

RESEARCH

Open Access



Novel variants in *TUBB8* gene cause multiple phenotypic abnormalities in human oocytes and early embryos

Tingwenyi Hu^{1,2†}, Chong Li^{1,2†}, Sen Qiao³, Weiwei Liu^{1,2}, Wei Han^{1,2}, Wei Li³, Rong Shi³, Xia Xue³, Juanzi Shi^{3*}, Guoning Huang^{1,2*} and Tingting Lin^{1,2*}

Abstract

Background The genotype-phenotype relationships between *TUBB8* variants and female infertility are difficult to clearly define due to the complex inheritance patterns and the highly heterogeneous phenotypes. This study aims to identify novel *TUBB8* variants and relevant phenotypes in more infertile females.

Methods A total of 35 females with primary infertility were recruited from two reproductive centers and investigated for identifying variants in *TUBB8*. Pedigree analysis, *in-silico* analysis and molecular remodeling were performed to assess their clinical significance. The effects of the variants on human oocytes and embryos as well as HeLa cells were analyzed by morphological observations, immunostaining and Western blot.

Results We totally identified five novel variants (p.G13R, p.Y50C, p.T136I, p.F265V and p.T366A) and five previously reported variants (p.I4L, p.L42V, p.Q134*, p.V255M and p.V349I) in *TUBB8* from 9 unrelated females with primary infertility. These variants were rare and highly conserved among different species, and were inherited in autosomal dominant/recessive patterns, or occurred *de novo*. In vitro functional assays in HeLa cells revealed that exogenous expression of mutant *TUBB8* proteins caused different degrees of microtubule structural disruption. The existence of these pathogenic *TUBB8* variants finally induced oocyte maturation arrest or morphological abnormalities, fertilization failure, cleavage failure, embryonic development defects and implantation failure in the affected females.

Conclusion These findings enriched the variant spectrum of *TUBB8* gene and could contribute to optimize genetic counselling and clinical management of females with primary infertility.

Keywords Primary female infertility, Oocyte maturation arrest, *TUBB8* gene, Genetic counselling

[†]These authors contribute equally to this work.

*Correspondence:

Juanzi Shi
shijuanzi123@126.com
Guoning Huang
gnhuang217@sina.com
Tingting Lin
yuting9263@163.com

¹Chongqing Key Laboratory of Human Embryo Engineering, Center for Reproductive Medicine, Women and Children's Hospital of Chongqing Medical University, Chongqing 400010, China

²Chongqing Clinical Research Center for Reproductive Medicine, Chongqing Health Center for Women and Children, Chongqing 400010, China

³Reproductive Center, Northwest Women's and Children's Hospital, Xi'an 710003, Shaanxi, China



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Background

Infertility is a disease of reproductive system defined by the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse [1]. According to the World Health Organization, around 17.5% of the adult population, roughly 1 in 6 worldwide, experience infertility [2]. Assisted reproductive technology (ART) has been widely applied for helping infertile couples to get progenies through in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). However, some couples suffered from repeated IVF and/or ICSI failures due to, for instance, oocyte maturation arrest and early embryonic development defects, and the strategies for improving reproductive outcomes are limited [3, 4].

From oocyte maturation to early embryo formation is a prolonged and highly organized process consisting of germinal vesicle (GV) breakdown, meiosis I, meiosis II, fertilization, cleavage, and early embryonic development, and its developmental block occurs at any stage, such as GV, metaphase I (MI), metaphase II (MII), fertilization, cleavage, and early embryonic development, termed as oocyte/zygote/embryo maturation arrest (OZEMA) [5, 6]. Intriguingly, increasing number of studies have demonstrated the important role of genetic factors in OZEMA, such as *TUBB8*, *PATL2*, *ZP1*, *ZP2*, *ZP3*, *CDC20*, *TRIP13* and *BTG4* [7]. The *TUBB8* gene (MIM: 616,768), which is located at chromosome 10p15.3 and encodes a predicted 444-amino acid protein, had been firstly described of genotype-phenotype relationship with OZEMA (OZEMA2, MIM: 616,780) in 2016 [8]. *TUBB8* protein is a primate-specific isotype of β -tubulin, forming a heterodimer with α -tubulin to constitute the error-prone spindle of oocyte and early embryo [9, 10]. To date, more than 100 variants in *TUBB8* gene have been identified and related to approximately 30% of infertile females with OZEMA in complex inheritance patterns, such as autosomal dominantly inheriting from father or arising *de novo*, or autosomal recessively inheriting from father and mother, or inheriting from mother with incomplete penetrance [11–13]. Subsequently, the disruption of microtubule formation and spindle assembly in oocyte and early embryo induced repeated ART failures with highly heterogeneous phenotypes, including GV arrest, MI arrest, oocytes with abnormal morphology, fertilization failure, cleavage failure, early embryonic development arrest and implantation failure [12–18]. These studies further highlight the essential role of *TUBB8* in oocyte maturation as well as early embryonic development and demonstrated the necessity of genetic counseling for high genetic and phenotypic heterogeneity of *TUBB8* variants and disease phenotypes. Consequently, further verification of phenotypes exhibited by the reported *TUBB8* variants and identification of novel *TUBB8* variants remain necessary.

In our previous study, we had identified 11 *TUBB8* variants from 15 unrelated families with complicated inheritance patterns and variable phenotypes [13]. In this study, we focused on continuously recruiting females with primary infertility from two reproductive centers to detect *TUBB8* variants and phenotypes. Through Sanger sequencing, nine missense variants and one loss-of-function variant, including five novel variants and five previously reported variants, were identified in nine unrelated families. In vitro functional assays with HeLa cells expressing wild-type or mutant *TUBB8* proteins were performed to assess their effect on protein stability and microtubule structure. The phenotypes caused by these *TUBB8* variants and the outcomes of IVF or ICSI were investigated. These findings enriched the variant spectrum of *TUBB8* gene and could contribute to optimize the genetic counselling and clinical management of infertile females.

