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ORIGINAL ARTICLE

Identification of a novel *SUOX* pathogenic variants as the cause of isolated sulfite oxidase deficiency in a Chinese pedigree

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Abstract

Background: Isolated sulfite oxidase deficiency (ISOD) is a life-threatening rare autosomal recessive disorder caused by pathogenic variants in *SUOX* (OMIM 606887) gene. The aim of our study was to establish a comprehensive genetic diagnosis strategy for the pathogenicity analysis of the *SUOX* gene within a limited time and to lay the foundation for precise genetic counseling, prenatal diagnosis, and preimplantation genetic diagnosis.

Methods: Two offspring from one set of parents were studied. Next-generation sequencing (NGS) was used to screen for disease-causing gene variants in a family with ISOD. Then, Sanger sequencing was performed to verify the presence of candidate variants. Sulfite, homocysteine and uric acid levels were detected in the patients. According to the ACMG/AMP guidelines, the pathogenicity level of novel variants was annotated.

Results: The nonsense pathogenic variant (c.1200C > G (p.Y400*)) and a duplication (c.1549_1574dup (p.I525 Mfs*102)) were found in the *SUOX* gene in the proband. The nonsense mutation (c.1200C > G (p.Y400*), pathogenic, isolated sulfite oxidase deficiency, autosomal recessive) has been reported as pathogenic and the duplication (c.1549_1574dup (p.I525 Mfs*102), pathogenic, isolated sulfite oxidase deficiency, autosomal recessive) was novel, which was classified as pathogenic according to the ACMG/AMP Standards and Guidelines.

Conclusion: We established the pathogenicity assessment in ISOD patients based on ACMG/AMP Standards and Guidelines and this is the first ISOD patient reported in mainland China. We also discovered that ISOD is caused by *SUOX* gene duplication mutation, which enriches the spectrum of *SUOX* pathogenic variants.

KEYWORDS
homocysteine, isolated sulfite oxidase deficiency, novel mutation, pathogenic identification, sulfite oxidase, *SUOX*

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1 | INTRODUCTION

Isolated sulfite oxidase deficiency (ISOD, OMIM 272300) is a rare life-threatening autosomal recessive disorder caused by loss-of-function variants in sulfite oxidase (*SUOX*) gene (OMIM 606887) (Garrett et al., 1998). So far, about 50 cases of ISOD have been reported in the world. ClinVar Database lists 67 *SUOX*-associated variants and The Human Gene Mutation Database (Professional) lists 28 ISOD-associated variants of *SUOX* gene including missense/nonsense pathogenic variants (20), small deletions (7), and a small insertion (1). There is no long-term effective treatment, and patients often die in infancy. Therefore, for families with probands, it is crucial to clearly identify pathogenic variants to avoid the birth of new sick infants.

Sulfite oxidase encoded by *SUOX* catalyzes the oxidation of sulfite to sulfate, which is the final step in the catabolism of sulfur-containing amino acids methionine and cysteine. The enzyme is also involved in the detoxification of exogenous sulfites and sulfur dioxide (Feng et al., 2007; Macleod et al., 1961). The enzyme is localized to the mitochondrial membrane gap and consists of two identical subunits (Davis et al., 2014). Each subunit possesses two redox centers located in the Mo and heme domains and contains a small N-terminus cyt b5 heme, a large central Mo domain, and a large C-terminus interface domain (Kisker et al., 1997). The currently recognized catalytic mechanism is proposed by Hille et al. (Brody & Hille, 1999; Johnson-Winters et al., 2010). Sulfite oxidase uses cytochrome c (cyt c) as a terminal electron receptor in humans (Equation 1). The sulfite oxidase catalyzes the reaction of transferring an oxygen molecule from water into a sulfite to form a molecule of sulfate. After binding to the oxidation, the two electrons in the sulfite are transferred to the molybdenum center of the cofactor molybdopterin. Then after two steps of reaction, the two electrons are transferred to the heme iron atom in the cytochrome b5 domain and finally transferred to the cytochrome c (Cohen et al., 1972; Sullivan et al., 1993).

$$SO_3^{2-} + H_2O + 2(\text{cyt c})_{ox} \rightarrow SO_4^{2-} + 2H^+ + 2(\text{cyt c})_{red} \quad (1)$$

The main clinical symptoms of ISOD include axial hypotonia/peripheral hypertonia, (pharmacoresistant) seizures, abnormal movement, feeding difficulties, psychomotor retardation, progressive microcephaly, and ectopia lentis (Claerhout et al., 2018). Ventriculomegaly, thinning of the corpus callosum, cystic white matter changes, cerebral and cerebellar atrophy are neuroimaging features of the disease (Schwarz, 2016). ISOD diagnosis includes the following metabolic symptoms: sulfite-positive in urine, reduced total homocysteine and normal methionine in plasma, low cystine and elevated S-sulfocysteine in urine and plasma, normal hypoxanthine and xanthine in plasma and urine, and normal uric acid levels in urine and plasma (Holder et al., 2014; Palacín et al., 2014).

