CASE REPORT

Genetic analysis of a novel SUMF1 variation associated with a late infantile form of multiple sulfatase deficiency

Jingjing Zhang | Dingyuan Ma | Gang Liu | Huasha Zeng | Yuguo Wang | Chunyu Luo | Ping Hu | Zhengfeng Xu

Department of Prenatal Diagnosis, Nanjing Maternity and Child Health Care Hospital, Women’s Hospital of Nanjing Medical University, Nanjing, China

Correspondence
Zhengfeng Xu, 123 Tianfei Street, Qinhua District, Nanjing 210004, China. Email: zhengfeng_xu_nj@163.com

Abstract
Background: Multiple sulfatase deficiency (MSD) (MIM#272200) is an ultra-rare autosomal recessive lysosomal storage disorder caused by mutation of the Sulfatase Modifying Factor 1 (SUMF1) gene.
Methods: Herein, we report an eight-year-old boy with a late infantile form of multiple sulfatase deficiency. A combination of copy-number variation sequencing (CNV-seq) and whole-exome sequencing (WES) were used to analyze the genetic cause for the MSD patient.
Results: Our results, previously not seen in China, show a novel compound heterozygous mutation with one allele containing a 240.55 kb microdeletion on 3p26.1 encompassing the SETMAR gene and exons 4–9 of the SUMF1 gene, and the other allele containing a novel missense mutation of c.671G>A (p.Arg224Gln) in the SUMF1 gene. Both were inherited from the proband’s unaffected parents, one from each. Bioinformatics analyses show the novel variation to be “likely pathogenic.” SWISS-MODEL analysis shows that the missense mutation may alter the three-dimensional (3D) structure.
Conclusions: In summary, this study reported a novel compound heterozygous with microdeletion in SUMF1 gene, which has not been reported in China. The complex clinical manifestations of MSD may delay diagnosis; however, molecular genetic analysis of the SUMF1 gene can be performed to help obtain an early diagnosis.

KEYWORDS
multiple sulfatase deficiency, mutation, SUMF1 gene

1 | INTRODUCTION

Multiple sulfatase deficiency (MSD) (MIM#272200) is a rare lysosomal storage disease, inherited in an autosomal recessive pattern. Since it was first described by Austin in 1964, <100 patients with the disease have been reported globally. The Sulfatase Modifying Factor 1 (SUMF1) gene (NM_182760.3) was identified as responsible for MSD. This gene encodes a formylglycine-generating enzyme (FGE) that is responsible for the post-translational modification of cysteine necessary the formation of formylglycine. This step is

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critical for the promotion of enzymatic function in all sulfatases. The clinical findings of MSD form a wild spectrum and combine symptoms due to each sulfatase deficiency. The common presentations of MSD are neurological deterioration, skeletal abnormalities, intellectual disability, ichthyosis, hepatosplenomegaly, and hearing loss. MSD can be classified into three types based on the age of onset, namely neonatal, late infantile, and juvenile. The neonatal type is the most severe and onset of multiple signs at birth. The late infantile is the most common type and subdivided into severe and mild forms based on the onset before or after 2 years old. The juvenile is very rare with late onset and slow progression.

Herein, we discuss an eight-year-old male patient affected by MSD, who presented atypical symptoms of a late infantile type of the disease caused by novel variations in SUMF1 gene.

2 | MATERIALS AND METHODS

2.1 | Patient description

The proband was the first child of healthy non-consanguineous Chinese parents. He was born at 39 weeks' gestation via normal spontaneous vaginal delivery. His development was normal until 13 months. He was hospitalized because of nystagmus, macular degeneration, corneal clouding, and myopia (Figure 1). A Brain MRI at the age of two confirmed bilateral symmetrical leukodystrophy. Additional symptoms were observed at the age of four, including psychomotor delay, lack of speech development, impaired vision, and macular degeneration. Hepatosplenomegaly was revealed by an ultrasound scan of the abdomen. The patient was 8 years old at the time of our study. Currently, he mainly suffers from impaired vision, developmental delay, and dry, thickened scaly skin. Written informed consent was obtained from the patient’s guardians.

2.2 | Biochemical analysis

Peripheral blood samples were collected from the patient. Sulfatase activities were measured in leukocytes and plasma following standard protocols.

2.3 | CNV-seq

Copy-number variation sequencing (CNV-seq) was performed as reported previously. Briefly, 50–100 ng genomic DNA was extracted from the blood and fragmented to construct DNA libraries by PCR amplification. The DNA libraries were sequenced using an Ion Proton Sequencer (Thermo Fisher Scientific, MA, USA), and approximately 4–5 million 200-bp raw reads were generated. Then, the processed sequences were aligned to the University of California Santa Cruz (UCSC) Human Genome Build 19 (Hg19/GRCh37) using the Burrows–Wheeler algorithm to obtain the copy-number value of each chromosome. The tested data were analyzed by the latest ClinVar, ClinGen, DECIPHER, DGV, HGMD, and OMIM databases.

