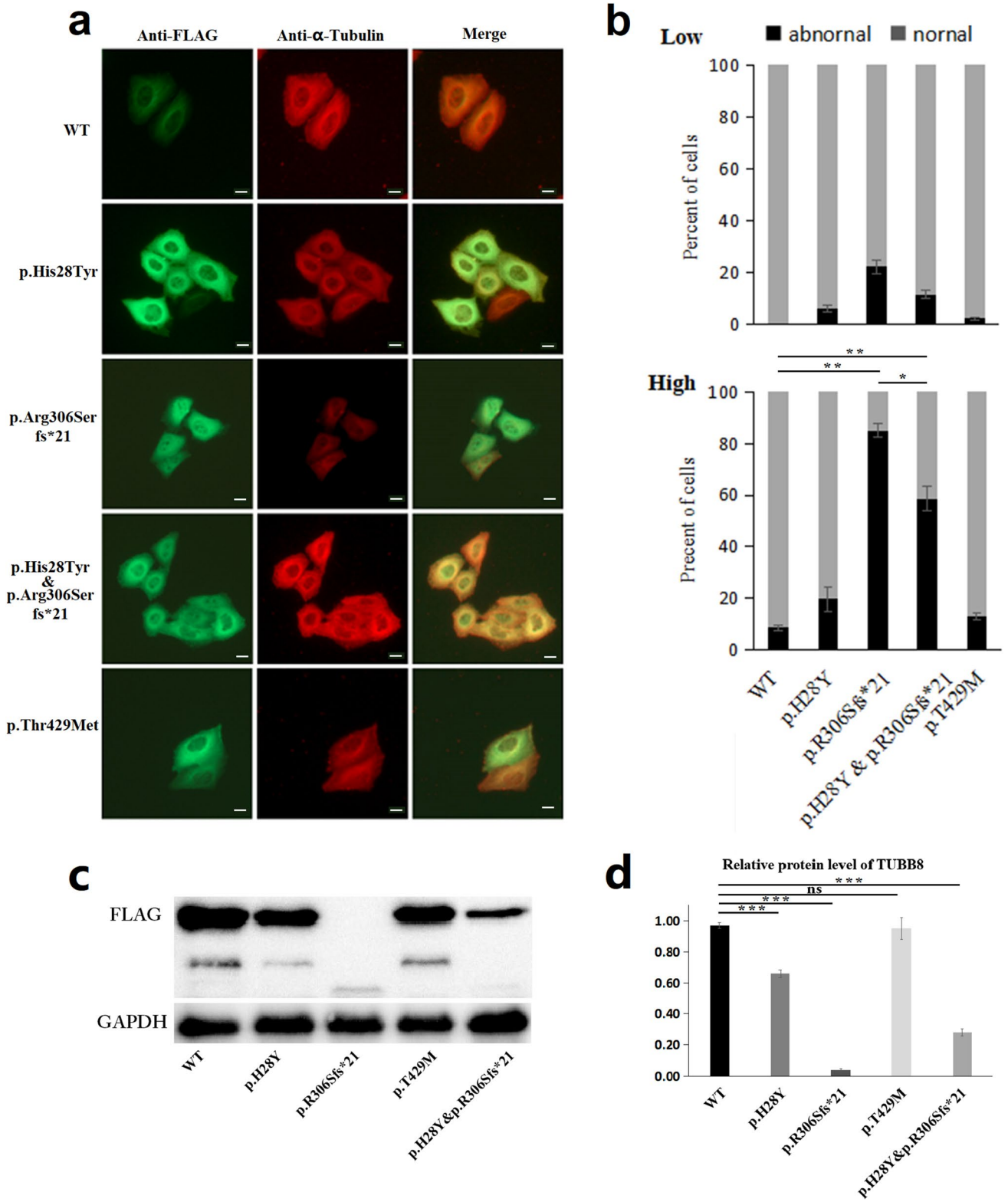
Discussion

Here, we identified a new compound heterozygous variant and a benign heterozygous variant in *TUBB8* in four female patients from two families. All the three sisters harboring the compound heterozygous c.915_916delCC and c.82C > T variant were infertile, two of whom had undergone failed IVF/ICSI attempts due to early embryonic development arrest. The infertile patient with the heterozygous c.1286C > T variant obtained high-quality embryos, failed in the first FET cycle, but conceived from the second FET cycle. These results expand the mutational and phenotypic spectrum of *TUBB8*.

According to the clinical information of 11 compound heterozygous mutations reported in 11 patients (Tables 3 and 4), compound heterozygous mutations in *TUBB8* mainly cause compound phenotypes including meiotic arrest, poor fertilization, complete cleavage failure, and embryonic arrest but not total MI arrest. Consistent with these reports, the compound heterozygous mutations reported here mainly caused early embryonic developmental arrest.