ISOD is pan-ethnic, which found in almost all ethnic groups and regions. The population of mainland China accounts for one-fifth of the world's population but there have been no reports of ISOD before. It is likely due to the difficulty of ISOD diagnosis. In the present study, we discovered two pathogenic variants in the *SUOX* gene of the ISOD-presenting proband in mainland China. One allele is a missense mutation previously linked to ISOD, the second allele is a novel duplication event that changes the reading frame of the *SUOX* mRNA. Our results enriched the *SUOX* gene mutation spectrum, which provides a theoretical basis for future prenatal diagnosis and preimplantation genetic diagnosis.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

The ethical approval of this study was obtained from the Ethics Committee of Sun Yat-Sen University. Informed consents were taken from the parents of the proband.

2.2 | Patient

The proband (female) is the second child from a non-related healthy couple of Chinese Han ethnicity and the couple's first child was a boy, who developed fever, convulsions and brain dysplasia 3 days after birth and died about 1 month after birth (Figure 1). At 38 weeks and 4 days of normal pregnancy, the proband was born by cesarean section. The proband had no history of intrauterine distress and asphyxiation. Apgar score was 10 points for 1, 5, and 10 min after birth. The proband was 48 cm in height (normal value: 44.7 cm~55.0 cm), 2330 g in weight (normal value: 2260~4650 g), 33 cm in head circumference (normal value: 30.4~37.5 cm), and had normal head shape.

2.3 | Next-generation sequencing (NGS) and candidate variants screening

Genomic DNA was extracted from peripheral whole blood using a Qiagen DNA extraction kit (Qiagen, Hilden, Germany). The purity and quality of DNA were checked by 1% agarose electrophoresis and Qubit, respectively. After passing the above test, the DNA was sequenced by NGS. All exons of the proband and her biological parents were sequenced by the HiSeq 2500 Illumina Genome Analyzer II platform, with an average coverage depth of

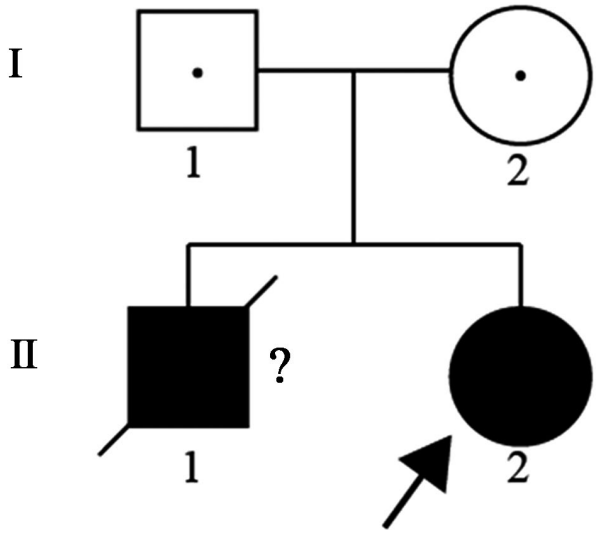

FIGURE 1 Pedigree of *SUOX* affected family. II_2 indicates the proband with compound heterozygotes variants at the *SUOX* gene: c.1200C > G (p.Y400*) and c.1549_1574dup (p.I525 Mfs*102). I_1 indicates the proband's mother who is a carrier for c.1200C > G (p.Y400*) variant. I_2 indicates the proband's father who is a carrier for c.1549_1574dup (p.I525 Mfs*102) variant. II_1 indicates the proband's older brother(deceased), who developed fever, convulsions and brain dysplasia 3 days after birth, and died about 1 month after birth. Question mark indicates no genetic verification

356 ± 226×. The variants were analyzed using the following strategy: (1) Sifting of variants against the Human Genome Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/index.php), NCBI SNP database (http://www.ncbi.nlm.nih.gov/snp), and The Exome Variant Server, an analysis program that automatically ignores minor allele frequencies >.01 (Richards et al., 2015); (2) Filtering out variants which do not coding protein and do not alter splice sites as predicted by BDGP (http://www.fruitfly.Org/seq_tools/splice.html); (3) Removing synonymous variants that do not alter amino acids; (4) Combining clinical symptoms and genetic characteristics to remove variations in which the phenotype doesn't match the patient's symptoms.

