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ORIGINAL ARTICLE

# A novel heterozygous *ERCC6* variant identified in a Chinese family with non-syndromic primary ovarian insufficiency

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## Abstract

**Background:** Premature ovarian insufficiency (POI) is a clinical syndrome occurring in women before 40 with decreased ovarian function. Up to 25% of POI cases result from genetic factors that remain largely unknown. The Excision repair cross-complementing, group 6 (*ERCC6*) variant has been found to cause POI, which is hardly ever diagnosed in adolescents.

**Methods:** Whole-exome sequencing was performed on a 19-year-old proband with non-syndromic POI and her parents. Sanger sequencing was used to confirm the identified variant. The effect of the variant on the protein was analyzed in silico and Swiss-MODEL.

**Results:** A novel heterozygous missense variant, c.2444G > A (p. GLy815Asp) of *ERCC6* was identified in the proband who inherited the variant from her father. The variant was confirmed in another POI patient from the pedigree and was absent in the proband's mother and sister who presented normally. In silico analysis predicted this variant was deleterious. Swiss-Model revealed that the mutant amino acid formed multiple H-bonds with adjacent residues, which may lead to a dysfunction of ERCC6 protein.

**Conclusion:** We firstly diagnosed an adolescent POI case associated with a novel heterozygous *ERCC6* variant. The results expanded the variants spectrum of *ERCC6* and provided guidance for POI diagnosis and genetic counselling.

**KEYWORDS**
adolescents, *ERCC6*, heterozygous variant, premature ovarian insufficiency

## 1 | INTRODUCTION

Premature ovarian insufficiency (POI) is featured by abnormal menstruation (amenorrhea or oligomenorrhea) and an elevated serum follicle-stimulating hormone (FSH > 25 U/L) on two occasions separated by 4 weeks or more in women before the age of 40. The prevalence of POI is about 1% in the general population and only 0.01% in people under the age of 20, which increases the challenge of POI diagnosis in adolescents (Webber et al., 2016). POI is highly clinical heterogeneous with variable manifestations. It can be divided into syndromic and non-syndromic categories depending on whether there are other complications such as mental retardation

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or cardiovascular diseases. According to the absence or presence of spontaneous menstruation, POI falls into two groups: primary and secondary.

The aetiology of POI is complex and genetic, immune, metabolic and infectious factors all contribute to the occurrence of POI. Genetic factors including chromosomal abnormalities and single-gene variations may account for up to 25% of POI cases (Qin, Jiao, et al., 2015; Shen et al., 2021), however, a large proportion of cases remain unexplained. In the early stage, chromosomal analysis has been recommended for POI diagnosis because chromosomal abnormalities which mainly include numerical and structural defects were considered to cause approximately 10~13% cases (Baronchelli et al., 2011; Kalantari et al., 2013). More recently, whole-exome sequencing (WES) has been proved to be a powerful approach to identify pathogenic gene variants that contribute to POI (Jolly et al., 2019). Over 100 genes involved in various pathways and biologic processes have been found to cause POI and largely expanded the genetic aetiology of POI (Franca & Mendonca, 2022). Amongst them, about 20 monogenic variants have been considered to be associated with non-syndromic POI, such as *BMP15* (Di Pasquale et al., 2006), *NR5A1* (Jaillard, Sreenivasan, et al., 2020), *NOTCH2* (Li et al., 2020), *WT1* (Wang et al., 2022) and *ERCC6* (Qin, Guo, et al., 2015).