Results

Identification of *TUBB8* variants from infertile females with OZEMA

We identified nine missense and one loss-of-function variants of *TUBB8* from nine unrelated females, including five novel variants (c.37G>A, p.G13R; c.149 A>G, p.Y50C; c.407 C>T, p.T136I; c.793T>G, p.F265V; c.1096 A>G, p.T366A) and five previously reported variants (c.10 A>C, p.I4L; c.124 C>G, p.L42V; c.400 C>T, p.Q134*; c.763G>A, p.V255M; c.1045G>A, p.V349I) [11, 19–22] (Fig. 1a). These variants presented as heterozygous state in all families, while two of them (c.400 C>T, p.Q134*; c.793T>G, p.F265V) coexisted in the proband of Family 7 (Table 1). Variants in Family 1, 6 and 8 occurred *de novo*, while variants in Family 3, 4 and 9 were inherited from the fathers. The variants' inheritance pattern in Family 2, 5 and 7 were unknown due to the unavailability of DNA samples from their parents. TA cloning revealed that the two heterozygous *TUBB8* variants of Family 7 were *in trans*, indicating that it was inherited probably in an autosomal recessive pattern (Fig. 1b). *In silico* analysis indicated that almost all the variants disrupted the function of *TUBB8*, as predicted by PolyPhen-2, SIFT, MutationTaster, and CADD, and they all have rare frequencies or were absent in the gnomAD database (Table 1). Thus, we speculated that these likely pathogenic variants of *TUBB8* were the primary cause of OZEMA.

Bioinformatic analysis of *TUBB8* variants

TUBB8 gene (NM_177987.2) is mapped to chromosome 10p15.3 and consists of 4 coding exons. As shown in Fig. 2a, amid the ten variants we identified, two were located in exon 1, two in exon 2, and six in exon 4. The amino acid changes caused by these variants were

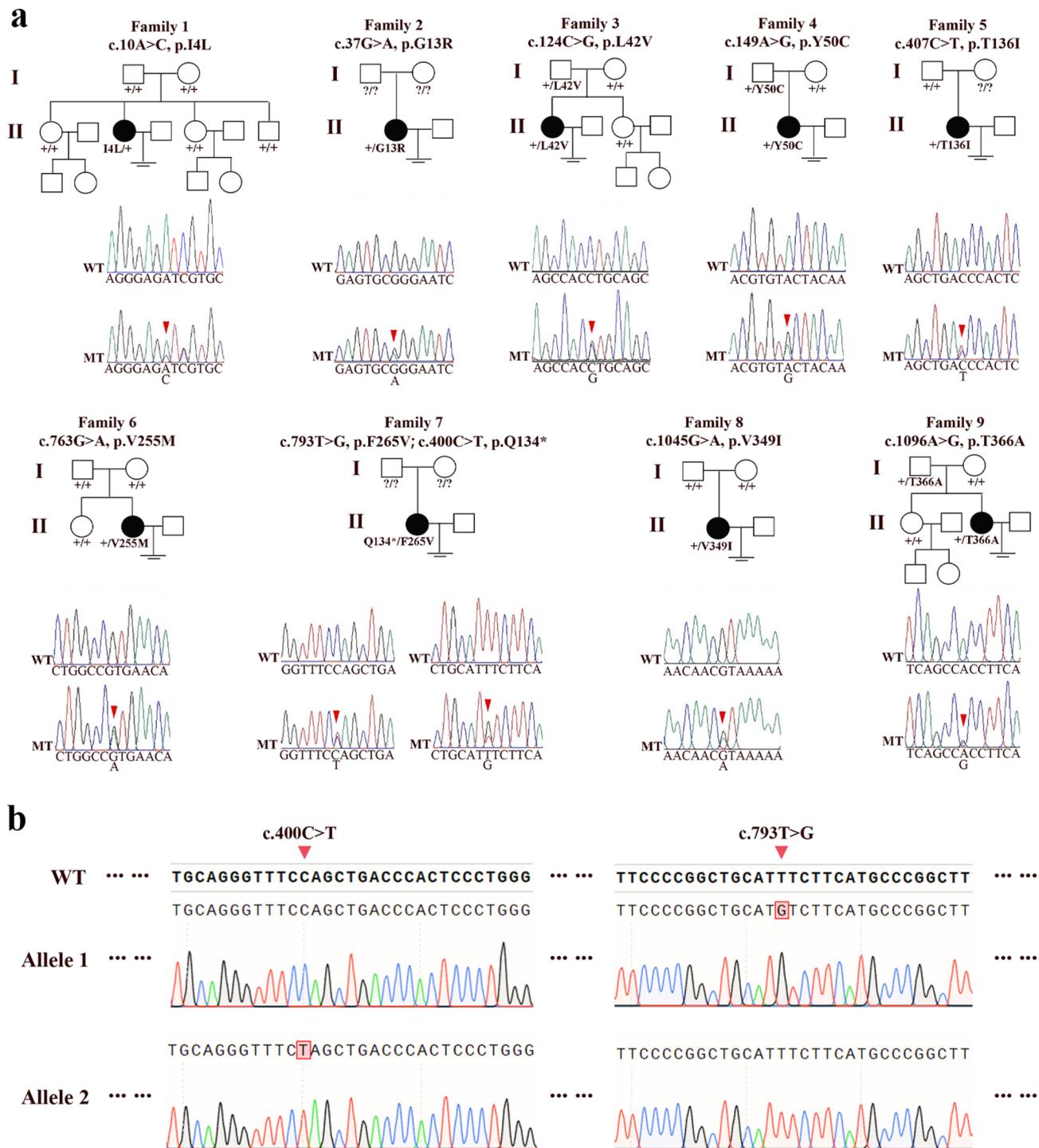


Fig. 1 Pedigrees and *TUBB8* variants of 9 families. **a** Pedigrees and Sanger sequencing chromatograms of 9 families. The “≡” sign indicates infertility, the black circles represent affected individuals, the question marks indicate absence of DNA samples, ‘+’ means wild-type, and the red arrows indicate mutation loci. MT, mutant allele; WT, wild-type allele. **b** TA cloning of Family 7, the red arrows indicate mutation loci; WT, wild-type allele

distributed over the full length of *TUBB8* protein. All the ten altered amino acids at variant positions were highly conserved among different species (Fig. 2a). Additionally, we predicted the effect of these variants on the structure of *TUBB8* protein using three-dimensional

protein structure based on PyMol software (Fig. 2b). The variants p.G13R, p.L42V and p.V255M occurred in α -helix region, while the variants p.I4L, p.Y50C, p.Q134*, p.T136I, p.F265V, p.V349I and p.T366A occurred in β -folding region. The p.Q134* variant caused premature