2.4 | Sanger sequencing

The variations obtained from NGS were verified by Sanger sequencing. The whole exons of *SUOX* (NG_008136.1) gene were amplified by PCR using the designed primers listed in Table 1. The protocol for PCR amplification was as follows: 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, (50–60°C) for 30 s, 72°C for 45 s, and 1 cycle at 72°C for 5 min. The size of the PCR product was confirmed by 1.5% agarose gel electrophoresis. The amplified product was sequenced by Majorbio Co, China. Sequencing results were compared with the reference sequence (NM_000456.2) on the NCBI.

2.5 | Quantitation of urinary and plasma metabolites

2.5.1 | Sulfite detection

The sulfite test strips were used to detect levels of Sulfite in the patient's urine. The test paper (QUANTOFIX Sulfite, Germany) was immersed in the patient's fresh urine for 1 s, and then placed on the test bench for 20 s to observe the color change. Normal urine was used as a negative control, and normal urine (100 mg/L) supplemented with sodium sulfite was used as a positive control.

2.5.2 | Uric acid test

The uricase method was used to detect uric acid levels. We got the proband's blood under fasting. The blood was centrifuged at 3000 r/min for 10 min. Then the supernatant was taken and detected by an Olympus AU640 automatic biochemical analyzer. The kit was purchased from Mindray (Shenzhen, China).

2.5.3 | Homocysteine detection

The enzymatic cycling assay was used to determine the level of homocysteine in the patient (Roberts & Roberts, 2004). The patient serum was obtained as described in 2.5.2 and detected by an Olympus AU640 automatic biochemical analyzer. The homocysteine detection kit was purchased from Medicalsystem (Zhejiang, China).

2.6 | Bioinformatics analysis

To identify the pathogenicity of the novel *SUOX* variants based on the ACMG/AMP Standards and Guidelines, we first queried the single nucleotide sequence polymorphism (dbSNP) database (http://www.ncbi.nlm.nih.gov/snp) and the Human Genome Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk), and reviewed all the previously published articles about *SUOX* gene. We then used Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) to estimate the degree of evolutionary conservation of the mutated domains (Fang et al., 2016). Finally, we used SWISS-MODEL (https://swissmodel.expasy.org/) to predict and compare the tertiary structure of wild-type and mutant SUOX proteins (Waterhouse et al., 2018).

TABLE 1 *SUOX* gene primers used for Sanger sequencing

Primer	Sequence	Tm (°C)	Product length (bp)
E1-F	5′-TCTTCTCCCAGGCTTCTTAG-3′	59	324
E1-R	5′-TGTTGCCCTTGAGGTTCG-3′		
E2-F	5′-GCCTCCACTCCTTCTGTC-3′	59	490
E2-R	5′-CTGCCCTGCAAGTTTCTT-3′		
E3-F	5′-TGATCCTCCTTCCTTGGC-3′	55	592
E3-R	5′-GCTGCTGCTCACTCTTCG-3′		
E4-F	5′-GAATGGAAGATGGGAGAA-3′	56	494
E4-R	5′-CAGGGATAGGTGGGAAA-3′		
E5-F	5′-CAGGGAGAAAGAAGTAGACC-3′	57	633
E5-R	5′-GGTTGCCTGTGGAGTTTA-3′		
E6-1F	5′-GTCTTGAACTCCCGACCTT-3′	58	327
E6-1R	5′-GAGCCAGTAACTCACGCACAT-3′		
E6-2F	5′-GGCCTTCAAAGCTGATG-3′	50	360
E6-2R	5′-AGACAGTGACTGACCC-3′		
E6-3F	5′-TGGATCCAGACACCTAT-3′	58	383
E6-3R	5′-CATCTCATATGCCAGC-3′		
E6-4F	5′-ACTGGACTCAGACCCTAC-3′	58	373
E6-4R	5′-GGTCACCTCCCCTGAT-3′		
E6-5F	5′-CCAGAGGAAAGTTACAGC-3′	58	359
E6-5R	5′-ACAATGTTCAGTTCCTTT-3′		
E6-6F	5′-CTCTGGATGGGGGCCTAA-3′	59	370
E6-6R	5′-TTGGGTATGGCTTAGGAAGG-3′		
E6-7F	5′-CAGTACACGGTGCATCCATT-3′	61	443
E6-7R	5′-ACGGGCATTAACACCTAGCA-3′		