*ERCC6* (Excision repair cross-complementing, group 6, OMIM#609413#) gene is located on chromosome 10q11 region and encodes a member of the SWI2/SNF2 DNA-dependent ATPase superfamily. The encoded protein, which can interact with a variety of transcription factors and excision repair proteins, is essential for transcription-coupled DNA double-strand break repair (Batenburg et al., 2017; Sin et al., 2016). Variants in *ERCC6* have newly been found to be related to non-syndromic POI and were first described in a Chinese cohort by Qin et al. They identified three novel heterozygous variants in *ERCC6* including c.643G>T (p. Glu215X), c.2237G>A (p. Gly746Asp) and c.3166G>A (p. Val1056Ile) (Qin, Guo, et al., 2015), followed by another two studies in which three *ERCC6* variants, including c.2510G>T (p. Arg837His), c.1389G>T (p. Gln463His) and c.2027T>G (p. Val676Gly), were reported (Jaillard, Bell, et al., 2020; Jin et al., 2020). In a recent study, another heterozygous *ERCC6* variant, c.1769C>T (p. Pro590Leu) was identified by next-generation sequencing in 74 sporadic POI patients (Shen et al., 2021). So far, only these four studies mentioned above have reported *ERCC6* variants in POI.

In this study, we performed whole exome sequencing on a non-syndromic POI family. A novel missense variant of *ERCC6*, c.2444G>A (p. GLy815Asp) was identified in the proband and was recurrent in her relative from the pedigree, suggesting that this variant may be responsible for the genetic aetiology of POI for this family.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients

The proband was a 19-year-old girl coming from a non-consanguineous Chinese Han family. She was enrolled in our reproductive medicine centre because of irregular menstruation for more than half a year. Her menstruation began at the age of 13. She encountered menstrual irregularity and developed into amenorrhea at the age of 17. She had no history of marriage or childbirth. During her visit to our centre, detailed physical, clinical and laboratory examinations, including sex hormones and AMH (Anti-Mullerian hormone) levels, were performed, as well as karyotype analysis. An inquiry on familial history was also made. Additional 100 matched controls were recruited. The diagnosis of POI was made according to European Society for Human Reproduction and Embryology (ESHER) guidelines. This study was approved by the Ethics Committee of Xinhua Hospital affiliated with Shanghai Jiao Tong University School of Medicine. Informed consent was obtained for clinical information collection and publication.

### 2.2 | Genomic DNA extraction

Genomic DNAs were extracted from peripheral blood samples following the standard procedures of the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). The concentration and quality of DNA were determined by Nanodrop 2000 (Thermo) and agarose gel electrophoresis.

### 2.3 | Whole-exome sequencing

Whole-exome sequencing was performed on the proband and her parents on the MGISEQ 2000 platform (BGI) for variants detection. DNA reads were mapped against the human genome reference from UCSC (hg19/GRCh37) utilizing the BWA (Burrows-Wheeler Alignment) tool. Variants calling was carried out as previously (McKenna et al., 2010). Variants located in exons and adjacent splicing regions were chosen for further annotation. Candidate variants were screened according to the following criteria: (1) the minor allele frequency (MAF) of the variant was lower than 0.1% in public databases including 1000

Genomes (http://www.1000genomes.org/variation-pattern-finder), Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org) and Genome Aggregation Database (gnomAD); (2) the variant wasn't synonymous; (3) the variant was related to phenotype based on previous studies or animal models.

### 2.4 | Sanger sequencing

The potential pathogenic variant identified by whole exome sequencing was confirmed in the proband and other family members except I-1 and I-2 whose DNA samples were unavailable by Sanger sequencing. Validation of the variant in 100 healthy controls was also performed. Primers used for Sanger sequencing were designed on Primer3 (version 0.4.0) and listed as follows: Forward primer 5'-CCTCCTTGCCTAGGGAATCT-3′; Reversed primer 5'-CACTCACCTGCCTTGACTGA-3′. The reference sequence NM_000124.4 of *ERCC6* was used and raw sequence data were analyzed with Lasergene DNA Star (Madison. WI, USA).

### 2.5 | In silico and pathogenicity analysis on identified variant

The ClustalW2 program was utilized to evaluate the conservation of amino acid residue where the identified variant occurred on ERCC6 protein amongst species. To analyze the effect of variant amino acid residue on the three-dimensional structure of the protein, the wild type and mutant protein structures were modelled in the Swiss PDB viewer based on the credible template structure obtained by SWISS-MODEL (https://swissmodel.expasy.org). SIFT (http://sift.jcvi.org/), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/), PROVEAN (http://provean.jcvi.org/index.php) and Mutation Taster (http://www.mutationtaster.org) were used to predict the effect of the identified variant on ERCC6 protein function. We finally categorized the identified variant into pathogenic, likely pathogenic, uncertain significance (VUS), likely benign, and benign groups according to the guideline of the American College of Medical Genetics and Genomics (ACMG) (Richards et al., 2015).