Table 1 Details of *TUBB8* variants identified in this study

| Family no. | Age | Chromosome localization | Exon/ Intron | cDNA change | Protein change | Mutation zygosity | Inheritance | Known/ novel | Allele frequency | | In silico bioinformatics prediction | | | |
|------------|-----|-------------------------|--------------|-------------|----------------|-------------------|-------------|--------------|------------------|------------|-------------------------------------|-----------------|----------|-----------|
| | | | | | | | | | gnomAD | gnomAD-EAS | PolyPhen-2 | MutationTaster | SIFT | CADD |
| Family 1 | 29 | chr10:95169 | Exon 1 | c.10 A>C | p.I4L | Het | de novo/AD | Known | 0.00000419 | 0 | Benign | Disease-causing | Damaging | Tolerable |
| Family 2 | 34 | chr10:95142 | Exon 1 | c.37G>A | p.G13R | Het | NA/AD | Novel | 0.00001549 | 0 | Probably-damaging | Disease-causing | Damaging | Damaging |
| Family 3 | 30 | chr10:94786 | Exon 2 | c.124 C>G | p.L42V | Het | Father/AD | Known | 0.0001 | 0.0012 | Probably-damaging | Disease-causing | Damaging | Tolerable |
| Family 4 | 35 | chr10:94761 | Exon 2 | c.149 A>G | p.Y50C | Het | Father/AD | Novel | ND | ND | Probably-damaging | Disease-causing | Damaging | Damaging |
| Family 5 | 26 | chr10:93925 | Exon 4 | c.407 C>T | p.T136I | Het | NA/AD | Novel | ND | ND | Probably-damaging | Disease-causing | Damaging | Damaging |
| Family 6 | 31 | chr10:93569 | Exon 4 | c.763G>A | p.V255M | Het | de novo/AD | Known | 0.00002163 | 0 | Probably-damaging | Disease-causing | Damaging | Damaging |
| Family 7 | 30 | chr10:93539 | Exon 4 | c.793T>G | p.F265V | Het | NA/AR | Novel | ND | ND | Probably-damaging | Disease-causing | Damaging | Damaging |
| Family 8 | 35 | chr10:93287 | Exon 4 | c.400 C>T | p.Q134* | Het | NA/AR | Known | 0 | 0 | NA | Disease-causing | NA | Damaging |
| Family 9 | 29 | chr10:93236 | Exon 4 | c.1045G>A | p.V349I | Het | de novo/AD | Known | ND | ND | Probably-damaging | Disease-causing | Damaging | Tolerable |
| Family 9 | 29 | chr10:93236 | Exon 4 | c.1096 A>G | p.T366A | Het | Father/AD | Novel | ND | ND | Probably-damaging | Disease-causing | Damaging | Tolerable |

Het, heterozygous; AD, autosomal dominant; NA, not available; ND, not detected; AR, autosomal recessive

termination of protein translation and suggested its strong deleterious effect. The p.G13R variant added the hydrogen bonds interacting with T136 and T7, which may alter the folding and thus affect microtubule assembly. The variants of p.Y50C and p.T136I resulted in the loss of hydrogen bonds interacting with Q134 and G13, respectively, which could destabilize its folding and thereby affect microtubule stability. Herein, these variants probably disrupted the normal function of TUBB8 protein in microtubules.

Effect of wild-type and mutant TUBB8 proteins on microtubule network in HeLa cells

To further investigate the pathogenic potential of the identified *TUBB8* variants, we transfected HeLa cells with plasmids expressing wild-type or mutant TUBB8 proteins and conducted functional assays. Western blot analysis revealed that the expression level of mutant TUBB8 proteins were significantly decreased than that of wild-type TUBB8 (Fig. 3a and b), indicating that these variants induced different degrees of instability of TUBB8 proteins. The variant of p.Q134* resulted in the complete degradation of TUBB8 protein without any truncated protein detected (Fig. 3a). Immunofluorescence assay revealed that when exogenous TUBB8 protein was expressed at a relatively low level, the wild-type and mutant proteins exerted little effects on the microtubule network (Fig. 3c and d). However, when expression at a relatively high level, both wild-type and mutant proteins exerted obvious effects on the microtubule network triggering severe structural damage (Fig. 3c and d). Meanwhile, all the mutant proteins had a significantly greater propensity than the wild-type protein to cause microtubule abnormalities in both low and high expression cases (Fig. 3d). These results suggested that the identified *TUBB8* variants could affect the stability of TUBB8 protein as well as the endogenous microtubule structure.

Oocyte and embryo phenotypes of probands harboring TUBB8 variants

A detailed description of the clinical characteristics and outcomes of ART cycles of the nine affected females is presented in Table 2. The nine probands were aged between 26 and 35 years, suffered from primary infertility for 3 to 8 years, and had experienced 1–3 cycles of IVF or ICSI failure during ART treatment. Consistent with our previous findings [13], females harboring different *TUBB8* variants exhibited variable phenotypes in oocyte maturation and morphology, fertilization, cleavage, as well as early embryonic development. All oocytes obtained from the probands in Family 9 with p.T366A and in Family 4 with p.Y50C were arrested at GV or MI stage with no MII oocytes (Fig. 4a). For the probands with p.I4L, p.L42V or p.V349I variants in Family1, 3 and

