

## ORIGINAL ARTICLE

# Two novel mutations in the *MCM8* gene shared by two Chinese siblings with primary ovarian insufficiency and short stature

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**Abstract**

**Background:** Minichromosome maintenance complex component 8 (*MCM8*) is responsible for homologous recombination and DNA double-strand breaks (DSBs) repair and is the cause of primary ovarian insufficiency (POI), which is seldom diagnosed in adolescents and children.

**Methods:** Whole-exome sequencing was performed in a 13-year-old girl, and Sanger sequencing was used to identify potentially pathogenic variants in her sister (aged 6 years and 7 months) and parents. To identify potential pathogenic mutations, DSBs were induced by mitomycin C (MMC), and the DNA repair capacity was evaluated by the histone H2AX phosphorylation level.

**Results:** Two novel mutations of *MCM8*, i.e., c.724T>C (p.C242R) and c.1334C>A (p.S445\*), were identified in a 13-year-old girl with POI who exhibited disappeared bilateral ovaries and short stature (height standard difference score [HtSDS] = -3.05), and her sister (aged 6 years and 7 months) with progressive POI whose ovary size decreased from normal to unclear and height growth gradually slowed. In the functional experiments, compared with the wild-type, HeLa cells overexpressing mutant p.C242R and p.S445\* showed a higher sensitivity to MMC. Furthermore, the mutant p.S445\* has a more deleterious effect on DNA damage repair.

**Conclusion:** Our results reveal that affected children with the novel pathogenic mutations p.C242R and p.S445\* in the *MCM8* gene are characterized by POI, short stature, cancer susceptibility, and genomic instability.

**KEYWORDS**

adolescents, DNA repair, *MCM8*, primary ovarian insufficiency, short stature

## 1 | INTRODUCTION

Minichromosome maintenance 8 (*MCM8*, OMIM: 608187), which is a newly discovered member of the minichromosome maintenance (*MCM*) family, is an autosomal gene recently implicated in causing hypergonadotropic hypogonadism

when inherited in an autosomal recessive fashion in rare consanguineous families. *MCM8*, which is located at 20p12.3 and comprises an amino-terminal DNA-binding domain and an AAA core domain, encodes a DNA helicase protein and participates in homologous recombination, meiosis and DNA replication (Bouali et al., 2017; Dou et al., 2016). *MCM8*

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knockout mice display damaged homologous recombination-mediated DNA repair during gametogenesis, growth defects and chromosome breakage (Lutzmann et al., 2012).

Recently, whole-exome sequencing (WES) has provided evidence regarding the possible role of several genes, including *MCM8*, in the pathogenesis of female hypergonadotropic hypogonadism (AlAsiri et al., 2015; Fauchereau et al., 2016; Qin, Jiao, Simpson, & Chen, 2015; Tenenbaum-Rakover et al., 2015; Wood-Trageser et al., 2014). Furthermore, studies have confirmed that the mutant *MCM8* gene is related to autosomal recessive primary ovarian insufficiency (POI) (AlAsiri et al., 2015; Bouali et al., 2017; Dou et al., 2016). Rajkovic and his colleagues used WES to detect homozygous mutations in the *MCM8* genes in consanguineous families with POI (primary amenorrhea). The homozygous mutant c.446C>G (p.P149R) in *MCM8* inhibited the recruitment of MCM8 to DNA damage sites, resulting in genomic instability (AlAsiri et al., 2015). Rs16991615, a single nucleotide polymorphism in *MCM8*, was also associated with the natural menopausal age in a previously discussed GWAS (Chen et al., 2014). POI affects approximately 0.01% of women aged under 20 years (excluding patients with Turner syndrome or other known chromosomal abnormalities) (Gordon, Kanaoka, & Nelson, 2015; Kanj, Ofei-Tenkorang, Altaye, & Gordon, 2018). Although POI is less common in adolescents, it presents significant challenges to children's gonadal development and well-being. The clinical phenotypes of *MCM8* are characterized by POI (primary amenorrhea), hypothyroidism, and hypergonadotropic hypogonadism (AlAsiri et al., 2015; Tucker, Grover, Bachelot, Touraine, & Sinclair, 2016). Compared with *MCM9* mutant in MCM family (Fauchereau et al., 2016), two clinical phenotypes, growth retardation (Wood-Trageser et al., 2014) and colorectal cancer (Goldberg, HubertA, Cohen, Plesser-Duvdevani, & Meiner, 2015), are rarely mentioned. Although it is known that the phenotype of short stature in female hypergonadotropic hypogonadism involves *SHOX*, *MCM9*, *GALT*, *BLM*, and *RECQL4* (Qin et al., 2015; Wood-Trageser et al., 2014).

