Isolated sulfite oxidase deficiency: a founder mutation

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Abstract Isolated sulfite oxidase deficiency is a rare autosomal recessive inborn error of sulfur metabolism. Clinical features generally include devastating neurologic dysfunction, ectopia lentis, and increased urinary excretion of sulfite, thiosulfate, and S-sulfocysteine. Missed diagnosis is not unusual because of variability in the sensitivity of the urinary sulfite and thiosulfate screening test. We present clinical, biochemical, and molecular data on two unrelated patients with isolated sulfite oxidase deficiency. The two patients belong to an Indigenous genetic isolate in Manitoba, Canada. Both patients (one male and one female, both now deceased) developed neonatal seizures and demonstrated progressive neurodevelopmental delay. Based on increased urinary excretion of sulfite, thiosulfate, and S-sulfocysteine and normal serum uric acid levels, sulfite oxidase deficiency was suspected. Both patients have a homozygous 4-bp deletion, 1347–1350delTTGT in the sulfite oxidase gene (*SUOX*), predicting a premature termination of the sulfite oxidase protein leading to absence of the carboxy-terminal third portion of the protein. This domain contains most of the contact sites essential for enzyme dimerization. This deletion mutation resulted in sulfite oxidase deficiency with early-onset severe clinical phenotype.

CASE PRESENTATION

The clinical and biochemical data on the two patients are presented in Tables 1 and 2, respectively.

TECHNICAL ANALYSIS

Genomic DNA was isolated from cultured fibroblasts and/or blood samples, and the gene encoding *SUOX* was amplified by polymerase chain reaction (PCR) using standard conditions. PCR primers spanning the entire coding region (CCTCAAGGATCTGCATTCAGGCC and AAGGGGTGGAGGTGGCTCCTTTCC) were included at a concentration of 1 μ molar, and the reaction was cycled for 30 sec at 55°, for 5 min at 68°, and for 30 sec at 95° for a total of 35 cycles. The entire reaction mixture was run on an agarose gel and the band at ~2.4 kb, corresponding to the sulfite oxidase gene with its single intron, was extracted from the gel and sequenced in both the forward and reverse directions. Automated DNA sequencing was performed at the Duke University Comprehensive Cancer Center facility using a PerkinElmer/ABI 377 DNA Sequencer and Big Dye sequencing chemistry. Numbering of nucleotides is based on the cDNA sequence, with the A of the ATG initiator methionine codon

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Ontology terms: increased urinary sulfite; increased urinary thiosulfate

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Table 1. Clinical findings

Patient	Age at presentation	Sex	Symptoms	Lens dislocation	Family history	Ethnic background	Current status
1	First week	F	Intractable seizures, severe developmental delay, spastic quadriplegia	Not assessed	Negative	Indigenous	Deceased at 9 yr
2	5 d	M	Lethargy, hypotonia, severe developmental delay, intractable seizures	Not assessed	Possible affected older sibling based on history	Indigenous	Deceased at 15 mo

denoted nucleotide +1. Numbering of the amino acids is also from the initiator methionine, which is the first amino acid in the 22-residue leader sequence of sulfite oxidase.

VARIANT INTERPRETATION

Both patients have a homozygous 4-bp deletion, 1347–1350delTTGT in the sulfite oxidase gene (*SUOX*), predicting a premature termination of the sulfite oxidase protein leading to absence of the carboxy-terminal third portion of the protein. This domain contains most of the contact sites essential for enzyme dimerization. The parents are heterozygous for this variant. This novel variant in *SUOX* likely is a founder mutation, given its presence in two unrelated Indigenous children from the same genetic isolate (Table 3).

Isolated sulfite oxidase deficiency (MIM 272300) is an autosomal recessive disorder caused by mutations in the sulfite oxidase gene (*SUOX*), GenBank accession number AY056018. The gene is located on Chromosome 12, in the region of 12q13.2, and the coding sequence contains a single intron. The gene product, sulfite oxidase, is a molybdohemoprotein comprised of 466 amino acids. It is synthesized with a 22-residue leader sequence that directs it to the mitochondrial intermembrane space. The mature protein lacks this presequence. The native enzyme is a dimer of identical subunits, each of which contains three domains—the amino-terminal heme domain, the central molybdenum domain, and a carboxy-terminal domain with key residues at the dimer interface (Johnson and Rajagopalan 1976; Kisker et al. 1997). The enzyme catalyzes the oxidation of sulfite to sulfate, the terminal reaction in the degradation of sulfur-containing amino acids, and transfers the electrons derived from substrate oxidation to cytochrome c on the inner mitochondrial membrane.