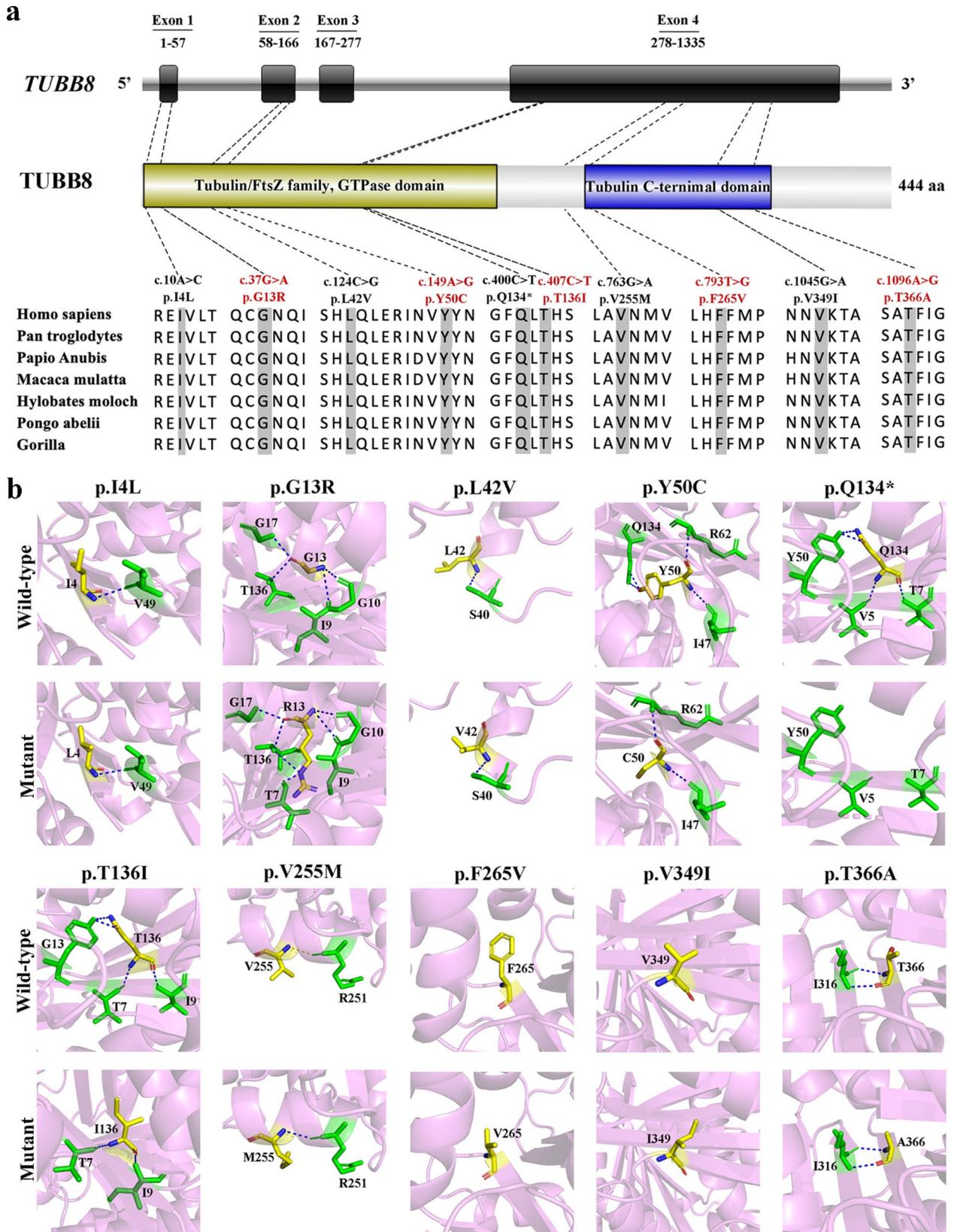


Fig. 2 Bioinformatic analysis of TUBB8 variants. **a** Locations and conservation analysis of the identified variants in TUBB8 protein. The red font indicates novel variants and the black font indicates previously reported variants. **b** Protein conformation predictions of TUBB8 variants. Blue dashed lines represent hydrogen bonds