In our study, two novel mutations of *MCM8* are identified by WES in a 13-year-old girl with POI and short stature, and the same mutations are identified in her sister (aged 6 years and 7 months) with progressive POI and height growth retardation. Furthermore, functional characterization reveals that two novel mutations are pathogenic. This is the first family with *MCM8* mutation identified in adolescence and childhood, which expands the genotype and phenotype spectrum of the disease.

## 2 | MATERIALS AND METHODS

### 2.1 | Subjects

This study was approved by the Committee of Chinese Medical Ethics of Shanghai Children's Hospital. We obtained written

informed consent from all participants in this study. A 13-year-old girl from a Chinese family presenting with delayed puberty and short stature presented at the Department of Endocrinology at the Shanghai Children's Hospital. After obtaining her detailed history and performing a physical examination, several tests were conducted, including the growth hormone provocative test; serum luteinizing hormone (LH), follicle stimulating hormone (FSH) and estradiol (E2) level tests; uterine and ovarian ultrasonography; karyotype analysis; adrenal function; immune function; and even bilateral gonadal biopsy. Her sister (aged 6 years and 7 months) also underwent sex hormone detection and gynecological ultrasound examinations.

### 2.2 | WES and Sanger sequencing

The sequencing libraries were constructed using a modification of the KAPA Library Preparation Kit (Kapa, Inc.). Fragments were purified using AMPureXP beads (Beckman Coulter Inc.) to remove small products. Then, the DNA fragments were subjected to the following three enzymatic steps: end repair, A-tailing, and ligation to Illumina paired-end indexed adapters. The libraries were subjected to minimal PCR cycling and quantified using a Qubit 2.0 Fluorometer (Thermo Fisher). The libraries were combined into pools for solution phase hybridization using a NimbleGen SeqCap EZ MedExome Target Enrichment Kit (Roche NimbleGen Inc). The captured libraries were analyzed using an Agilent 2100 Bioanalyzer, and the DNA concentrations were measured using a Qubit 2.0 Fluorometer and then sent for sequencing to generate  $2 \times 150$  bp paired-end reads using a HiSeq X Ten platform (Illumina, Inc.). The quality control of the WES is illustrated in Table 1 (Zhang et al., 2020).

The variants identified by WES were selected for data interpretation with a minor allele frequency  $<0.01$  in dbSNP (<https://www.ncbi.nlm.nih.gov/SNP>), Human Gene Mutation Database (<http://www.hgmd.cf.ac.uk/ac/index.php>), the ExAC consortium (<http://exac.broadinstitute.org/>), the 1000 Genome Project (<http://www.1000genomes.org/>) database, Clin Var (<https://www.ncbi.nlm.nih.gov/clinvar/>), and BGI database with ~50,000 Chinese Han samples. We also classified the identified variations into pathogenic, likely pathogenic, VUS, likely benign and benign groups according to the variant interpretation guidelines of the American College of Medical Genetics and Genomics (ACMG). Finally, we also analyzed the novel variants by the OMIM database and previously published literature. The detailed data interpretation pipeline for WES were based on previously reported methods (Han et al., 2020).

The WES results of the proband were verified, and potentially pathogenic variants were confirmed in her family members and matched controls by Sanger sequencing using an ABI 3100 Sequencer (Applied Biosystems). Sanger sequencing was performed with the following

**TABLE 1** Quality control data of whole-exome sequencing

Total	
Raw reads (All reads)	88,201,718
QC Fail reads	4,829,452
Raw data (Mb)	13318.46
Paired reads	83,372,266
Mapped data (Mb)	12153.47
Mapped reads	83,262,132
Fraction of mapped data	91.25%
Fraction of mapped reads	99.86%
Unique Mapped	67,963,307
Unique Mapped Reads on Target Regions	52,665,801
No-mismatch Mapped	58,342,073
No-mismatch Reads on Target Regions	46,054,616
Mismatch alignment bases rate	30.02%
≥Q30 bases	88.83%
Target	
Target reads	64,400,519
Fraction of target reads in all reads	77.24%
Fraction on Target Regions Covered by Reads	99.18%
Average depth	110.564
Coverage (>0x)	99.18%
Coverage (≥4x)	97.31%
Coverage (≥10x)	95.5%
Coverage (≥25x)	89.22%
Coverage (≥50x)	72.36%

primers: F7 5'-GCTCCTAAAAGGAACTTGTGAA-3', R7 5'-ATTCGGGGTGATGAGTAGTGG-3'; F12 5'-TTGTTT CCTTAGGCAGCGTG-3', R12 5'-AACTTACTGGTTAG GACTTTGC-3'; the reference sequence NM\_001281522.1 of *MCM8* was used. The mutations were identified by sequence alignment with the NCBI Reference Sequence (NM\_001281522.1) using DNASTAR software version 5.0 (DNASTAR). The nomenclature of the mutations and variants followed the guidelines of the Human Genome Variation Society ([www.hgvs.org/mutnomen](http://www.hgvs.org/mutnomen)).