### 2.6 | Review of *ERCC6* variants in the aetiology of POI

We conducted an overall review of the literature that reported POI cases associated with *ERCC6* variants through searching the NCBI PubMed database. The collected information was re-analyzed and summarized.

## 3 | RESULTS

### 3.1 | Clinical diagnosis

A 19-year-old girl from a Chinese non-consanguineous family was defined as the proband (III-1) (Figure 1a). She had menarche at the age of 13. Menstrual irregularity began and amenorrhea was found at the age of 17. Laboratory sex hormone testing showed evaluated FSH level (>25 IU/L) on two occasions more than 4 weeks apart and extremely low AMH level (<0.01 ng/ml), indicating a loss of ovarian function (Table 1). Physical examination indicated a normal stature and weight. Her intelligence and karyotype were normal. No obvious abnormality was found in her uterus and bilateral ovaries. Histories of radiotherapy, chemotherapy and ovarian surgery were excluded. Other complications were not found. According to the diagnostic criteria of POI mentioned in the ESHER guideline, the proband was diagnosed as non-syndromic POI. The investigation of familial members revealed that there is another female (II-1) with POI, who

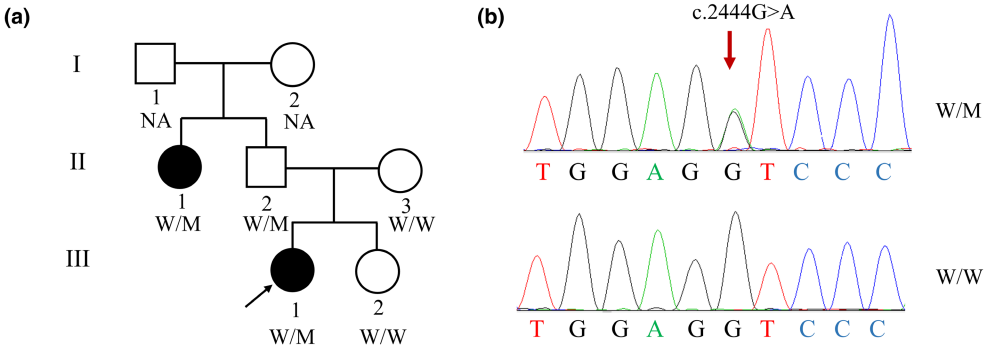


**FIGURE 1** Identification of a heterozygous *ERCC6* variant in a Chinese family with non-syndromic POI. (a) Pedigree of the POI family. The proband was represented with a black arrow. (b) Sanger sequencing validation of the identified heterozygous *ERCC6* variant in the family. NA, DNA samples were not available; W, wild type; M, mutant. The red arrow indicated the position of the *ERCC6* variant

suffered secondary amenorrhea and was diagnosed outside the hospital at the age of 27 years and 30 years, respectively. Other family members presented with normal phenotypes.

**TABLE 1** Clinical features of the proband with non-syndromic POI

| Features | First visit | Second visit |
|---|---|---|
| Age (years) | 17 years and 5 months | 18 years |
| Menarche (years) | 13 years | / |
| Amenorrhea (years) | 17 years | / |
| FSH (IU/L) | 119.32[a] | 176.99[a] |
| LH (IU/L) | 43.04 | 59.55 |
| E2 (pmol/L) | 229.41 | 102.76 |
| PRL (mlU/L) | 173.10 | 171.90 |
| P (nmol/L) | 0.89 | 0.6 |
| T (nmol/L) | 1.39 | 1.29 |
| AMH (ng/ml) | <0.01[a] | <0.01[a] |
| Karyotype | 46, XX | / |

Abbreviations: AMH, Anti-Mullerian hormone; E2, Estradiol; FSH, Follicle-Stimulating Hormone; LH, Luteinizing Hormone; P, Progesterone; PRL, Prolactin; T, Testosterone.