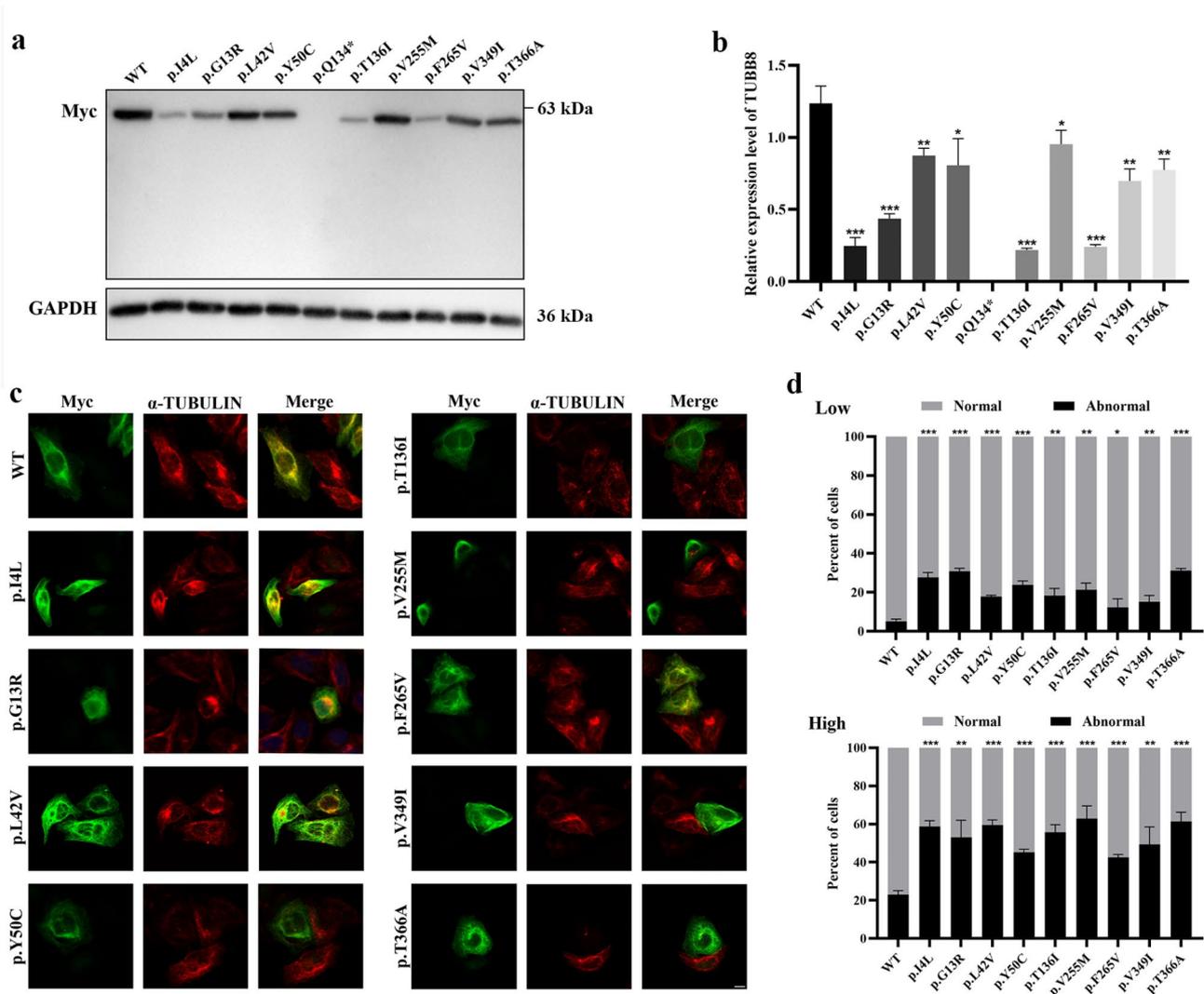


Fig. 3 Expression of wild-type and mutant TUBB8 proteins in HeLa cells. **a,b** The protein expression level of TUBB8 in HeLa cells transfected with plasmids encoding Myc-tagged wild-type and mutant TUBB8 proteins. WT, wild-type. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. **c,d** Microtubule phenotypes induced by the expression of wild-type and mutant TUBB8 proteins in HeLa cells. Cells were immunoassayed with an antibody against Myc epitope (green) to visualize transgene and counterstained with an antibody against α -TUBULIN (red) to visualize endogenous microtubule network. Scale bar, 10 μ m. WT, wild-type. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

8, 20-41.7% of the oocytes developed into MII stage but failed to be fertilized normally, presenting with no pronucleus or more than two pronuclei (Table 2; Fig. 4a). Some oocytes from the proband in Family 8 were with abnormal morphologies of large or more than one polar body. The most oocytes of 25 obtained from proband with p.T136I in Family 5 were at GV stage (n=3), MI stage (n=5), MII stage (n=13), and with abnormal morphologies of large polar body (n=4), respectively (Table 2; Fig. 4a). Only 38.5% (5/13) of the MII oocytes were successfully fertilized and cleaved normally, but developed into unusable embryos on day 3 (Fig. 4b). For proband in Family 6 with p.V255M variant, half of the retrieved 6 oocytes at MII stage were normally fertilized but with abnormal cleavage pattern (Table 2).

Furthermore, probands in Family 2 with p.G13R variant and in Family 7 with compound heterozygous variants (p.F265V, p.Q134*) both had a high proportion of MII oocytes, 8 of 14 MII oocytes from proband with p.G13R were successfully fertilized, and 2 usable embryos were obtained. For proband with p.F265V and p.Q134*, 3 of 13 MII oocytes were successfully fertilized and 1 usable embryo was obtained. Unfortunately, both two probands failed to conceive after fresh embryo transfer (Table 2). Immunostaining of the MI oocyte from normal control showed that both spindle and DNA were visible, and the DNA was regularly arranged on both sides of the equatorial plate. In the MI oocytes from proband with p.T366A in Family 9, the spindles were invisible, and the DNA was disorderly organized (Fig. 4c). Thus, we speculated that

Table 2 Clinical characteristics of patients and their ART cycles

| Family no. | Age (years) | Duration of infertility (years) | Previous ART cycles | Total no. of oocytes retrieved | GV oocyte | MII oocyte | MII oocyte | Oocyte with abnormal morphology | Degenerated oocyte | Fertilized oocyte | Cleaved embryo | Usable embryo | Outcome of embryo transfer |
|------------|-------------|---------------------------------|---------------------|--------------------------------|-----------|------------|------------|---------------------------------|--------------------|-------------------|----------------|---------------|----------------------------|
| Family 1 | 29 | 5 | 2 | 12 | 0 | 7 | 5 | 0 | 0 | 0 | 0 | 0 | / |
| Family 2 | 34 | 7 | 3 | 15 | 1 | 0 | 14 | 0 | 0 | 8 | 7 | 2 | Failure |
| Family 3 | 30 | 7 | 1 | 9 | 5 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | / |
| Family 4 | 35 | 8 | 1 ^a | 10 | NA | NA | NA | NA | NA | NA | NA | 0 | / |
| Family 5 | 26 | 7 | 2 | 25 | 3 | 5 | 13 | 4 | 0 | 5 | 5 | 0 | / |
| Family 6 | 31 | 6 | 1 | 6 | 0 | 0 | 6 | 0 | 0 | 3 | 2 | 0 | / |
| Family 7 | 30 | 7 | 1 | 13 | 0 | 0 | 13 | 0 | 0 | 3 | 1 | 1 | Failure |
| Family 8 | 35 | 5 | 2 | 10 | 0 | 1 | 2 | 6 | 1 | 0 | 0 | 0 | / |
| Family 9 | 29 | 3 | 1 | 6 | 1 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | / |

ART, assisted reproductive technology; GV, germinal vesical; MII, metaphase II; NA, not available

^a This ART cycle was conducted in other hospital

the *TUBB8* variants obstructed the oocyte maturation and early embryonic development by disrupting the normal function of spindle.

Discussion

In this study, we recruited 35 females with primary infertility and identified ten different *TUBB8* variants in nine unrelated females, including nine missense variants (p.I4L, p.G13R, p.L42V, p.Y50C, p.T136I, p.V255M, p.F265V, p.V349I and p.T366A) and one loss-of-function variant (p.Q134*), respectively. These variants were inherited in dominant or recessive patterns, and even occurred *de novo*. Furthermore, we identified five novel variants and provided additional evidence for the clinical significance of five previously reported variants. The phenotypes caused by these variants were diverse, including abnormalities in oocyte maturation and morphology, fertilization failure, cleavage failure, embryonic development arrest and implantation failure. Through functional assay *in vitro*, we found that mutant *TUBB8* proteins induced different degrees of microtubule structural disruption after exogenous expression in HeLa cells. These findings expanded the mutant spectrum of *TUBB8* gene and could facilitate the optimization of genetic counseling in primary infertile females.