### 2.3 | Multiple sequence alignment and molecular structural modeling

To understand the evolutionary conservation of the wild-type amino acids of the two novel mutations in the *MCM8* gene, Clustal X1.8 software was used to compare the human wild-type (WT) *MCM8* protein sequence with its orthologues in *Homo sapiens*, *Mus musculus*, *Rattus norvegicus*, *Bos taurus*, *Gallus gallus*, *Xenopus tropicalis*, and *Danio rerio*. To understand the effect of the two novel mutants on the *MCM8* protein structure, in silico protein structural analysis was performed. We identified feasible template structures for *MCM8*

using SWISS-MODEL (<http://www.swissmodel.expasy.org>). Three-dimensional (3D) protein modeling of the wild-type and mutant *MCM8* proteins was performed using Swiss-PdbViewer 4.1 molecular visualization software.

### 2.4 | Construction of a green fluorescent protein-MCM8 plasmid and mitomycin C sensitivity assay

The coding sequence of the wild-type protein was amplified by PCR (forward primer: 5'-ACAGACCTGATCTGAG TAAAACCACA-3'; reverse primer: 5'-TTCCCTTTCTTTC TATTCATCCTTG-3'). Using the constructed plasmid pLVX-IRES-Puro-MCM8-green fluorescent protein (GFP) as a template, the mutants p.C242R and p.S445\* were introduced with the following primers: C242R-F 5'-CGTGCT GCATGTGGAGAAATTCAGAG-3', C242R-R 5'-AAGAA AAGCCATCTTGGTGCAAAGAG-3'; S445\*-F 5' AAAAA GATAGTTCTCTGGAGATTTT-3', S445\*-R 5'-AAAGAG TTACCGTCAGACCAGAGGTG-3'. The mutant plasmids were amplified, cyclized, transformed and sequenced. All plasmids were confirmed by Sanger sequencing.

HeLa cells (a human cervical carcinoma cell line) were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum at 37°C. After 12 hr of incubation with wild-type *MCM8*, the mutant p.C242R and p.S445\* plasmids were transiently transfected into HeLa cells with Lipofectamine 2000 Transfection Reagent (Invitrogen) and cultured for 48 hr. Then, HeLa cells overexpressing wild-type or mutant *MCM8* were exposed to 600 nM mitomycin C (MMC) for 21 hr to induce DNA double-strand breaks (DSBs) and harvested immediately or after recovery for 2 hr in fresh and complete medium culture medium at 37°C. The cells were used for western blot (WB) detection. The protein lysates were separated by 15% twelve sodium sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane. The membrane with incubated with a  $\gamma$ -H2AX antibody (rabbit, Cell Signaling, 1:1,000 dilution) overnight at 4°C and washed with TBST. The samples were incubated with an HRP-conjugated Affinip ure Goat Anti-Rabbit IgG (H+L) (1:10,000 dilutions) secondary antibody for 1 hr at room temperature (25°C). An anti- $\beta$ -actin antibody (rabbit, Abbkine, 1:50,000 dilutions) was used as a control. The fluorescence intensity of the film surface was detected by strong chemiluminescence.

## 3 | RESULTS

### 3.1 | Clinical evaluation of the two siblings

A 13-year-old girl (height of 138 cm, a height standard difference score (HtSDS) of -3.05 (<-2SD), weight of 36.1 kg,

body mass index of 18.96 kg/m<sup>2</sup>, and growth velocity of 3 cm/year) had multiple facial nevi, peltate chest, breasts Tanner I, and PH1, similar to Turner syndrome. At birth, she weighed 2,900 g with a height of 50 cm. She had no hearing or visual loss, mental retardation or ataxia. As shown in Table 2, multiple sex hormones examinations revealed high FSH (86.34 IU/L) and LH (17.14 IU/L) levels and low estradiol (77 pg/ml) and anti-Mullerian hormone (AMH) (0.07 ng/ml) plasma levels, indicating a diagnosis of hypergonadotropic hypogonadism. The functions of the thyroid gland, adrenal gland and immune system were normal. The hypothalamic pituitary MRI showed no abnormalities. She had a 3-year bone age delay (BA = 10 years). The growth hormone provocative test indicated that the peak value of growth hormone was 10.3 ng/ml (normal range: >10 ng/ml). The plasma level of IGF-1 was within the following normal range: 350.00 (180–850) ng/ml. Bilateral ovaries were not found through ultrasonography. Furthermore, the bilateral gonadal biopsy confirmed fibrous hyperplasia without follicular tissue (Figure 1). The initial dose of estrogen (estradiol valerate) replacement was 0.25 mg/day, and the dose was gradually increased to 0.75 mg/day over one and a half years. In combination with calcium and vitamin D3, her height increased to 148.7 cm (HtSDS = −2.02) by the age of 15 years, the bilateral breasts enlarged to Tanner II, and the size of uterus by ultrasonography increased from 10 × 17 × 6 mm to 24 × 38 × 19 mm, her menarche was established.