[a]Abnormal results according to reference values.

## 3.2 | Identification of a novel heterozygous *ERCC6* variant

Whole-exome sequencing was performed on the proband and her parents. Based on the filtration criteria mentioned above, a heterozygous variant, c.2444G > A (p. GLy815Asp) in *ERCC6* (NM_000124.4) was identified as the potential pathogenic variant for this family. It was located in exon 13 of *ERCC6* cDNA and led to a replacement of Gly by Asp at amino acid 815 in the ATPase domain of ERCC6 protein (Figure 2a,c). This variant was absent in 1000 Genomes, ExAC or gnomAD databases, indicating it's a novel variant (Table 2). Sanger sequencing suggested that the proband's father harboured the identified heterozygous variant, whilst her mother and younger sister who present normal did not carry this variant, consistent with the autosomal dominant inheritance pattern of POI caused by *ERCC6* variants (Figure 1). The variant was recurrent in another family member (II-1) with POI and was not found in 100 healthy controls, furtherly strengthening that the identified variant was highly correlated with the POI phenotype in this family.

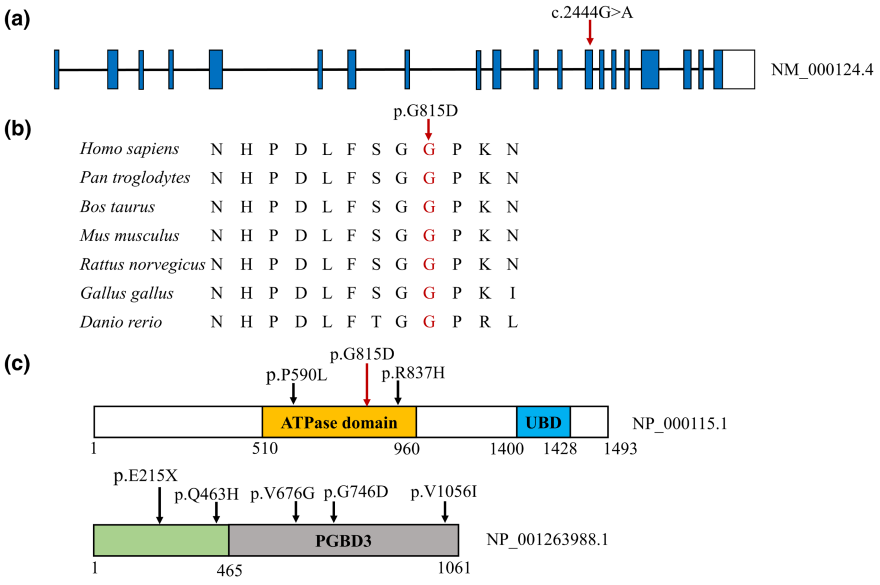


**FIGURE 2** Analysis of the identified *ERCC6* variant. (a) Schematic diagram of *ERCC6* transcript NM_000124.4. Exons of *ERCC6* were represented with rectangles. The blue and blank regions indicated the coding and non-coding regions of exons, respectively. The variant lay in exon 13 of *ERCC6* indicated by the red arrow. (b) Alignment of ERCC6 amino acid sequence amongst species. The variant G815D was highly conserved across different species. Sequences used were as follows: Homo sapiens, NP_000115.1; Pan troglodytes, XP_009438633.3; Bos taurus, NP_001178272.1; Mus musculus, NP_001074690.1; Rattus norvegicus, NP_001100766.1; Gallus gallus, XP_004942197.2; Danio rerio, XP_688972.2. (c) Schematic illustration of ERCC6 protein. The yellow and blue rectangles represented ATPase domain and Ubiquitin-binding domain (UBD) of ERCC6 protein (NP_000115.1), respectively. Green and grey rectangles in an ERCC6-PGBD3 fusion protein (NP_001263988.1) represented the exons 1–5 of ERCC6 and PGBD3, respectively. Numbers refer to amino acid positions. The red and black arrows indicated the position of the *ERCC6* variant identified in this study and previously reported, respectively