3 | RESULTS

3.1 | Clinical features and phenotype-genotype correlation

On the first day after the proband's birth, there was no obvious inducement to cry, limb jitter, and no binocular gazing. On the second day after birth, the head computed tomography (CT) and magnetic resonance imaging (MRI) scans showed low white matter density, unclear basal ganglia structure, and the narrowed bilateral lateral ventricles. The signals of bilateral lenticular nucleus and caudate nucleus were abnormal: slightly higher T2 weighted imaging (T2WI) signal, slightly higher fluid attenuated inversion recovery (FLARI) signal, significantly higher diffusion weighted imaging (DWI) signal, and significantly lower apparent diffusion coefficient (ADC) signal. In addition, the bilateral frontal white matter had slightly lower T2 dark fluid signal. After repeated use of sedatives (Phenobarbital), the limbs are still dancing, rowing and cycling-like mechanical stereotypes. On the 4th and 14th days after birth, dynamic electroencephalogram (EEG) showed a mild abnormal neonatal EEG and prolonged interburst interval duration (IBI). Four months after birth, patient's brain MRI revealed decrease in brain white matter, dysplasia of corpus callosum, slightly widened bilateral ventricles and widening of the subarachnoid space at the bilateral frontal top (Figure S1). At the time of writing, the child is 1 year and 6 months old and bedbound, with progressive encephalopathy, abnormal muscle tone, and intractable seizures (Figure 2, Video S1). During follow-up examination, the proband was also found to have history of recurrent pneumonia, umbilical hernia, and inguinal oblique femoral hernia symptoms. It is the supporting evidence PP4 that the patient's phenotype is highly specific for ISOD with a single genetic etiology (Richards et al., 2015).

3.2 | Mutation detection results

As described in the above method (2.3), the heterozygous *FIG4*, *RYR1* and the compound heterozygous *SUOX* are variations related to the patient's symptoms by screening the NGS sequencing results. The heterozygous *FIG4* was excluded because its pathogenesis of the autosomal recessive hereditary diseases had not been confirmed, so was the heterozygous *RYR1*. The results of Sanger sequencing of *SUOX* gene are shown in the following figure (Figure 3). The proband was identified as a

compound heterozygote, who not only inherited a known pathogenic variant *SUOX* c.1200C > G (p.Y400*), which was previously reported in ISOD case (HGMD ID CM021349)

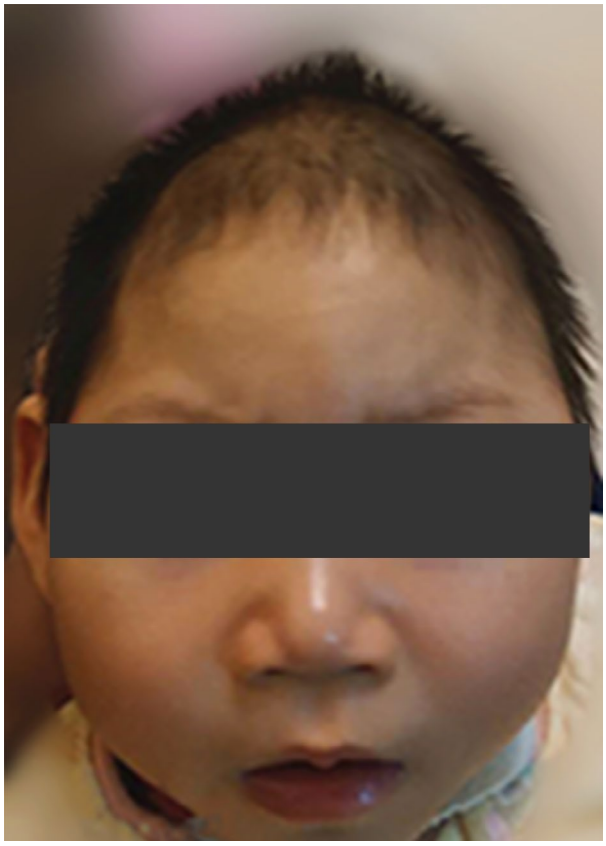

FIGURE 2 Progressive microcephaly. The head circumference is 38 cm (compared to age-matched population average of 46.4 cm) at 1 year and 6 months old

(Johnson et al., 2002), from his mother, but also inherited the novel *SUOX* duplication (c.1549_1574dup (p.I525 Mfs*102) from his father. The novel *SUOX* variant is a frameshift mutation in the causal gene where loss of function is a known mechanism of isolated sulfite oxidase deficiency. It is a very strong evidence of pathogenicity (PVS1) (Richards et al., 2015). For recessive disorders, detected in trans with a pathogenic variant can be considered as moderate evidence of pathogenicity (PM3) (Richards et al., 2015).