Her sister (aged 6 years and 7 months) had a shorter height than normal (114.5 cm, HtSDS = −1.07), and the sizes of her uterus and bilateral ovaries and the levels of LH and FSH were normal (Table 2). Interestingly, approximately one year later, her height was 119 cm (HtSDS = −1.83); the gynecological ultrasound re-examination revealed that the uterus shrank to 12 × 16 × 4 mm, and the bilateral ovaries were not clear. Moreover the levels of LH and FSH were lower than those obtained in the first test results, and the level of serum AMH was still low (0.05 ng/ml). It is noteworthy that alpha fetoprotein (AFP) levels were as high as that of her elder sister (Table 2). She did not receive any treatments and only followed up closely.

### 3.2 | WES and Sanger sequencing identified of two heterozygous novel mutations of *MCM8*

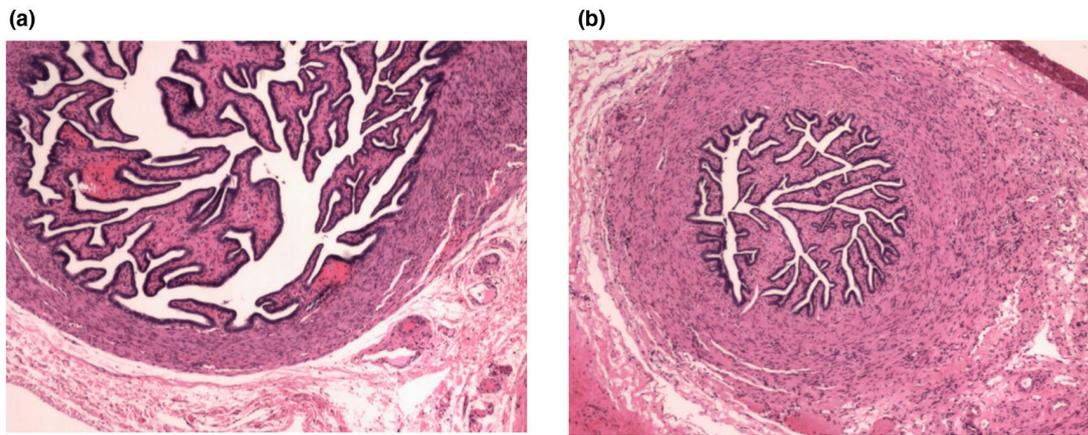
The results showed that two heterozygous novel mutations c.724T>C and c.1334C>A were identified in *MCM8* gene. The c.724T>C (rs5939307) in exon 7 leads to the change of amino acid 242 from cysteine to arginine, and the c.1334C>A (rs5958601) in exon 12 leads to the early termination of translation at Serine 445. We did not find these two mutations in 100 ethnically matched normal healthy control individuals. The two novel variants have not been reported in