The *TUBB8* c.82C > T mutation has been found in two other reported compound heterozygous mutations, c.82C > T/c.148_154delins and c.82C > T/c.398 T > C. In the c.82C > T/c.148_154delins mutation, c.82C > T was from the patient's father, and c.148_154delins was from her mother. The inheritance pattern of the c.82C > T/c.398 T > C mutation is unknown. Since both parents in family 2 were deceased and only the c.915_916delCC mutation was detected in the brother, we presumed the inheritance pattern of the c.915_916delCC/c.82C > T mutations in our three patients was compound heterozygosity. According to our results, although the expression of p.His28Tyr is lower than that of wile-type, it did not result in abnormal microtubule networks in vitro, and there is no report of a heterozygous c.82C > T mutation causing female infertility, so the pathogenicity of c.82C > T needs further study to confirm.

The *TUBB8* c.915_916delCC mutation is a novel mutation that clearly disturbed the cytoplasmic microtubule network in cultured cells, and its effect was even worse than c.82C > T and c.915_916delCC co-transfection. p.Arg306Serfs*21 shows a diffuse mottled stain throughout the cytoplasm, suggesting that it cannot form functional microtubules with α-tubulin. The productive folding of α-tubulin and β-tubulin and their integration into heterodimers require the interaction of newly synthesized polypeptides with several molecular chaperones. These include prefoldin; the cytosolic chaperonin, CCT; and five tubulin specific chaperones termed TBCA-E that function in concert as a GTP-dependent heterodimer assembly nanomachine [8]. Western blot analysis confirmed that p.R306Sfs*21 plasmid produced a truncated and trace protein, which implied the p.Arg306Serfs*21 variant resulted in a severely impaired protein function following a loss-of-function mechanism. We predict that it was degraded due to the change


a
Anti-FLAG
Anti-α-Tubulin
Merge
WT
p.His28Tyr
p.Arg306Ser fs*21
p.His28Tyr & p.Arg306Ser fs*21
p.Thr429Met
b
Low
abnormal
nornal
Percent of cells
High
Precent of cells
WT
p.H28Y
p.R306Sfs*21
p.H28Y & p.R306Sfs*21
p.T429M
c
FLAG
GAPDH
WT
p.H28Y
p.R306Sfs*21
p.T429M
p.H28Y&p.R306Sfs*21
d
Relative protein level of TUBB8
ns
WT
p.H28Y
p.R306Sfs*21
p.T429M
p.H28Y&p.R306Sfs*21

◂**Fig. 3** HeLa cells were transfected with constructs engineered to express FLAG-tagged TUBB8 (wild-type and mutant). **a** The results of immunostaining with antibodies targeting the FLAG epitope to detect expression of the transgene (green) and antibody to α-tubulin to detect the endogenous microtubule network (red). Scale bar = 5 μm. **b** Quantitative analysis of the microtubule phenotypes shown in **a**. Approximately 200 transfected cells expressing either wild-type or mutant TUBB8 were examined in three separate experiments. Bars indicate standard deviations. $^{*}P < 0.05$, $^{**}P < 0.01$. **c** The expression level of WT, p.H28Y, p.R306Sfs*21, p.T429M, and p.H28Y & p.R306Sfs*21 TUBB8 protein in Hela cells detected by Western Blot. **d** Quantitative analysis of expression level of wild-type and mutant TUBB8 shown in **c**. Bars indicate standard deviations. $N = 3$ independent transfections. $^{***}P < 0.001$; ns, no significance

of three-dimensional structure that cannot combine with molecular chaperones, like another truncated TUBB8 protein, T143Dfs*12 [8]. Consistent with previous studies, the loss-of-function variants in *TUBB8* usually affect female fertility in a homozygous or compound heterozygous pattern, implying the haploinsufficiency mechanism, while the missense variants mostly present as heterozygous pattern, implying the dominant-negative mechanism of *TUBB8* variant-related phenotypes.

Our expanded analysis of family 1 showed that the patient's grandma, father, and younger aunt were all fertile, even when the c.1286C > T heterozygous variant was present, i.e., a heterozygous c.1286C > T mutation did not cause infertility in this family. Furthermore, immunofluorescence analysis confirmed that the c.1286C > T variant did not cause significant microtubule network abnormality in vitro. Extended pedigree segregation and mutational effect assays are indispensable for determining the true pathogenicity of mutants when patients acquire usable embryos, especially high-quality embryos. Therefore, we reported to the patient in family 1 that the *TUBB8* c.1286C > T variant does not affect fertility and advised that her oocytes were suitable for assisted reproductive treatment.