3.3 | Quantitative results of urine and plasma metabolites

The patient's urine sulfite experiment was positive and homocysteine level was extremely low at 1.1 μmol/L, compared to expected 5-15 μmol/L in healthy subjects. At the same time, uric acid levels were normal at 196 μmol/L, compared to the healthy range of 155-375 μmol/L. The increase in creatine kinase (500, 41-186 U/L), creatine kinase isoenzyme (58, 0–24 U/L) and lactate dehydrogenase (392,109-245 U/L) was found in the 4th and 18th months of the patient's examination.

3.4 | Bioinformatical analysis of identified *SUOX* pathogenic variants

3.4.1 | The frequency of the novel variant in control populations

The novel *SUOX* duplication (c.1549_1574dup (p.I525 Mfs*102) was not found in the controls from 1000 Genomes Project (http://browser.1000genomes.org), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and gnomAD (https://gnomad.broadinstitute.org) (Until July 1, 2020). Because the novel *SUOX* duplication (c.1549_1574dup (p.I525 Mfs*102) absents from the controls in 1000 Genomes, gnomAD and ClinVar, it is considered as moderate evidence of pathogenicity (PM2) (Richards et al., 2015).

3.4.2 | Conservation analysis and protein three-dimensional structure prediction

We analyzed amino acid sequence conservation of the location where the novel *SUOX* duplication mutation (c.1549_1574dup (p.I525 Mfs*102)) was found in the proband. We performed multiple alignment of amino acid sequences at this locus from the following species: *Danio rerio* (Zebrafish), *Xenopus tropicalis* (Western clawed frog), *Equus caballus* (Horse), *Bos taurus* (Bovine), *Mus musculus* (Mouse), *Oryctolagus cuniculus* (Rabbit), *Canis lupus familiaris* (Dog), *Macaca fascicularis* (Cynomolgus monkey), *Homo sapiens* (Human), and *Pan troglodytes* (Chimpanzee) (Figure 4). From the figure we can see that p. I525 and subsequent amino acids are highly conserved in vertebrates. In addition, predicted the amino acid sequence of the c.1549_1574dup (p.I525 Mfs*102) mutant protein and constructed its putative tertiary structure using SWISS-MODEL (Figure 5B). Comparison of the predicted tertiary structure in the wild type (Figure 5A) and mutant (Figure 5B) of SUOX proteins revealed significant changes in protein structure (Figure 5C,D). Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary etc.), providing the supporting evidence PP3 (Richards et al., 2015).

Our results in the study indicated that the novel *SUOX* duplication (c.1549_1574dup (p.I525 Mfs*102) should be classified as a pathogenic variant causing isolated sulfite oxidase deficiency with the evidences (PVS1, PM2, PM3,