**TABLE 2** Clinical and hormonal characteristics of the study participant

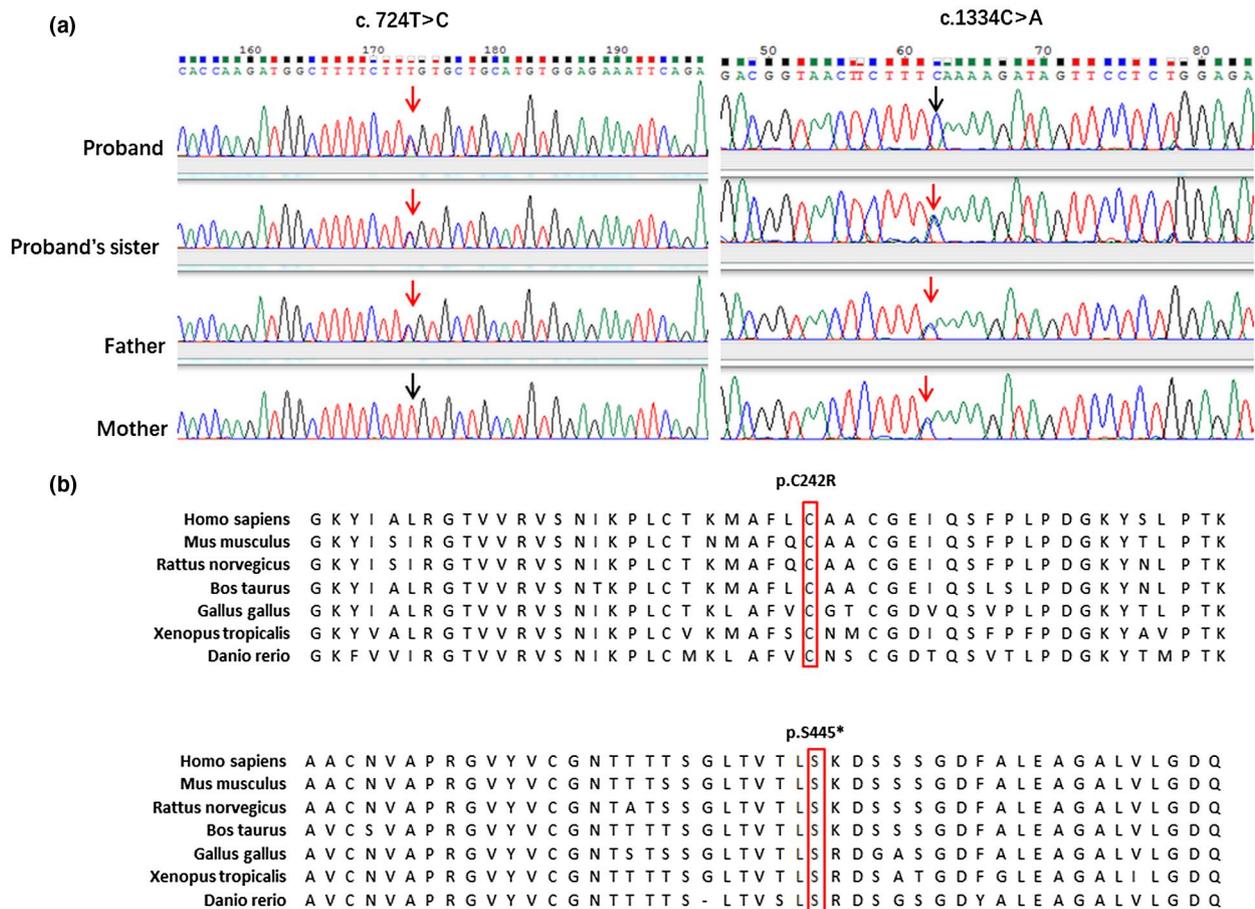
	Proband		Proband's Sister	
	First visit	Final visit	First visit	Final visit
Age (years)	13 years	15 years	6 years 7 months	7 years 10 months
Height (cm)	138	148.7	114.5	119
HtSDS	−3.05	−2.02	−1.07	−1.83
Weight (kg)	36.1	39	19	21.8
Tanner stage	B1, PH1	B2, PH2	B1, PH1	B1, PH1
FSH (mIU/ml)	86.34	103	2.51	0.74
LH (mIU/ml)	17.14	13.7	0.44	0.17
E2 (pg/ml)	77	215	<73	<73.4
T (ng/ml)	1.01	<0.69	<0.69	<0.69
AMH (ng/ml)	0.07	0.01	0.01	0.05
AFP (ng/ml)	17.32	21.22	15.02	20.32
BA (years)	10	12	7	8
Ultrasound imaging	U (10 × 17 × 6 mm) Bilateral ovaries not found	U (24 × 38 × 19 mm) Bilateral ovaries not found	U (14 × 20 × 7 mm) ROV (11 × 22 × 10 mm) LOV (9 × 21 × 9 mm)	U (12 × 16 × 4 mm) Bilateral ovaries not visible
Karyotype	46,XX	/	46,XX	/

Note: Normal ranges: FSH (mIU/mL)—follicular phase: 3.85–8.78; LH (mIU/ml)—follicular phase: 2.12–10.89; E2 (pg/ml)—follicular phase: 0–447.74; T (ng/ml): 0–2.6; AMH (ng/ml): 0.39–6.67 (0–10 years); 1.52–9.41 (11–18 years); AFP (ng/ml): 0–7.

Abbreviations: AFP, alpha fetoprotein; AMH, anti-Mullerian hormone; FSH, follicle stimulating hormone; HtSDS, height standard difference score; LH, luteinizing hormone.



**FIGURE 1** Ovarian biopsy pathology. (a) Right ovary; (b) Left ovary. Microscopically, in tubal tissue without follicular tissue, only fibrous tissue proliferation exists



**FIGURE 2** Family pedigree and *MCM8* mutation sequencing. (a) Mutation information of families with *MCM8* variants (references NM\_001281522.1). Missense mutation (c.724T>C) and compound heterozygous termination mutation (c.1334C>A) in affected individuals (Proband: 13-year-old girl; Proband's sister: aged 6 years and 7 months) and the heterozygous mutations from unaffected parents. (b) Protein alignment analysis shows that the cysteine residue at 242 and the serine residue at 445 in *MCM8* are conserved across seven species

any previous study, and it was not listed in dbSNP, HGMD, ExAC, ClinVar, or the 1000 Genome project database and BGI's database which is consisting of ~50,000 Chinese Han samples. Sanger sequencing validated that the proband were homozygous for this variant and that their parents were

heterozygous carriers (Figure 2a), conformed to the autosomal recessive inheritance pattern. According to the variant interpretation guidelines of ACMG, these two variants (c.724T>C and c.1334C>A) were classified as "pathogenic" variants. Hence, both mutations could be regarded as potential

pathogenic mutations causing disease in the proband in a compound heterozygous manner.