### 3.3 | In silico and pathogenetic analysis of the *ERCC6* variant

Multiple sequences alignment in ClustalW2 showed that the Gly residue at 815 was strictly conserved amongst different species (Figure 2b), suggesting that Gly815 was located in a critical functional domain and variant at this locus may be harmful. Further analysis revealed that the mutant amino acid residue Asp815 was negatively charged, and had a longer side chain compared to the uncharged wild type, which may change the structure of ERCC6 protein. To clarify this, we modelled and analysed the 3D structure of ERCC6 in a Swiss PDB viewer. As results show, the mutant Asp815 was predicted to form multiple H-bonds with adjacent Pro816 and Lys817, which was not found in wild type and may affect the folding and formation of ERCC6 protein (Figure 3). This variant was predicted to be deleterious by PolyPhen2, PROVEAN and Mutation Taster (Table 2). According to the variant interpretation guidelines of ACMG (2015), variant c.2444G > A (p. GLy815Asp) of *ERCC6* was defined as likely pathogenic (PM1 + PM2 + PP1 + PP3).

### 3.4 | Review of *ERCC6* variants in the aetiology of POI

It's well-known that heterozygous variants of *ERCC6* are correlated with non-syndromic POI. About 43 million years ago, the PiggyBac transposable element derived 3 (*PGBD3*) integrated into the intron 5 of *ERCC6* and generated an evolutionally conserved fusion gene known as *ERCC6-PGBD3*. As a result of alternative splicing, two products were generated. One was the original transcript encoding the full-length ERCC6. The other one encoded a fusion protein comprised the first 5 exons of ERCC6 and the entire PGBD3 transposase (Newman et al., 2008). Here, we reviewed the PubMed database and summarized all POI cases caused by *ERCC6* or *ERCC6-PGBD3* variants in the literature (Table 3; Figure 2c). Amongst the all reported variants, one was frameshift, and the others were missense, indicating that missense variants of *ERCC6* were the major causes of non-syndromic POI. The variant identified in our study further emphasized the role of missense variants of *ERCC6* in the aetiology of non-syndromic POI and expanded the variants spectrum of *ERCC6*.

**TABLE 2** In silico analysis of the identified variant in *ERCC6*

| Nucleotide change[a] | Amino acid change[b] | charged change | ExAC[c] | gnomAD[d] | 1000 Genomes[e] | SIFT[f] | PolyPhen2[g] | PROVEAN[h] | Mutation Taster[i] |
|---|---|---|---|---|---|---|---|---|---|
| c.2444G > A | p. G815D | None→ negatively | 0 | 0 | 0 | Tolerated | Probably damaging | Deleterious | Disease causing |

*Notes*: Minor allele frequencies in public databases including [c]ExAC, [d]gnomAD and [e]1000 Genomes; [a]cDNA and [b]protein reference sequences were NM_000124.4 and NP_000115.1, respectively; [f]SIFT, [g]PolyPhen2, [h]PROVEAN and [i]Mutation Taster were used to predict the impact of identified variant on ERCC6 function.