2.4 | Whole-exome sequencing

To obtain genomic DNA, 2 ml peripheral blood from the patient and the parents was collected. Genomic DNA was extracted in accordance with the protocol of the QIAamp DNA Mini Kit (QIAGEN). The qualifying genomic DNA was randomly broken into fragments and an Agilent SureSelect Human ALL ExonV6 kit (Agilent Technologies) was used to enrich, hybridize, and capture the obtained fragments following the manufacturer's protocol. Sequencing was performed by Illumina HiSeq 4000 PE150. The average depth and 20x coverage were approximately 100x and 99.8%, respectively. SpeedSeq was used to perform sequence alignment against the reference human genome (Hg19/GRCh37). Data filtering was performed based on the strategies of a previous study. Finally, the pathogenicity of the potential mutation was evaluated in accordance with the 2015 American College of Medical Genetics (ACMG) guidelines. Sanger sequencing was performed to confirm the candidate disease-causing mutations.

2.5 | Bioinformatics analysis

Software programs from the 1000 genomes, ExAC, GnomAD, Polyphen-2, SIFT, and MutationTaster were used to predict the potential pathogenicity of the mutation. Vertebrate Multiz

FIGURE 1 Fundus photographs of the eyes (A), left eye; (B), right eye) showing macular degeneration (black arrows).
Alignment and Conservation and phyloP in UCSC were used to evaluate the amino acid conservation.\textsuperscript{15}

Three-dimensional structural models of SUMF1 were predicted using a known template (RCSB PDB:1y1h.1.A) on Swiss-model web (https://swissmodel.expasy.org/interactive). Protein structure images were generated using the PDB file and PyMOL. PyMOL (http://pymol.org/) was also used to predict hydrogen bond changes in mutant protein to assess the stability.\textsuperscript{16,17}

3 RESULTS

The activities of five different lysosomal sulfatases were absent or significantly reduced in the patient’s blood, namely arylsulfatase A, arylsulfatase B, iduronate-2-sulfatase, heparan-N-sulfatase, and galactosamine 6-sulfatase (Table 1).

\textbf{TABLE 1} Biochemical features of the patient

| Parameter               | Result | Reference range | Comment          |
|-------------------------|--------|-----------------|------------------|
| Arylsulfatase A         | 2.2\textsuperscript{a} | 3.7–28.2\textsuperscript{a} | Reduced activity |
| Arylsulfatase B         | 2.5\textsuperscript{b} | 8.2–41.2\textsuperscript{b} | Deficient activity |
| Iduronate-2-sulfatase   | 4.0\textsuperscript{c} | 42.8–405.8\textsuperscript{c} | Deficient activity |
| Heparan-N-sulfatase     | 27.4\textsuperscript{d} | 55.4–246.4\textsuperscript{d} | Reduced activity |

\textsuperscript{a}nmol/mg/h.
\textsuperscript{b}nmol/g/min.
\textsuperscript{c}nmol/g/h.

CNV-seq revealed a 240.55 kb deletion on 3p26.1 (chr3:4229096-4469643). The deletion encompasses two genes including SETMAR gene and the exons 4–9 of SUMF1 gene (Figure 2). According to the 2015 ACMG standards and guidelines for the interpretation of sequence variants,\textsuperscript{12} this deletion is predicted as “pathogenic”(PVS1+PM2+PP4). Our whole-exome sequencing (WES) results show a missense mutation, c.671G>A (p.Arg224Gln) in exon 5 of SUMF1 gene (Figure 3). This c.671G>A (p.Arg224Gln) mutation’s population frequency is 0 in the 1000 Genomes Project and ExAC databases, and 0.00001193 in GnomAD. Polyphen-2, SIFT, and MutationTaster predicted this variant to be likely damaging. An adjacent, previously reported mutation, c.670C>T (p.Arg224Trp), can also cause MSD.\textsuperscript{18} According to the 2015 ACMG guidelines, the novel mutation c.671G>A (p.Arg224Gln) can be classified as “likely pathogenic”(PM2+PM5+PP3+PP4).

Arg224 is extremely conserved in the homologous proteins of different species (Figure 4A). We used SWISS-MODEL to predict changes to the SUMF1 3D structure caused by this mutation. In the wild-type model, there is one hydrogen bond between Arg224 and Leu225, three hydrogen bonds between Arg224 and Glu230, two hydrogen bonds between Arg224 and Ala 366, and two hydrogen bonds between Arg224 and Asp368 (Figure 4B). When the Arg at position 224 is mutated to Gln, the alkaline amino acid changes to a neutral amino acid, and the six hydrogen bonds disappear, altering the 3D structure, and possibly affecting protein stability (Figure 4C).