### 3.3 | Multiple sequence alignment and molecular structural modeling prediction

In the proband, WES identified two heterozygous mutation, i.e., c.724T>C (p.C242R) and c.1334C>A (p.S445\*). The multiple sequence alignment showed that two mutated amino acids Cys242 and Ser445 are evolutionarily highly conserved among different species (Figure 2b), indicating the severity of two mutations in both the structure and functions of the wild-type MCM8 protein.

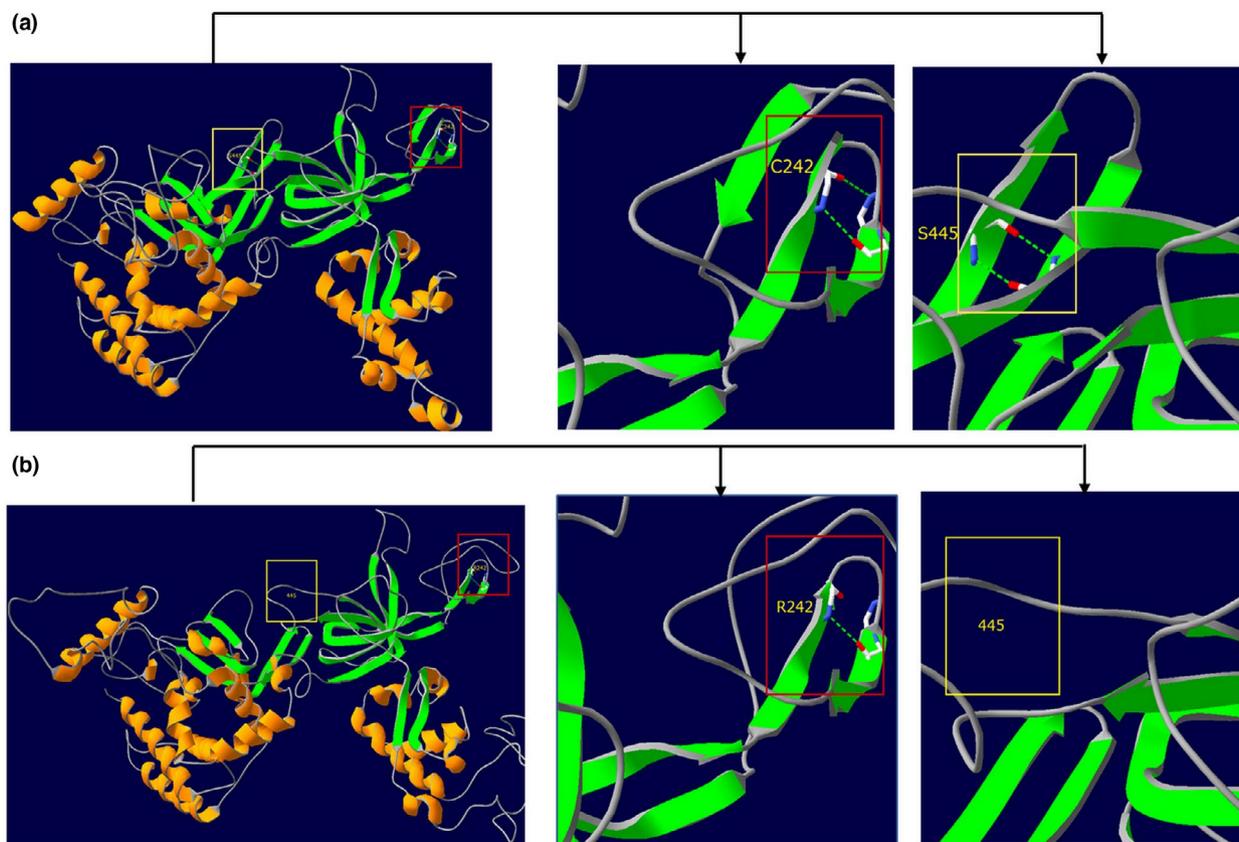
A 3D structural model of MCM8 was predicted using SWISS-MODEL to determine whether the novel p.C242R and p.S445\* mutations in this Chinese family affect the structure of MCM8. The sequence identity between the target and template was 27.91%. Using Swiss-Pdb Viewer 4.1 (Figure 3), the mutant p.C242R was predicted to lose one hydrogen bond between Cys242 and Gly246; Cys242 only has one other hydrogen bond with Glu247, possibly perturbing

the amino acid side chain. The mutant p.S445\* was predicted to lose two H-bonds between Ser445 and Ala454, resulting in amino acids that could not be translated and a change in conformation.