So far, a total of 140 different variants in *TUBB8* gene have been reported, as summarized in Table S1. The vast majority of variant types in *TUBB8* were missense (87.9%, 123/140), and the rest were loss-of-function variants, including 14 frameshift (10%), 2 nonsense (1.4%) and 1 deletion (0.7%). The missense variants of *TUBB8* usually cause female infertility in a heterozygous pattern via dominant-negative effects, while loss-of-function variants are usually pathogenic when present in a compound heterozygous or homozygous pattern [15, 19, 23–28]. Consistent with previous studies, the novel *TUBB8* variants p.G13R, p.Y50C, p.T136I and p.T366A identified in this study presented in heterozygous pattern, implying their dominant-negative effects. For the compound heterozygous variant of p.F265V and p.Q134* in Family 7, given the pathogenic characteristics of the *TUBB8* loss-of-function variants mentioned above, we speculated that this compound heterozygous variant was *in trans* and confirmed it through TA cloning. It is worth noting that the phenotypes resulting from the heterozygous variant of p.F265V in *TUBB8* remains unknown.

Despite high heterogeneity is the hallmark of *TUBB8* variants, there are still some variants that have been reported repeatedly. Combining our present study with previously reported studies about *TUBB8* variants, we identified 5 candidate mutant-hotspots, namely p.I4L (n=7), p.A313V (n=7), p.V255M (n=5), p.G98R (n=5) and p.E108K (n=5). Based on the reported studies we further reviewed the phenotypes of these candidate

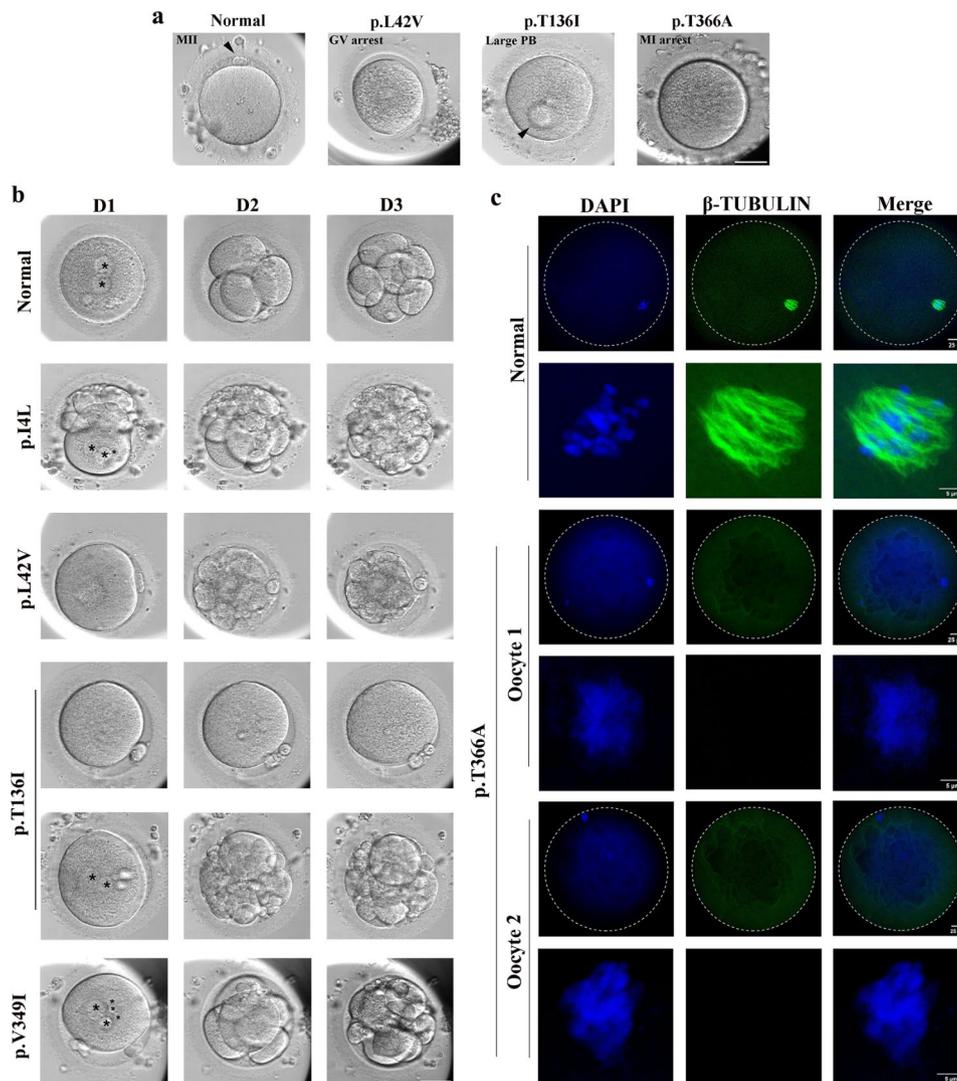


Fig. 4 Phenotypes of oocytes and embryos from probands with *TUBB8* variants. **a** Morphology of oocytes from a normal control and three probands on day 0. The black arrows indicate polar bodies. Scale bar, 50 μ m. **b** Morphology of zygotes on day 1, and embryos on day 2 and day 3 from a normal control and four probands. The black stars indicate pronucleus. Scale bar, 50 μ m. **c** β -TUBULIN/DNA immunostaining of a normal MI oocyte and two MI oocytes from Family 9. Oocytes were immunoassayed with an antibody against β -TUBULIN (green) to visualize spindle and counterstained with DAPI (blue) to visualize DNA.

mutant-hotspots and found that the variants p.I4L and p.E108K appeared to affect the entire process from oocyte maturation to early embryonic development, accompanied by the phenotypes of GV arrest, MI arrest, oocytes with abnormal morphology, fertilization failure, cleavage failure and early embryonic development arrest [11, 13, 15, 19, 21, 22, 29]. The patients harboring variant p.A313V were reported to have conflicting ART results [16, 22, 30], nevertheless, our previous study showed that three patients with heterozygous p.A313V have good prognosis of ART outcomes and recent follow-up informed us that all of them had successful deliveries, so we speculated that it may be a benign variant [13]. The variant of p.V255M was reported to induce MI arrest in oocytes and early embryonic development arrest [17–19,

31], our study complemented the phenotypes of fertilization failure with p.V255M variant. The phenotypes have been reported for the variant p.G98R of *TUBB8* included MI arrest, fertilization failure and oocytes with abnormal morphology [11, 13, 19, 22, 32].