Few *TUBB8* variants have been examined in functional assays for classification according to the American College of Medical Genetics and Genomics (ACMG) guidelines, making them suitable as genetic markers for counseling [23]. For patients with likely pathogenic mutations, oocyte donation may be the most feasible infertility treatment approach at present, while the pathogenicity of other *TUBB8* variants undergoes further definitive evaluation. When other variants in *TUBB8* or other OOMD genes (*PADI6* [24], *WEE2* [25], *PATL2* [26], *PANX1* [27], *TRIP13* [28], *LHX8* [29], *CDC20* [30], *ZP1* [31], *ZP2* [32], *ZP3* [33], *BTG4* [34], *TLE6*[35], *NLRP2* [36], *NLRP5* [37], *KHDC3L* [38], and *REC114* [39]) of uncertain significance are detected in female infertile patients who fail ART, receptive endometrium and synchronized development between the embryo and the endometrium

Table 3 Overview of the 11 reported compound heterozygous variants in TUBB8

cDNA alteration	Amino acid alteration	Inheritance pattern	Phenotypes				
			MA	PF	CCF	EA	NA
c.580G > A/c.1245G > A (18)	p.E194K/p.M415I	De novo/de novo	√			√	
c.322G > A/c.426dupG (16)	p.E108K/p.T143Dfs*12	Inherited-pa/inherited-ma		√	√		
c.1286C > T/c.1301_1327del (16)	p.T429M/p.434-442del	Inherited-ma (both are from her mother)					√
c.1103 T > C/c.382dup (19)	p.I368T/p.D128Gfs*27	Inherited-ma/inherited-pa	√	√			
c.1103 T > C/c.80_100del (19)	p.I368T/p.E27_A33del	Unknown/inherited-ma	√		√		
c.82C > T/c.148_154delins (19)	p.H28Y/p.Y50_N52delins	Inherited-pa/inherited-ma	√				
c.400C > T/c.353A > G (19)	p.Q134*/p.D118G	Inherited-ma/inherited-pa	√		√		
c.82C > T/c.398 T > C (19)	p.H28Y/p.F133S	Unknown				√	
c.722G > C/c.1190_1192dup (17)	p.R241P/p.W397dup	Inherited-ma/inherited-pa	√			√	
c.269dupT/c.426dupG (17)	p.I91Hfs*35/p.T143Dfs*12	Unknown/inherited-pa	√	√	√		
c.322G > A/c.966dupC (17)	p.E108K/p.M323Hfs*6	Inherited-pa/inherited-ma			√		

Abbreviation: MA, oocyte meiotic arrest; PF, poor fertilization; CCF, complete cleavage failure; EA, embryonic arrest; NA, not available

Table 4 Clinical characteristics of the eleven reported patients with compound heterozygous mutations in TUBB8

cDNA alteration	Age (years)	Duration of infertility (years)	Previous IVF/ICSI cycles	Total no. of oocyte retrieved	GV oocyte	MI oocyte	PB1 oocyte	Oocyte with abnormal morphology	Immature oocyte (unknown stage)	Fertilized oocyte	No. of cleavage embryos	Embryos arrested at early stage	Usable embryos	Outcome of ET
c.580G > A/c.1245G > A (18)	28	2	2	19	0	0	3	0	16	3	3	3	0	
c.322G > A/c.426dupG (16)	39	12	2	27	1	4	20	2	0	2	0	/	0	
c.1286C > T/c.1301_1327del (16)	Had no clinical information													
c.1103 T > C/c.382 dup (19)	30	4	2	13	0	9	2	0	2	0	0	0	0	
c.1103 T > C/c.80_100del (19)	26	/	1	10	0	9	1	0	0	1	0	0	0	
c.82C > T/c.148_154delins (19)	35	9	4	31	0	0	11	0	20	11	8	/	2	Failure
c.400C > T/c.353A > G (19)	31	7	3	51	0	1	0	44	50	44	0	/	0	
c.82C > T/c.398 T > C (19)	33	11	1	12	0	2	8	2	0	3	1	1	0	
c.722G > C/c.1190_1192dup (17)	34	9	2	36	16	15	4	0	0	4	3	3	0	
c.269dupT/c.426dupG (17)	24	4	1	30	0	9	9	8	4	2	0	/	0	
c.322G > A/c.966dupC (17)	28	4	1	15	2	0	13	0	0	11	0	/	0	

should both be taken into account together with use of high-quality embryos to optimize outcomes [40].