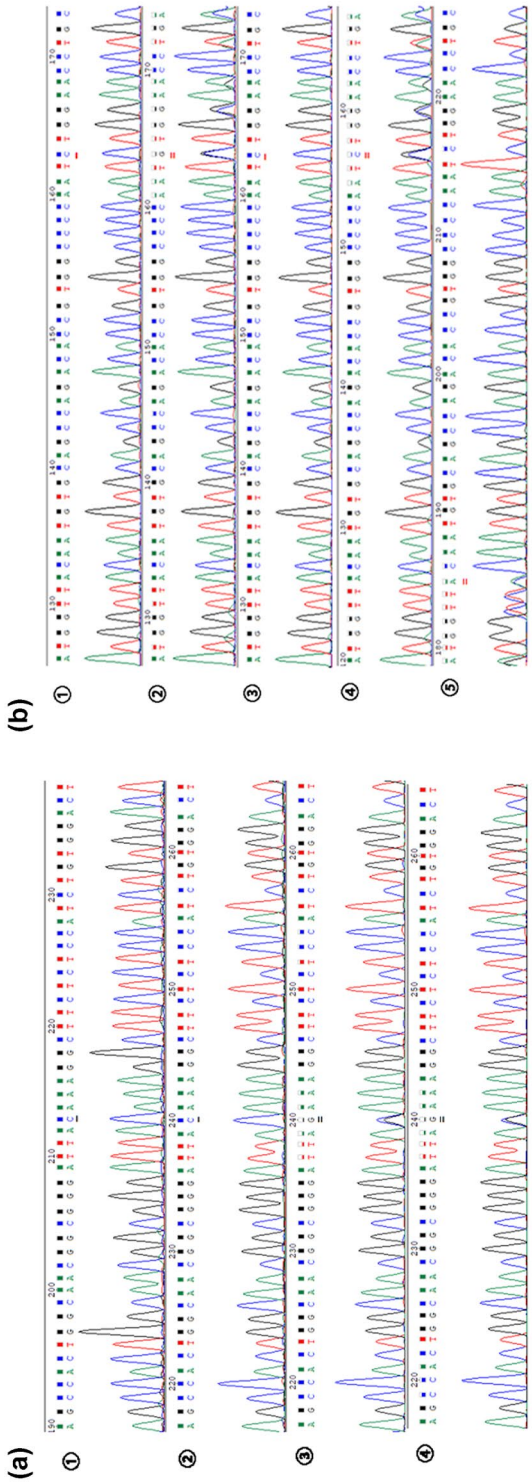

FIGURE 3 (A) Sequencing of the c.1200 area of SUOX gene.① Healthy unrelated control, C/C at c.1200; ② Father, C/C at c.1200; ③ Mother, C/G at c.1200; ④ Proband C/G at c.1200. (B) Sequencing of the c.1549_1574 area of SUOX gene. ① Unrelated healthy control, single line shows reference sequence; ② Father, carrier of c.1549_1574dup (p.I525 Mfs*102); ③ Mother, comparable to reference sequence; ④ The proband's forward sequencing and ⑤ The proband's reverse sequencings, carrier of c.1549_1574dup (p.I525 Mfs*102)

PP3, and PP4) based on the ACMG/AMP Standards and Guidelines.

4 | DISCUSSION

In human body, sulfite oxidase oxidizes sulfite to sulfate (Rocha et al., 2014). The molybdenum cofactor is required for the biological function of sulfite oxidase (Brumaru et al., 2017). Sulfite oxidase is encoded by the *SUOX* gene. The normal function of the *MOCS1*, *MOCS2*, *MOCS3*, and *GEPH* genes was required for the synthesis of molybdenum cofactors (Bayram et al., 2013). Defects in the synthesis of sulfite oxidase or in the synthesis of molybdenum cofactors lead to the loss of sulfite oxidase activity. In order to distinguish sulfite oxidase deficiency caused by different causes, the disease caused by *SUOX* gene mutation is called isolated sulfite oxidase deficiency, and the disease caused by defects in molybdenum cofactor synthesis is called molybdenum cofactor deficiency (MoCD). They are all autosomal recessive genetic diseases with similar neuroimaging findings resembling hypoxic-ischemic injury (Hobson et al., 2005; Hoffmann et al., 2007). Defects in the synthesis of molybdenum cofactors can also lead to the defects in xanthine dehydrogenase and aldehyde oxidase, which leads to reduced levels of uric acid in plasma, and elevated levels of xanthine and hypoxanthine in urine (Armstrong & Que, 2012). Therefore, in order to exclude MoCD, patients with suspected ISOD need to check the level of uric acid and (or) xanthine and (or) hypoxanthine. Due to the rarity of ISOD, most of its biochemical indicators cannot be detected in hospitals. However, homocysteine is a common indicator of disease diagnosis, so most hospitals have carried out. In the past, people often pay attention to the significance of its higher than normal level, and often ignore the lower than normal level of homocysteine, which may indicate the occurrence of ISOD. For example, among the reported cases, there were eight examined patients with obviously lower homocysteine level than normal controls with (Claerhout et al., 2018). According to the results of 3.3, we confirmed that the patient has ISOD disease.

In order to fully understand the patient's genetic information, we used NGS to obtain the full exome information of the patient's family. NGS technology can provide a large amount of data quickly, but these data have a relatively high error rate (0.1%-15%), and the read length of the NGS platform is much shorter than the traditional Sanger sequencing platform (Hu et al., 2008; Xu et al., 2016). Therefore, the variation obtained from NGS needs to be verified by Sanger sequencing, especially in clinical applications (Rohrbach & Giunta, 2012). We found a heterozygous mutation in the *SUOX* gene that matches the patient's condition, including a known pathogenic nonsense mutation (c.1200C > G (p.Y400)) and a novel duplications mutation c.1549_1574dup (p.I525 Mfs*102) In addition

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c.1549_1574dup,    LWQLKAPVPAGQKELNIVCKAVDDGYNVQPDTVAPMCSQTPWPQSGTCE
Danio              LWEIDVPLPDGAQELEIVCKAVDNSYNTQPDTVGPIWNLRGVLSNAWHR
Xenopus            LWQLNVPFPSNISDFTIICKAVDSSYNVQPDTVAPIWNLRGVLNNAWHR
Equus              LWELQAPVPAGQKELNIVCKAIDDSYNVQPDTMAPIWNLRGVLSNAWHR
Bos                LWHLQAPLPAGIKELNIVCKAVDESYNVQPDTVAPIWNLRGVLNNAWHR
Mus                IWQLKAQVPAEQKELNIICKAVDDSYNVQPDTVAPIWNLRGVLSNAWHR
Oryctolagus        LWQLQAPVPAGQKELHIVCKAVDESYNVQPDTVAPIWNLRGVLSNAWHR
Canis              LWQLQASVPAGKKELNIVCKAVDDSYNVQPDTVAPIWNLRGVLSNAWHR
Macaca             LWQLQAPVPAGQKELNIVCKAVDDGYNVQPDTVAPIWNLRGVLSNAWHR
Homo               LWQLKAPVPAGQKELNIVCKAVDDGYNVQPDTVAPIWNLRGVLSNAWHR
Pan                LWQLKAPVPPGQKELNIVCKAVDDGYNVQPDTVAPIWNLRGVLSNAWHR
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FIGURE 4 Species conservation analysis of c.1549_1574dup (p.I525 Mfs*102). p. I525 and subsequent amino acids are highly conserved among the 10 species investigated