In this study, none of the females carrying *TUBB8* variants had successful pregnancy, neither by IVF nor ICSI. Through reviewing previously published studies, successful deliveries have only been reported in females with p.A313V and p.T429M [12, 13], implying the likely benign significance of these two variants, but it needs to be verified by more data. Thus far, there is no effective treatment strategies for female infertility caused by *TUBB8* variants, donor egg appears to be the most feasible treatment strategy for the females

carrying pathogenic variants of *TUBB8*. Nevertheless, Jia et al. found that additional expression of wild-type *TUBB8* cRNA in the mouse oocytes expressing mutant *TUBB8* could rescue the embryonic development defects of resulting embryo and produce full-term offspring [33]. This study provides a possible means of treating female infertility caused by *TUBB8* variants and it seems to be a very promising direction for subsequent research.

Conclusions

In conclusion, our study identified five novel variants to expand the mutant spectrum of *TUBB8* gene and described the phenotypes of five previously reported variants to strengthen the genotype–phenotype correlation of *TUBB8* variants. In light of the unfavorable reproductive outcomes in females harboring pathogenic *TUBB8* variants, genetic screen of *TUBB8* is of great importance to females diagnosed with primary infertility, and genetic counselling should be conducted cautiously.

Methods

Human subjects

From June 2022 to May 2023, 35 females diagnosed with primary infertility and experiencing IVF or ICSI failure due to disorders in oocyte maturation, fertilization, zygotic cleavage and early embryonic development from the Center for Reproductive Medicine, Women and Children's Hospital of Chongqing Medical University (Chongqing, China) as well as the Reproductive Center, Northwest Women's and Children's Hospital (Xi'an, China.) were recruited. Through Sanger sequencing, a total of nine females harboring ten different *TUBB8* variants were finally enrolled in this study. A written informed consent was signed before the collection of blood or oocyte. Besides, the blood samples were collected from all females and their family members when possible.

Sanger sequencing, TA cloning and variant analysis

Sanger sequencing was performed as previously reported [13]. Briefly, the amplicons of specific primers listed in Table S2 were sequenced with ABI 3500 (Thermo Fisher Scientific, Waltham, MA, USA) and analyzed with Chromas 2.6.5 (Technelysium Pvt. Ltd., South Brisbane, Australia). For TA cloning, the coding region of *TUBB8* were amplified via polymerase chain reaction, the amplicons were then cloned into pMD19T vector through TA cloning kit (TAKARA, Japan) according to the instruction. The gnomAD database (<http://gnomad-sg.org/>) was used to analyze the variants' frequencies. The potential pathogenicity of all identified variants was evaluated by *in silico* analysis with Polymorphism Phenotyping v2 (PolyPhen-2, <http://genetics.bwh.harvard.edu/pph2/index.shtml>), Sorting Intolerant from Tolerant (SIFT, <https://sift.bii.a-star.edu.sg/>), MutationTaster (<https://www.mutationtaster.org/>), and Combined Annotation Dependent Depletion (CADD, <https://cadd.gs.washington.edu/>).

MutationTaster (<https://www.mutationtaster.org/>), and Combined Annotation Dependent Depletion (CADD, <https://cadd.gs.washington.edu/>).

Molecular modelling and amino acid conservation analysis

The diagram images of *TUBB8* gene and protein were modeled by Illustrator for Biological Sequences (IBS, <http://ibs.biocuckoo.org/online.php>). The amino acid conservation analysis of *TUBB8* protein was performed among different species via the Clustal Omega tool (<http://www.clustal.org/omega/>). Wild-type and mutant *TUBB8* protein structures were predicted using the SWISS-MODEL software (<https://swissmodel.expasy.org>) based on the 7rro.28.pdb template and further mapped to the atomic model using PyMol (<http://www.pymol.org>).

Evaluation of oocytes and early embryos

The morphologies of retrieved oocytes were examined by light microscopy. Embryos were cultured after insemination using a time-lapse monitoring system (Embryoscope Plus, Vitrolife, Sweden) to limit exposure of embryos to suboptimal conditions. For the immunostaining, oocytes were first fixed in 4% paraformaldehyde for 30 min, then incubated in 0.5% Triton X-100 for 15 min to permeabilize the membrane and in 3% bovine serum albumin for 1 h to block. Oocytes were stained with an anti- β -TUBULIN FITC antibody (1:500 dilution, F2043, Sigma-Aldrich) and DAPI (P0131, Beyotime) to label the meiotic spindle and DNA, respectively. The images were captured on a confocal laser-scanning microscope (TCS SP8, Leica, Germany).

Expression of WT and mutant *TUBB8* proteins in HeLa cells

A full-length *TUBB8* cDNA cloned in a pCDNA3.1-Myc-HisA vector with a CMV promoter was purchased from Tsingke Biotechnology Co., Ltd. Point mutation using quick-change polymerase chain reaction was performed for the generation of *TUBB8* mutants, including c.10 A>C, c.37G>A, c.124 C>G, c.149 A>G, c.400 C>T, c.407 C>T, c.763G>A, c.793T>G, c.1045G>A, c.1096 A>G. The plasmids expressing wild-type or mutant *TUBB8* proteins were separately transfected in HeLa cells pre-seeded on 24-well plate by Lipofectamine 3000 (Thermo Fisher Scientific, USA) according to the manufacturer's recommendations. After 48 h, the cells were fixed, permeabilized, and labeled with the antibodies against Myc epitope (1:500 dilution, AM926-1, Beyotime) to visualize transgene and α -TUBULIN (1:800 dilution, 5335s, CST) to visualize endogenous microtubule network. The next day, cells were incubated with Goat anti Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa FluorTM555 (1:500

dilution; A-21,428, Invitrogen, America) and Goat anti Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™488 (1:1000 dilution; A-11,001, Invitrogen, America). Cells were observed and photographed using confocal laser-scanning microscope (TCS SP8, Leica, Germany). For the quantification of microtubule phenotypes, approximately 200 cells expressing either wild-type or mutant TUBB8 were examined and classified according to the level of transgene expression (as judged by the fluorescence intensity) and microtubule structure (as judged by the microtubule appearance, filament density or diffuse mottled pattern of Myc and α -TUBULIN labels) in each of three independent experiments. In addition, in order to detect the expression level of wild-type and mutant proteins, the plasmids expressing wild-type or mutant TUBB8 were transfected in HeLa cells pre-seeded on 6-well plate. 48 h after transfection, the cells were collected and dissolved in RIPA lysis buffer (Beyotime, China) mixed with proteinase inhibitor to extract proteins, then the protein expression was detected by Western blot.