In conclusion, here we present a novel compound heterozygous mutation in *TUBB8* that causes familial female infertility and another benign heterozygous mutation, expanding the known mutational spectrum in *TUBB8* associated with early embryonic development arrest.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate The study was approved by the Institute Review Board of the Department of Reproductive Medicine, Central South University. Informed consent was obtained from all individual participants included in the study.

Consent for publication Patients signed informed consent regarding publishing their data and photographs.

Competing interests The authors declare no competing interests.

References

1. Dean J. Exacting requirements for development of the Egg. N Engl J Med. 2016;374(3)(1533–4406 (Electronic)):279–80.
2. Li R, Albertini DF. The road to maturation: somatic cell interaction and self-organization of the mammalian oocyte. Nat Rev Mol Cell Biol. 2013;14(3):141–52.
3. Manandhar G, Schatten H, Sutovsky P. Centrosome reduction during gametogenesis and its significance. Biol Reprod. 2005;72(1):2–13.
4. Schuh M, Ellenberg J. Self-organization of MTOCs replaces centrosome function during acentrosomal spindle assembly in live mouse oocytes. Cell. 2007;130(3):484–98.
5. Janke C, Magiera MM. The tubulin code and its role in controlling microtubule properties and functions. Nat Rev Mol Cell Biol. 2020;21(6):307–26.