Sulfite oxidase deficiency is classically characterized by severe neurological symptoms including seizures, often refractory to anticonvulsant medications, and rapidly progressing

Table 2. Biochemical findings

Analyte (normal range)	Urine S-sulfo-cysteine (<25 $\mu\text{mol}/\text{mmolCr}$)	Urine sulfite (not detected)	Plasma cystine (23–49 $\mu\text{mol}/\text{L}$)	Serum uric acid (130–330 $\mu\text{mol}/\text{L}$)	Xanthine hypoxanthine	Cranial MRI	Enzyme activity (liver) (7–28 μg)	Molecular diagnosis
Patient 1	Small peak	80–400 mg/L	5	162	Normal	Agenesis of corpus callosum	0	1347-1350delTTGT
Patient 2	131	40 mg/L	4	187	Normal	Giant cisterna magna	Not done	1347-1350delTTGT

Table 3. Genomic findings

Disease	Gene	Inheritance	HGVS cDNA	NM	Mutation	Protein	Variant interpretation
Sulfite oxidase deficiency	SUOX	Autosomal recessive	NC_000012.11: g.56398694_56398697delTTGT	NM_000456.2: c.1521_1524delTTGT	c.1521_1524delTTGT	p.C508Rfs*109	Pathogenic

to an encephalopathic state. A significant proportion of patients develop complete or partial ectopia lentis. Although milder cases have been described, in the classic presentation, death occurs at an early age, and none of the treatments tested to date improved the clinical outcome (Johnson and Duran 2001). The preliminary diagnosis is made by elevated urinary sulfites, increased excretion of thiosulfate, increase in S-sulfocysteine, and normal serum uric acid, xanthine, and hypoxanthine. Confirmatory diagnosis is made by measuring sulfite oxidase activity in liver or fibroblasts or identifying a disease-causing mutation in *SUOX*. To date there have been 21 missense/nonsense mutations, seven small deletions, and one small insertion in *SUOX* reported in individuals with isolated sulfite oxidase deficiency (HGMD). The mutation identified here resulted in severe sulfite oxidase deficiency with early onset and severe clinical manifestations.

SUMMARY

It is important to consider isolated sulfite oxidase deficiency/molybdenum cofactor deficiency particularly when dealing with severe seizures and developmental delay in infancy. Screening of fresh urine for sulfites is a simple test but has false positives and false negatives. Urine for thiosulfate can be falsely positive with sulfur containing antibiotics and some anti-convulsants. Urine screening for S-sulfocysteine is more reliable. Uric acid is normal in isolated sulfite oxidase deficiency as compared to molybdenum cofactor deficiency, in which it is usually low. Cranial MRI and EEG are usually abnormal although not diagnostic. The mutation described here of a 4-bp deletion 1347–1350delTTGT in *SUOX*, in two unrelated Indigenous patients predicts a premature termination of the sulfite oxidase protein. Carrier screening for this deletion mutation will be offered to the patients' extended families.

ADDITIONAL INFORMATION

Data Deposition and Access

The variant has been submitted to ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) and can be found under accession number SCV001450523.

Competing Interest Statement

The authors have declared no competing interest.

Referees

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Ethics Statement

Verbal consent for publication from the families was obtained. We did not require Research Ethics Board approval as the molecular testing reported in this manuscript was done as part of standard clinical care.

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Author Contributions

A.A.M., C.R.G., and C.P. oversaw patient care, data collection, data analysis, genetic interpretation, and writing the original draft preparation. R.R.S. assisted with the molecular analysis. E.L.S. and R.A. verified the accuracy of the molecular nomenclature and helped submit the variant to ClinVar. All coauthors read and approved the manuscript.

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