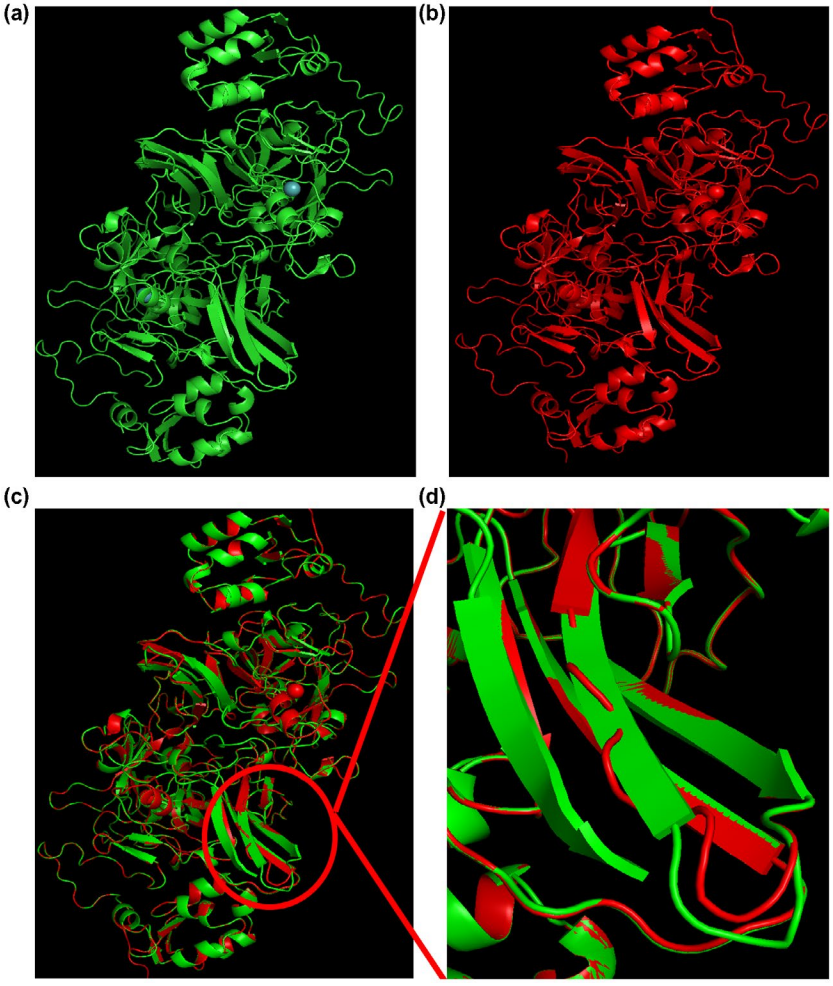

FIGURE 5 Predicted tertiary structures of wild-type (A) and p.I525 Mfs*102 mutant (B) *SUOX* protein. (C) Wild-type and mutant overlay. (D) Fine structure of variant sites. Comparing the tertiary structure of wild-type and novel mutant type proteins, it can be seen that the variant leads to significant changes in protein structure, including at least one shortening of the β sheet and the disappearance of a β sheet

to obtaining credible gene information, identification of the pathogenicity of suspicious genes is critical, especially novel variants. When a novel mutation occurs, multiple methods are needed to assess the degree of pathogenicity of the mutation. These include, but not limited to, co-segregation analysis of genes and diseases, species conservation analysis, pathogenicity assessment based on different biological algorithms (SIFT (Kumar et al., 2009), PloyPhen-2 (Adzhubei et al., 2010), Mutation Taster (Schwarz et al., 2010), PROVEAN (Choi & Chan, 2015), etc.) and prediction of protein structure (Ye et al., 2013). These methods can provide us with evidences to determine the pathogenicity of novel mutation.