6. Janke C. The tubulin code: molecular components, readout mechanisms, and functions. J Cell Biol. 2014;206(4):461–72.
7. Feng R, Sang Q, Kuang Y, Sun X, Yan Z, Zhang S, et al. Mutations in TUBB8 and human oocyte meiotic arrest. N Engl J Med. 2016;374(3):223–32.
8. Feng R, Yan Z, Li B, Yu M, Sang Q, Tian G, et al. Mutations in TUBB8 cause a multiplicity of phenotypes in human oocytes and early embryos. J Med Genet. 2016;53(10):662–71.
9. Sirajuddin M, Rice LM, Vale RD. Regulation of microtubule motors by tubulin isotypes and post-translational modifications. Nat Cell Biol. 2014;16(4):335–44.
10. Huang L, Tong X, Luo L, Zheng S, Jin R, Fu Y, et al. Mutation analysis of the TUBB8 gene in nine infertile women with oocyte maturation arrest. Reprod Biomed Online. 2017;35(3):305–10.
11. Yao Z, Zeng J, Zhu H, Zhao J, Wang X, Xia Q, et al. Mutation analysis of the TUBB8 gene in primary infertile women with oocyte maturation arrest. J Ovarian Res. 2022;15(1):38.
12. Cao T, Guo J, Xu Y, Lin X, Deng W, Cheng L, et al. Two mutations in TUBB8 cause developmental arrest in human oocytes and early embryos. Reprod Biomed Online. 2021;43(5):891–8.
13. Lu Q, Zhang X, Cao Q, Wang C, Ding J, Zhao C, et al. Expanding the genetic and phenotypic spectrum of female infertility caused by TUBB8 mutations. Reprod Sci. 2021;28(12):3448–57.
14. Yuan P, Zheng L, Liang H, Li Y, Zhao H, Li R, et al. A novel mutation in the TUBB8 gene is associated with complete cleavage failure in fertilized eggs. J Assist Reprod Genet. 2018;35(7):1349–56.
15. Jia Y, Li K, Zheng C, Tang Y, Bai D, Yin J, et al. Identification and rescue of a novel TUBB8 mutation that causes the first mitotic division defects and infertility. J Assist Reprod Genet. 2020;37(11):2713–22.
16. Chen B, Wang W, Peng X, Jiang H, Zhang S, Li D, et al. The comprehensive mutational and phenotypic spectrum of TUBB8 in female infertility. Eur J Hum Genet. 2019;27(2):300–7.
17. Zheng W, Hu H, Zhang S, Xu X, Gao Y, Gong F, et al. The comprehensive variant and phenotypic spectrum of TUBB8 in female infertility. J Assist Reprod Genet. 2021;38(9):2261–72.
18. Chen B, Li B, Li D, Yan Z, Mao X, Xu Y, et al. Novel mutations and structural deletions in TUBB8: expanding mutational and phenotypic spectrum of patients with arrest in oocyte maturation, fertilization or early embryonic development. Hum Reprod. 2017;32(2):457–64.
19. Zhao L, Guan Y, Wang W, Chen B, Xu S, Wu L, et al. Identification novel mutations in TUBB8 in female infertility and a novel phenotype of large polar body in oocytes with TUBB8 mutations. J Assist Reprod Genet. 2020;37(8):1837–47.
20. Liu Z, Xi Q, Zhu L, Yang X, Jin L, Wang J, et al. TUBB8 mutations cause female infertility with large polar body oocyte and fertilization failure. Reprod Sci. 2021;28(10):2942–50.
21. Sha Q, Zheng W, Feng X, Yuan R, Hu H, Gong F, et al. Novel mutations in TUBB8 expand the mutational and phenotypic spectrum of patients with zygotes containing multiple pronuclei. Gene. 2021;769:145227.
22. Yang P, Yin C, Li M, Ma S, Cao Y, Zhang C, et al. Mutation analysis of tubulin beta 8 class VIII in infertile females with oocyte or embryonic defects. Clin Genet. 2021;99(1):208–14.
23. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–24.
24. Xu Y, Shi Y, Fu J, Yu M, Feng R, Sang Q, et al. Mutations in PADI6 cause female infertility characterized by early embryonic arrest. Am J Hum Genet. 2016;99(3):744–52.
25. Sang Q, Li B, Kuang Y, Wang X, Zhang Z, Chen B, et al. Homozygous mutations in WEE2 cause fertilization failure and female infertility. Am J Hum Genet. 2018;102(4):649–57.
26. Chen B, Zhang Z, Sun X, Kuang Y, Mao X, Wang X, et al. Biallelic mutations in PATL2 cause female infertility characterized by oocyte maturation arrest. Am J Hum Genet. 2017;101(4):609–15.
27. Wang W, Qu R, Dou Q, Wu F, Wang W, Chen B, et al. Homozygous variants in PANX1 cause human oocyte death and female infertility. Eur J Hum Genet. 2021;29(9):1396–404.
28. Zhang Z, Li B, Fu J, Li R, Diao F, Li C, et al. Bi-allelic missense pathogenic variants in TRIP13 cause female infertility characterized by oocyte maturation arrest. Am J Hum Genet. 2020;107(1):15–23.
29. Zhao L, Li Q, Kuang Y, Xu P, Sun X, Meng Q, et al. Heterozygous loss-of-function variants in LHX8 cause female infertility characterized by oocyte maturation arrest. Genet Med. 2022;24(11):2274–84.
30. Zhao L, Xue S, Yao Z, Shi J, Chen B, Wu L, et al. Biallelic mutations in CDC20 cause female infertility characterized by abnormalities in oocyte maturation and early embryonic development. Protein Cell. 2020;11(12):921–7.
31. Huang HL, Lv C, Zhao YC, Li W, He XM, Li P, et al. Mutant ZP1 in familial infertility. N Engl J Med. 2014;370(13):1220–6.
32. Zhou Z, Ni C, Wu L, Chen B, Xu Y, Zhang Z, et al. Novel mutations in ZP1, ZP2, and ZP3 cause female infertility due to abnormal zona pellucida formation. Hum Genet. 2019;138(4):327–37.
33. Cao Q, Zhao C, Zhang X, Zhang H, Lu Q, Wang C, et al. Heterozygous mutations in ZP1 and ZP3 cause formation disorder of ZP and female infertility in human. J Cell Mol Med. 2020;24(15):8557–66.
34. Zheng W, Zhou Z, Sha Q, Niu X, Sun X, Shi J, et al. Homozygous mutations in BTG4 cause zygotic cleavage failure and female infertility. Am J Hum Genet. 2020;107(1):24–33.
35. Alazami AM, Awad SM, Coskun S, Al-Hassan S, Hijazi H, Abdulwahab FM, et al. TLE6 mutation causes the earliest known human embryonic lethality. Genome Biol. 2015;16:240.
36. Mu J, Wang W, Chen B, Wu L, Li B, Mao X, et al. Mutations in NLRP2 and NLRP5 cause female infertility characterised by early embryonic arrest. J Med Genet. 2019;56(7):471–80.
37. Li M, Jia M, Zhao X, Shi R, Xue X. A new NLRP5 mutation causes female infertility and total fertilization failure. Gynecol Endocrinol. 2021;37(3):283–4.
38. Wang X, Song D, Mykytenko D, Kuang Y, Lv Q, Li B, et al. Novel mutations in genes encoding subcortical maternal complex proteins may cause human embryonic developmental arrest. Reprod Biomed Online. 2018;36(6):698–704.
39. Wang W, Dong J, Chen B, Du J, Kuang Y, Sun X, et al. Homozygous mutations in REC114 cause female infertility characterised by multiple pronuclei formation and early embryonic arrest. J Med Genet. 2020;57(3):187–94.
40. Teh WT, McBain J, Rogers P. What is the contribution of embryo-endometrial asynchrony to implantation failure? J Assist Reprod Genet. 2016;33(11):1419–30.