The proband’s healthy parents were also analyzed by CNV-seq and Sanger sequencing. The results reveal that the father has...
the c.671G>A heterozygous mutation while the mother has the same heterozygous deletion as the proband on 3p26.1 (Figures 2B and 3B).

**4 | DISCUSSION**

We identify and report a case of MSD resulting from novel compound heterozygous mutations in SUMF1 not previously seen in any Chinese population. The presence of nystagmus was the focus of initial investigative work through an ophthalmology clinic for our patient. Examination of the patient’s eyes revealed nystagmus, macular degeneration, corneal clouding, and myopia, which have also presented in previous MSD cases. Developmental delay is the first clinical manifestation in many MSD cases; however, our patient differed from most cases because he showed developmental delay after visual regression. MSD phenotype is the combination of the multiple phenotypes, and many individuals experience a delay in diagnosis. Furthermore, sulfatase activities in the blood do not correlate with disease severity. However, blood biomarkers can be used to track MSD progression.

So far, about 80 mutations have been reported in all the MSD patients (Figure 5). In the study, a novel SUMF1 variation was identified. One allele presents a 240.55 kb deletion on 3p26.1 including SETMAR and exons 4–9 of the SUMF1 gene, and the other allele has a missense mutation in exon 5 of the SUMF1 gene. SETMAR is involved in essential networks regulating replication, transcription and translation. It also contributed to brain development during embryogenesis. In the study, the proband showed the heterozygote deletion of SETMAR and no point mutation was detected by whole-exome sequencing. Moreover, the Database of Genomic Variants displayed many individuals in the general population has heterozygous deletions in the part of their genome and implied that heterozygous deletions of SETMAR were not deleterious. Therefore, we concluded that the deletion of SETMAR has no impact to MSD.

The copy-number variation of the SUMF1 gene is rare in MSD families. One report discusses an MSD patient carrying a 28 kb deletion on 3p26.1 that includes exons 8–9 of the SUMF1 gene. Deletions such as these could easily lead to SUMF1 protein loss of function. The missense mutation we observed on the other allele is predicted to alter the SUMF1 protein 3D structure and may influence FGE function. To corroborate the pathogenic potential of this mutation, we performed a computational comparative analysis of the sequences through PolyPhen (Polymorphism Phenotyping, http://genetics.bwh.harvard.edu/pph). The result shows that the missense mutation, c.671G>A (p.Arg224Gln), is probably harmful. The MutationTaster (https://www.mutationtaster.org) analysis predicts it to be a disease-causing variant. In a previous study described a missense mutation at an adjacent position in the protein (c.670C>T, p.Arg224Trp) in an MSD patient with a moderate phenotype. The patient was 13 years old at the time of the research and had mild visceromegaly, dysostosis, and intellectual disability, but he could walk, speak, and write. This clinical manifestation is different from that of our patient. Based on the combination of the largely deleted allele and the missense variation on the other allele, the patient in our study may be expected to have a more severe phenotype.

Because MSD is a rare autosomal recessive inborn disorder and more common in families, genetic diagnosis is particularly important for MSD families. The patient’s parents have no clinical

![Figure 3](image_url)
manifestation and the SUMF1 genes were sequenced to find the heterozygous mutations. The paternal result shows the prospective missense mutation, while the maternal result shows the SUMF1 partial gene deletion. Our MSD family investigation reinforces the importance of not only point mutations but also copy-number variation in SUMF1 genetic studies. Because clinical and biochemical manifestations may not provide definitive results in early onset MSD, mutational analysis can help in early diagnosis and enable the possibility of prenatal diagnosis and preimplantation diagnosis of causal mutations.

In summary, we report novel SUMF1 variations in a Chinese patient, and provide genetic diagnosis for the family. Because MSD is a complicated and untreatable disease, earlier gene sequencing can lead to a less delayed MSD diagnosis. As genotype-phenotype
correlations accumulate for this disease, adding the novel variations to the responsible gene mutation spectrum will become ever more helpful for appropriate genetic counseling for at risk families and patients.26

AUTHOR CONTRIBUTIONS
Jingjing Zhang and Dingyuan Ma analyzed the data and wrote the manuscript. Gang Liu and Huasha Zeng performed the ES sequencing. Yuguo Wang and Chunyu Luo collected the clinical information. Ping Hu and Zhengfeng Xu designed the study and reviewed the manuscript. All the authors read and approved the final manuscript.

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CONFLICT OF INTEREST
The authors have no conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author on reasonable request.

ORCID
Zhengfeng Xu https://orcid.org/0000-0002-7824-7578

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