By simultaneously analyzing the parental genes, the patient inherited two pathogenic variants in *SUOX* gene from the father and mother, respectively. Additionally, the novel mutation was not found in the 1000 Genomes Project

(http://browser.1000genomes.org), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and gnomAD (https://gnomad.broadinstitute.org). Since the novel mutation occurred at the c-terminal of sulfite oxidase, we performed a conservative analysis including 10 species, and the results showed that the amino acids affected by the novel mutation were highly conserved among the 10 species. The degree of evolutionary conservation reflects whether the given amino acid sequence is important for protein function. If the amino acid is highly conserved, any changes will likely cause the loss of protein function. Therefore, it can be speculated that the amino acids affected by the novel mutation play an important role in loss of function of sulfite oxidase to cause ISOD. Furthermore, prediction of the tertiary structure of the protein revealed significant changes in the tertiary structure after the mutation, including at least one shortening of the β sheet and the disappearance of a β sheet. At the same time, we reviewed the *SUOX* gene-related articles and then found that there were two reported pathogenic variants in the affected amino acid sequence (c.1589C > T, p.R529* (Hoffmann et al., 2007) and c.1589G > A, p.G530D (Kisker et al., 1997)). Gel Filtration and Analysis Settling Equilibrium Ultracentrifugation studies indicated that recombinant human p.G530D mutant was present as a monomer in solution (Kisker et al., 1997). Thus, amino acids affected by the novel mutation may affect the formation of sulfite oxidase dimers, resulting in loss of function.

In 2015, the American College of Medical Genetics (ACMG), the Association of Molecular Pathologists (AMP) and the American Society of Pathologists (CAP) jointly proposed guidelines for interpreting germline variation. Standards and Guidelines for the Interpretation of Sequence Variants assigns the strength of various pathogenic evidence and classifies the variation into five categories based on intensity: pathogenic, likely pathogenic, uncertain significance, likely benign, or benign (Richards et al., 2015). In the present study, the novel variant (c.1549_1574dup (p.I525 Mfs*102) should be classified as pathogenic variant causing isolated sulfite oxidase deficiency, a autosomal recessive disease with 1 very strong pathogenic evidence (PVS1), 2 moderate ones (PM2 and PM3) and 2 supporting ones (PP3 and PP4) according to the ACMG/AMP Standards and Guidelines.

In summary, we consider that the diagnostic strategy of ISOD is as the follows: (1) Collecting clinical symptoms and signs (including axial hypotonia/peripheral hypertonia, (pharmacoresistant) seizures, abnormal movement, feeding difficulties, psychomotor retardation, progressive microcephaly and ectopia lentis and neuroimaging features (including ventriculomegaly, thinning of the corpus callosum, cystic white matter changes, cerebral, and cerebellar atrophy); (2) If symptoms are consistent, plasma homocysteine (widely available in hospitals), and urine sulfite levels (simple method, easy materials) should be tested; (3) If low homocysteine and positive sulfite are detected, plasma uric acid and levels of xanthine and hypoxanthine in urine should be detected; (4) If normal uric acid levels in plasma and normal xanthine and hypoxanthine in urine are detected, the patient can be diagnosed as ISOD; (5) When the patient is diagnosed as ISOD clinically, the *SUOX* gene should be detected for pathogenic variants. The classification of the pathogenic variants based on the ACMG/AMP Standards and Guidelines is described in the method section of the article.

5 | CONCLUSION

We discussed the general strategy for ISOD diagnosis and established a procedure for pathogenicity analysis according to the ACMG/AMP Standards and Guidelines. We first reported ISOD patients in mainland China and found a novel mutation. Our results enrich the *SUOX* gene mutation spectrum, which provides a theoretical basis for future genetic counseling, prenatal diagnosis, and preimplantation genetic diagnosis. Patient's plasma analysis revealed an increase in creatine kinase, creatine kinase isoenzyme, and lactate dehydrogenase. This is the first report of these symptoms in ISOD patients, possibly enriching the clinical features of ISOD and thus providing a possible reference for future clinical ISOD diagnosis. The proband also had recurrent episodes of pneumonia as well as umbilical hernia and inguinal oblique femoral hernia in follow-up. This gives us new insight into ISOD patient care.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

AUTHOR CONTRIBUTIONS

Peng Du conceived and designed the experiments, performed the experiments, performed the data analyses and wrote the manuscript; Reem N. Hassan performed the data analyses and revised the manuscript; Hualei Luo, Jie Xie, Yue Zhu Hu, Qiuyue and Jin Yan performed the experiments and analyzed the results; Weiying Jiang conceived and designed the experiments, and contributed reagents and materials, helped to perform the data analysis and revision of the manuscript. Weiying Jiang approved and made the decision to publish the paper.

DATA AVAILABILITY STATEMENT

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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