List of abbreviations

| | |
|-------|--|
| ART | assisted reproductive technology |
| IVF | in vitro fertilization |
| ICSI | intracytoplasmic sperm injection |
| GV | germinal vesicle |
| MI | metaphase I |
| MII | metaphase II |
| OZEMA | oocyte/zygote/embryo maturation arrest |

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13048-023-01274-3>.

Supplementary Material 1

Acknowledgements

We would like to thank all the participants enrolled in this study and all the researchers of included studies.

Authors' contributions

Study conception and design: Tingting Lin, Guoning Huang, Juanzi Shi; Data collection: Tingting Lin, Sen Qiao, Tingwenyi Hu; Bioinformatic analysis: Tingwenyi Hu, Rong Shi, Xia Xue; Oocyte immunofluorescence: Chong Li; Oocyte morphological analysis: Weiwei Liu, Wei Han, Wei Li; Functional assay in cells: Tingwenyi Hu, Chong Li; Writing and revising of the manuscript: Tingwenyi Hu, Tingting Lin. All authors have reviewed the manuscript and approved of its submission.

Funding

This work was supported by the Chongqing Natural Science Foundation [CSTB2023NSCQ-MSX0518] and Chongqing medical scientific research project (Joint project of Chongqing Health Commission and Science and Technology Bureau, 2023MSXM008 and 2023MSXM054).

Data availability

The analyzed data generated during this study are available from the corresponding author on reasonable requests.

Declarations

Ethics approval and consent to participate

This study was approved by the Clinical Application and Ethics Committee of Human Assisted Reproductive Technology of Chongqing Health Center for Women and Children (2022-RGI-01, 9 June 2022) and the Ethics Review Board of the Northwest Women's and Children's Hospital (2022020). The informed consent was obtained from all participants.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 20 July 2023 / Accepted: 3 September 2023

Published online: 25 November 2023

References

- Carson SA, Kallen AN. Diagnosis and management of infertility: a review. *JAMA*. 2021;326:65.
- Infertility prevalence estimates, 1990–2021. Geneva: World Health Organization; 2023. <https://www.who.int/publications/i/item/978920068315>.
- Hart RJ. Physiological aspects of female fertility: role of the Environment, Modern Lifestyle, and Genetics. *Physiol Rev*. 2016;96:873–909.
- Yatsenko SA, Rajkovic A. Genetics of human female infertility†. *Biol Reprod*. 2019;101:549–66.
- Li R, Albertini DF. The road to maturation: somatic cell interaction and self-organization of the mammalian oocyte. *Nat Rev Mol Cell Biol*. 2013;14:141–52.
- Coticchio G, Dal Canto M, Mignini Renzini M, Guglielmo MC, Brambillasca F, Turchi D, et al. Oocyte maturation: gamete-somatic cells interactions, meiotic resumption, cytoskeletal dynamics and cytoplasmic reorganization. *Hum Reprod Update*. 2015;21:427–54.
- Solovova OA, Chernykh VB. Genetics of Oocyte Maturation defects and early embryo development arrest. *Genes*. 2022;13:1920.
- 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;374:223–32.
- Sirajuddin M, Rice LM, Vale RD. Regulation of microtubule motors by tubulin isotypes and post-translational modifications. *Nat Cell Biol*. 2014;16:335–44.
- Yan L, Yang M, Guo H, Yang L, Wu J, Li R, et al. Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells. *Nat Struct Mol Biol*. 2013;20:1131–9.
- 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;38:2261–72.
- Zhang J, Li S, Huang F, Xu R, Wang D, Song T et al. A novel compound heterozygous mutation in *TUBB8* causing early embryonic developmental arrest. *J Assist Reprod Genet* [Internet]. 2023 [cited 2023 May 17]; Available from: <https://link.springer.com/https://doi.org/10.1007/s10815-023-02734-x>.
- Lin T, Liu W, Han W, Tong K, Xiang Y, Liao H, et al. Genetic screening and analysis of *TUBB8* variants in females seeking ART. *Reprod Biomed Online*. 2023;46:244–54.
- Yu W, Zhang S, Yin B, Dong C, Zhang VW, Zhang C. Identification of *TUBB8* variants in 5 primary infertile women with multiple phenotypes in oocytes and early embryos. *Reprod Sci*. 2023;30:1376–82.
- 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;32:457–64.
- 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;15:38.
- Huo M, Zhang Y, Shi S, Shi H, Liu Y, Zhang L, et al. Gene spectrum and clinical traits of nine patients with oocyte maturation arrest. *Front Genet*. 2022;13:772143.

18. 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;53:662–71.
19. 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;27:300–7.
20. 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;37:1837–47.
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;99:208–14.
23. 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;35:305–10.
24. 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;43:891–8.
25. Xiang J, Wang W, Qian C, Xue J, Wang T, Li H, et al. Human oocyte maturation arrest caused by a novel missense mutation in *TUBB8*. *J Int Med Res*. 2018;46:3759–64.
26. 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;28:2942–50.
27. 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;28:3448–57.
28. Li W, Li Q, Xu X, Wang C, Hu K, Xu J. Novel mutations in *TUBB8* and *ZP3* cause human oocyte maturation arrest and female infertility. *Eur J Obstet Gynecol Reproductive Biology*. 2022;279:132–9.
29. 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;35:1349–56.
30. Yuan H, Chen J, Li N, Miao H, Chen Y, Lyu S, et al. Target-sequencing of female infertility pathogenic gene panel and a Novel *TUBB8* loss-of-function mutation. *Front Genet*. 2022;13:865103.
31. Lanuza-López MC, Martínez-Garza SG, Solórzano-Vázquez JF, Paz-Cervantes D, González-Ortega C, Maldonado-Rosas I, et al. Oocyte maturation arrest produced by *TUBB8* mutations: impact of genetic disorders in infertility treatment. *Gynecol Endocrinol*. 2020;36:829–34.
32. Wang A-C, Zhang Y-S, Wang B-S, Zhao X-Y, Wu F-X, Zhai X-H, et al. Mutation analysis of the *TUBB8* gene in primary infertile women with arrest in oocyte maturation. *Gynecol Endocrinol*. 2018;34:900–4.
33. 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;37:2713–22.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.