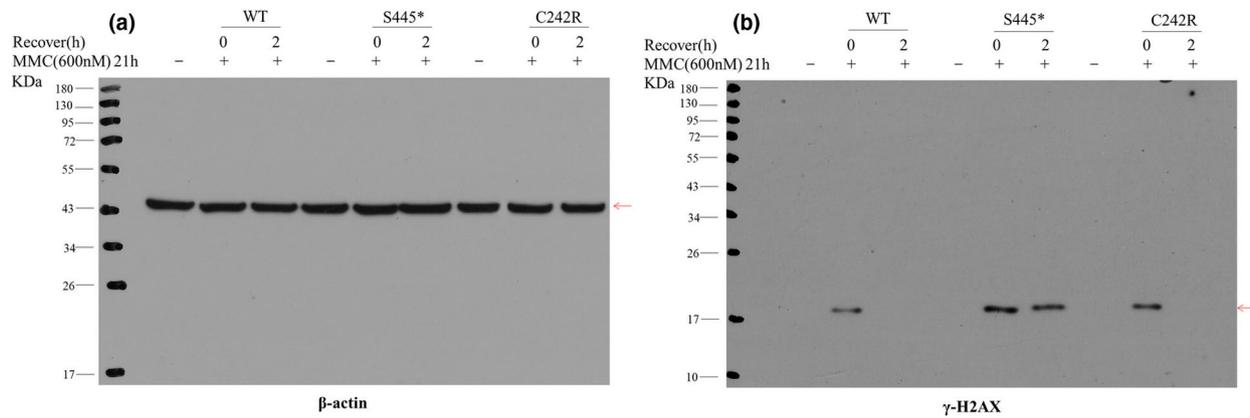
### 3.4 | Mitomycin C sensitivity assay

The WB results showed that wild-type MCM8 and the p.S445\* and p.C242R mutants expressing HeLa cells had up-regulated levels of  $\gamma$ -H2AX after incubation in the medium containing 600 nM mitomycin C (MMC) for 21 hr, indicating that HeLa cells with both MCM8-WT and MT (mutant) exhibited DSBs (Figure 4). The degree of mutant damage was slightly higher than that in the WT, indicating that mutants p.S445\* and p.C242R were more sensitive to the DNA damage inducer MMC.

The injured WT and MT-p.C242R were transferred to fresh medium (medium without MMC) for 2 hr, and the expression of  $\gamma$ -H2AX was significantly downregulated, while transferring MT-p.S445\* after 2 hr of repairing did not significantly downregulate the expression of  $\gamma$ -H2AX, suggesting that the



**FIGURE 3** Molecular modeling comparison of wild-type (a) and mutant-type (b) MCM8 protein. (a) In the wild-type protein Cys242 is linked to Gly246 and Glu247 by two H-bonds. The wild-type protein has two H-bonds between Ser445 and Ala454. (b) In the mutant protein Cys242 is predicted to lose one H-bond between Cys242 and Gly246 due to the substitution of cysteine to arginine at position 242. The mutant protein Ser445 is predicted to lose two H-bonds due to the translation termination at position Ser445. The sites of 242 and 445 are highlighted in red and yellow frame lines, respectively, and are locally zoomed



**FIGURE 4** Dysfunctional DSBs repair in mutants p.S445\* and p.C242R. (a) Detection of  $\beta$ -actin antibody as an internal reference signal. (b) Human cervix carcinoma cell line (HeLa) cells overexpressing wild-type (WT) or mutant MCM8 (MT) were exposed to MMC for 21 hr, and double-stranded breaks were induced. The level of  $\gamma$ -H2AX in cells overexpressing p.S445\* and p.C242R was increased compared with that in wild-type MCM8. After recovery for 2 hr,  $\gamma$ -H2AX disappeared from WT and MT p.C242R but  $\gamma$ -H2AX still existed after recovery in the cells overexpressing p.S445\*

mutant p.S445\* might destroy or delay the DNA damage repair function of the MCM8 protein (Figure 4).

