12-1-2020

Isolated sulfite oxidase deficiency: a founder mutation.

Aizeddin A Mhanni

Cheryl R Greenberg

Elizabeth L Spriggs

Ronald Agatep

Reena Ray Sisk

See next page for additional authors

COLD SPRING HARBOR
Molecular Case Studies

RAPID COMMUNICATION

Isolated sulfite oxidase deficiency: a founder mutation

Aizeddin A. Mhanni,[1] Cheryl R. Greenberg,[1,2] Elizabeth L. Spriggs,[1,2] Ronald Agatep,[2] Reena Ray Sisk,[3] and Chitra Prasad[4]

[1]Department of Pediatrics and Child Health, [2]Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, Manitoba R3A 1S1, Canada; [3]Saskatoon Health Authority, Saskatoon S7K 0M7, Canada; [4]London Health Sciences Centre, Western University, London, Ontario N6A 5A5, Canada

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.

Corresponding author:
amhanni@hsc.mb.ca

Ontology terms: increased urinary sulfite; increased urinary thiosulfate

Published by Cold Spring Harbor Laboratory Press

doi:10.1101/mcs.a005900

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 µmolar, 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

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*-sulfocysteine (<25 μmol/mmolCr)	Urine sulfite (not detected)	Plasma cystine (23–49 μmol/L)	Serum uric acid (130–330 μmol/L)	Xanthine hypoxanthine	Cranial MRI	Enzyme activity (liver) (7–28 μ/g)	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 anticonvulsants. 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

Andreas Schulze
Anonymous

Received October 12, 2020; accepted in revised form November 5, 2020.

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.

Acknowledgments

We thank Dr. K. Rajagopalan and Dr. J.L. Johnson from the Department of Biochemistry, Duke University Medical Center for the initial molecular studies. We thank the patients and their families for their cooperation.

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.

Funding

Work at Duke University Medical Center was supported by a National Institutes of Health (NIH) Grant 61444283.

REFERENCES

Kisker C, Schindelin H, Pacheco A, Wehbi WA, Garrett RM, Rajagopalan KV, Enemark JH, Rees DC. 1997. Molecular basis of sulfite oxidase deficiency from the structure of sulfite oxidase. *Cell* **91:** 973–983. doi:10.1016/S0092-8674(00)80488-2

Johnson JL, Duran M. 2001. Molybdenum cofactor deficiency and isolated sulfite oxidase deficiency. In *The metabolic and molecular bases of inherited disease*, 8th ed. (ed. Scriver CR, Beaudet AL, Sly WS, Valle D), pp. 3163–3177. McGraw-Hill, New York.

Johnson JL, Rajagopalan KV. 1976. Human sulfite oxidase deficiency: characterization of the molecular defect in a multicomponent system. *J Clin Invest* **58:** 551–556. doi:10.1172/JCI108500

Isolated sulfite oxidase deficiency: a founder mutation

Aizeddin A. Mhanni, Cheryl R. Greenberg, Elizabeth L. Spriggs, et al.

Cold Spring Harb Mol Case Stud 2020, **6:** a005900
Access the most recent version at doi:10.1101/mcs.a005900