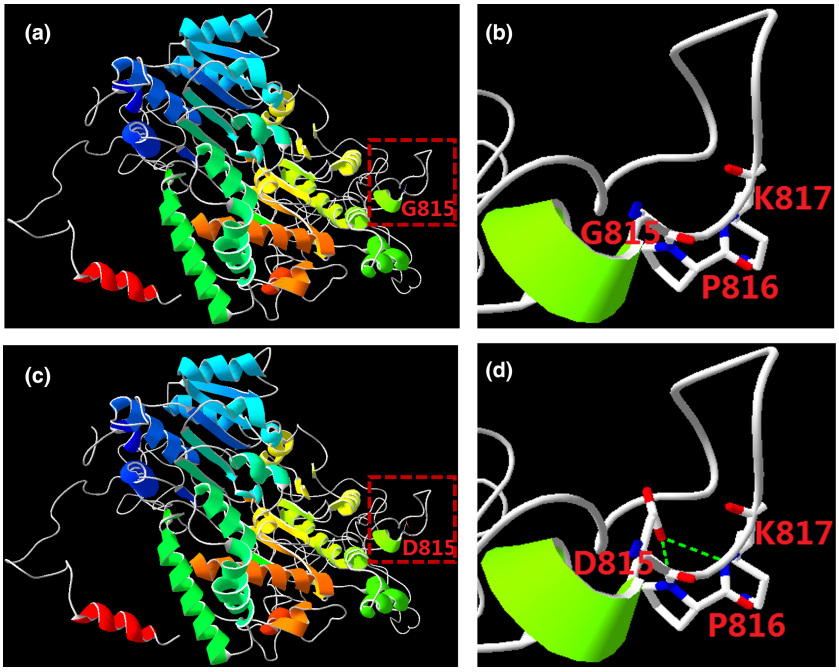


**FIGURE 3** 3D structure modelling of wild type and mutant ERCC6 protein. (a) In the wild type ERCC6 protein, G815 had no H-bond with adjacent P816 and K817 residues. (c) In the mutant ERCC6 protein, the amino acid residue at 815 was supposed to form H-bonds with P816 and K817 when substituted by Asp. (b) and (d) displayed magnification of the region within red dashed boxes in (a) and (c), respectively. The H-bond was indicated by green dotted lines

**TABLE 3** Summary of previously reported *ERCC6* variants in POI patients

| *ERCC6* variants (cDNA, protein) | Zygosity | Mutation type | Age (years) | Menarche (years) | Amenorrhea (years) | Age at diagnosis | Reproductive history | ACMG classification |
|---|---|---|---|---|---|---|---|---|
| c.2237G > A p. G746D[a] | Het[c] | missense | 66 | 14 | 18 | 29 | No | Likely Pathogenic |
| c.2237G > A p. G746D | Het | missense | 58 | 14 | 27 | 30 | No | Likely Pathogenic |
| c.2237G > A p. G746D | Het | missense | 36 | 14 | 37 | 37 | SV[f]:1 | Likely Pathogenic |
| c.2237G > A p. G746D | Het | missense | 28 | 13 | 23 | 28 | No | Likely Pathogenic |
| c.643G > T p. E215X[a] | Het | nonsense | 25 | 14 | 24 | 25 | No | Pathogenic |
| c.3166G > A p. V1056I[a] | Het | missense | 27 | 15 | 25 | 26 | No | Likely Pathogenic |
| c.1769G > T p. P590L[b] | Het | missense | 35 | 15 | 29 | 35 | No | Likely Pathogenic |
| c.2027T > G p. V676G[a] | Het | missense | NA[d] | –[e] | – | 20 | No | Likely Benign |
| c.1389G > T p. Q463H[a] | Het | missense | NA | 11 | NA | 29 | No | Uncertain Significance |
| c.2510G > T p. R837H[b] | Het | missense | NA | NA | NA | NA | NA | Likely Pathogenic |
| c.2444G > A p. G815D[b] | Het | missense | 32 | 14 | 27 | 30 | No | Likely Pathogenic |
| c.2444G > A p. G815D | Het | missense | 19 | 13 | 17 | 18 | No | Likely Pathogenic |

[a]Variants found in *ERCC6-PGBD3* chimeric transcript. The cDNA and protein reference sequences for them were NM_001277059.1 and NP_001263988.1, respectively;

[b]Variants found in original *ERCC6* transcript. Reference sequences of cDNA and protein for these variants, including the c.2444G > A reported in our study, were NM_000124.4 and NP_000115.1, respectively;

[c]Het, Heterozygous;

[d]NA, not available;

[e]no records since this patient was primary amenorrhea;

[f]SV, spontaneous vaginal birth.