## 4 | DISCUSSION

The term POI also allows for the inclusion of hypergonadotropic hypogonadism, premature ovarian failure, and ovarian dysgenesis. The *MCM8* gene involved in DNA damage repair have been found to be the cause of POI (AlAsiri et al., 2015; Desai et al., 2017; Qin et al., 2015; Tenenbaum-Rakover et al., 2015). Two novel MCM8 mutations were identified in a 13-year-old girl with POI and short stature and in her sister aged 6 years and 7 months with progressive POI by WES in this study. One mutation was missense mutation c.724T>C (p.C242R) in exon 7, which is located at the amino-terminal DNA binding domain of MCM8. Cysteine 242 only has one hydrogen bond after losing one bond, possibly perturbing the amino acid side chain. The other mutation is the termination mutation c.1334C>A (p.S445\*) in exon 12, located at the AAA<sup>+</sup> core domain encoding DNA helicase proteins. Serine 445 loses two hydrogen bonds and becomes the termination codon; subsequently, the amino acids, which are involved in homologous recombination, meiosis, and DNA replication, are no longer translated. These two novel mutations were derived from the genomes of her father and mother and conformed to the autosomal recessive inheritance pattern.

Functional experiments were performed to explore the deleterious effects of the identified mutations in our study. DNA DSBs can induce the phosphorylation of H2AX and the formation of  $\gamma$ -H2AX, which plays an important role in recruiting DNA damage response (DDR) proteins to DNA lesion sites and initiating DDR (Vallard et al., 2017; Zhang et al., 2016). The mutants p.C242R and p.S445\* were more

sensitive to the DSBs inducer MMC than the WT. After 2 hr of recovery, the  $\gamma$ -H2AX expression of the mutant p.S445\* was not significantly downregulated, revealing that it was more significantly impaired in its ability to repair DNA than the mutant p.C242R and the WT, leading to genomic instability and rendering the oocyte prone to apoptosis. The results also confirm that the mutant p.S445\* has a more deleterious effect on the homologous recombination-mediated DNA repair pathway and can explain its unique clinical manifestations.

The 13-year-old girl with these two novel *MCM8* mutations displayed delayed puberty, short stature and ovarian insufficiency, she had 46,XX hypergonadotropic hypogonadism and could be diagnosed with POI. In addition, her ovarian biopsy showed no follicular cell proliferation (Figure 1), suggesting that the *MCM8* mutations had a greater impact during the follicular phase. This finding can be attributed to the higher expression of MCM8 during the follicular phase of the menstrual cycle than during the ovulatory and luteal phases (Dondik, Lei, Gaskins, & Pagidas, 2019). Her younger sister (aged 6 years and 7 months) harbored the same *MCM8* mutations but had no symptoms at her first visit. Interestingly, when she visited at the age of 7 years and 10 months, her ovarian B-mode ultrasonography showed that the sizes of both ovaries had decreased from normal to unclear, indicating that ovarian failure was occurring. These two novel *MCM8* mutants seem to have more significant effects on ovarian development in children and adolescents, especially during the follicular phase. The levels of AFP were increased in both sisters, and the mutant p.S445\* increased the expression of  $\gamma$ -H2AX, which has been recognized as a common feature of DDR activation in precancerous lesions and cancer tissues (Nagelkerke & Span, 2016). This finding can be explained by cancer susceptibility, consistent with previous animal studies showing that *MCM8*<sup>-/-</sup> female mice

only have arrested primary follicles and frequently develop ovarian tumors (Lutzmann et al., 2012).

Moreover this 13-year-old girl had short stature (HtSDS =  $-3.05$ ,  $<-2SD$ ) in early adolescence, while the HtSDS of her sister (aged 6 years and 7 months) decreased from  $-1.07$  to  $-1.83$  a year later, which is consistent with a decline in ovarian function, revealing that the damage to MCM8 function could lead to height growth retardation. The reason for this effect may be related to the impaired DNA repair function of MCM8 and the low estrogen level, which is similar to the pathogenesis of MCM9 (Wood-Trageser et al., 2014). Early low-dose estrogen replacement therapy contributed to height growth in her older sister, as her HtSDS increased from  $-3.05$  to  $-2.02$ . Considering the cancer susceptibility, growth hormone therapy should not be used blindly.

## 5 | CONCLUSIONS

MCM8 mutation is one of the genetic causes of POI in children and adolescents. It is difficult to detect POI during childhood unless puberty is delayed, WES is helpful for early diagnosis and hormone replacement treatment. Estrogen replacement therapy, rather than starting growth hormone therapy before the identification of MCM8 mutations, is conducive to improving height growth in adolescence. The novel mutant p.S445\* has a greater influence on DNA damage repair than p.C242R. The affected individual with these two mutants had ovarian insufficiency, height growth retardation at the adolescent stage and cancer susceptibility. However, the specific mechanism of this condition needs to be elucidated in future studies.

## ACKNOWLEDGEMENTS

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## CONFLICTS OF INTEREST

None declared.

## AUTHOR CONTRIBUTIONS

This study was conceived and designed by FW, SG, and PL. The experiments were conducted by FW. Data were analyzed by FW and PL. PL and SG contributed clinical diagnosis and treatment of patients. The paper was written by FW and PL.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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