## 4 | DISCUSSION

POI refers to the disease found in women under the age of 40 and is a major threat to reproductive health. The prevalence of POI varies amongst races and populations, which is considered to be about 1% in the general population and 0.01% or less in people younger than 20 (Webber et al., 2016). Even though POI affected less in people under 20, it severely impacted the patient's quality of life and reproduction and it deserved more attention. In this study, we reported a 19-year-old patient with non-syndromic POI and performed whole exome sequencing on her family. A novel heterozygous variant, c.2444G > A (p. Gly815Asp) in *ERCC6* was identified to be probably responsible for the POI phenotype of this family. To our knowledge, *ERCC6* variants were seldom diagnosed in adolescents, and the patient we reported was the youngest ever in POI cases associated with *ERCC6* variants (Table 3), which broadened our understanding of POI and provided help for the clinical diagnosis of POI patients.

ERCC6, also known as Cockayne syndrome (CSB) protein, consists of 1493 amino acids. The functional regions of ERCC6 protein including the ATPase domain, ubiquitin-binding domain (UBD) and winged helix domain (WHD) make it critical to DNA excision repair (Batenburg et al., 2017). Genetic variants in *ERCC6* have been found to be in connection with multiple human diseases. In the early time, homozygous or compound heterozygous variants in *ECRR6* have been widely known to cause Cockayne syndrome and UV-sensitive syndrome (Calmels et al., 2018; Horibata et al., 2004). Additionally, other studies have reported that single nucleotide polymorphism (SNP) of *ERCC6* was associated with the susceptibility to age-related macular degeneration and lung cancer (Baas et al., 2010; Lin et al., 2008). As for heterozygous variants of *ERCC6*, they have been newly identified to cause non-syndromic in recent studies (Qin, Guo, et al., 2015; Shen et al., 2021). Up to now, only seven variants of *ERCC6* have been reported in non-syndromic POI patients. Therefore, the detection of more *ERCC6* variants not only enriched the genetic variants spectrum of POI but also furtherly emphasized the important role of DNA damage repair in ovarian function and the pathogenesis of POI. This may provide clues for future pathogenic variants screening in POI patients.

POI is highly clinical heterogenous that affected individuals often manifested variances in severity and the age of onset. The proband in our study was early onset. She presented with menopause at the age of 17. However, the other POI patient in the same family was less serious and late-onset that her mense stopped at the age of 28. There might be intergenic interactions, and epigenetic or environmental factors that modified the clinical presentation of POI (Bouilly et al., 2016). Due to the low incidence and high heterogeneity of POI, it brings uncertainty to clinical diagnosis. Hence, genetic testing is of great significance to provide diagnostic certainty and genetic counselling. The *ERCC6* variant identified in the proband was inherited from her father, who had a normal phenotype and was absent in her mother and sister who were phenotypically normal. This supported a female-specific autosomal dominant inherence pattern of non-syndromic POI caused by the *ERCC6* variant. It is well known that homozygous or compound heterozygous variants in *ERCC6* are related to Cockayne syndrome (CS), which is an autosomal recessive disease. A large collection of homozygous or compound heterozygous variants in *ERCC6* have been found to cause CS (Laugel, 2013). Amongst these CS-affected families, there existed number of women with heterozygous *ERCC6* variants (Jaakkola et al., 2010). However, no POI was observed in any of these CS families. It seems that heterozygous *ERCC6* variants may contribute to the aetiology of non-syndromic POI in a dominant-negative or gain-of-function manner. More efforts are needed to confirm the pathogenic mechanism in the future. Considering the patient's desire for childbearing, our findings could provide guidance for genetic counselling and fertility management.

In conclusion, we identified a novel heterozygous variant, c.2444G > A (p. Gly815Asp) of *ERCC6* in a non-syndromic POI family. The results highlighted the role of *ERCC6* variants in the aetiology of POI and provided instruction for clinical diagnosis and genetic counselling.

### AUTHOR CONTRIBUTIONS

Lele Kuang and Yuping Gao designed the study, Lele Kuang analyzed the data and wrote the manuscript; Bin Liu and Di Xi collected data and reviewed the manuscript.

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### CONFLICT OF INTEREST

None.

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