COLD SPRING HARBOR
Molecular Case Studies

RESEARCH REPORT

Postmortem whole-genome sequencing on a dried blood spot identifies a novel homozygous SUOX variant causing isolated sulfite oxidase deficiency

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Abstract Rapid whole-genome sequencing (rWGS) has shown that genetic diseases are a common cause of infant mortality in neonatal intensive care units. Dried blood spots collected for newborn screening allow investigation of causes of infant mortality that were not diagnosed during life. Here, we present a neonate who developed seizures and encephalopathy on the third day of life that was refractory to antiepileptic medications. The patient died on day of life 16 after progressive respiratory failure and sepsis. The parents had lost two prior children after similar presentations, neither of whom had a definitive diagnosis. Postmortem rWGS of a dried blood spot identified a pathogenic homozygous frameshift variant in the *SUOX* gene associated with isolated sulfite oxidase deficiency (c.1390_1391del, p.Leu464GlyfsTer10). This case highlights that early, accurate molecular diagnosis has the potential to influence prenatal counseling and guide management in rare, genetic disorders and has added importance in cases of a strong family history and risk factors such as consanguinity.

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Ontology terms: abnormality of urinary uric acid concentration; central hypotonia; episodic respiratory distress; episodic tachypnea; generalized tonic seizures; limb hypertonia

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INTRODUCTION

It has been estimated that 21% of infant deaths in intensive care settings are due to single-locus genetic diseases (Wojcik et al. 2019; Kingsmore et al. 2020). Rapid whole-genome sequencing can now provide a provisional diagnosis in <19 h (Clark et al. 2019) and has repeatedly demonstrated clinical utility in this population (Willig et al. 2015; Armes et al. 2018; Farnaes et al. 2018; French et al. 2019; Sanford et al. 2019; Australian Genomics Health Alliance Acute Care Flagship 2020). Although some genetic causes of infant mortality are common and well-characterized, such as trisomy 21 and 18, many single-locus diseases go undiagnosed, often leaving families of deceased infants uncertain as to the precise risk of recurrence. Additionally, the landscape of genetic diseases that contribute to infant mortality remains poorly characterized, and death certificates are often inaccurate in their accounting of cause of death (Mieno et al. 2016; McGivern et al. 2017). Accurate molecular diagnosis not

only provides the possibility to implement rapid precision medicine, it can also guide involvement of additional services such as palliative care, genetic counseling, and reproductive technology for future pregnancies. Therefore, a definitive diagnosis of a presumed lethal condition can provide critical guidance to families and clinicians (Petrikin et al. 2015). Here we present a case of isolated sulfite oxidase deficiency (ISOD) diagnosed postmortem causing mortality in a 2-wk-old infant. This infant had two older sisters who died after a similar presentation. This case highlights the role of whole-genome sequencing as a first-tier test in infants highly suspected of having a genetic disorder and the feasibility of the utilization of blood spot cards from newborn screening to perform rapid whole-genome sequencing (rWGS).

CLINICAL PRESENTATION AND FAMILY HISTORY

The patient was a female infant born at 41 wk weighing 3.31 kg to a 48-yr-old mother via spontaneous vaginal delivery. No abnormalities were identified on prenatal screening, and there were no complications during pregnancy. Her parents were first cousins and of Somalian descent. APGAR scores at birth were 8 and 9 at 1 and 5 min, respectively. At 14 h of life, she became tachypneic with a respiratory rate of 96. She was transferred to Rady Children's Hospital, San Diego (RCHSD) level IV neonatal intensive care unit (ICU) for a higher level of care. On admission, she was alert, active, pink, and well-perfused, and vital signs were within normal limits apart from isolated tachypnea. She had mild intercostal retractions, no grunting, and clear and equal breath sounds. No dysmorphic features were noted. Initial laboratory test values upon admission were within normal limits. Family history was notable for two deaths of older siblings—one at 1 wk of age and one at 22 mo of age—who had also been admitted to our institution with intractable seizures (Fig. 1A).

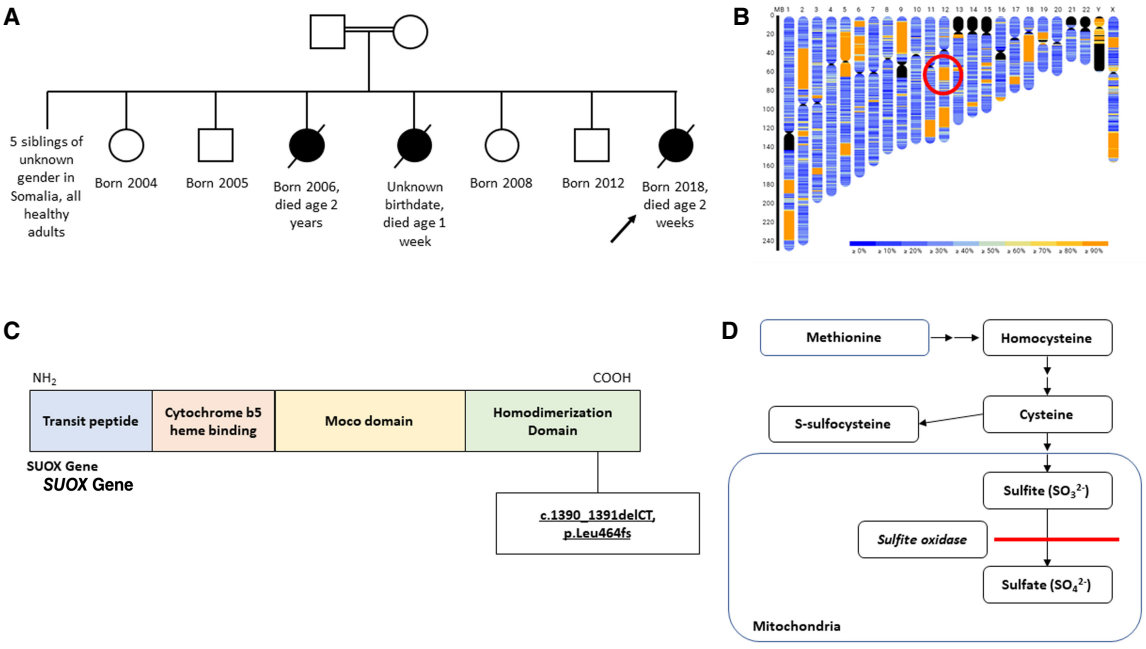

Figure 1. Variant and metabolic pathway information for sulfite oxidase deficiency. (*A*) Pedigree of presented case, (*B*) regions of homozygosity plot for presented case, (*C*) schematic of sulfite oxidase (*SUOX*) gene with homozygous variant identified, and (*D*) metabolic pathway of sulfite oxidase.

Table 1. Observed and expected phenotypic features

Expected phenotypes for sulfite oxidase deficiency (OMIM ID# 272300)	Observed in proband	Category
Generalized dystonia	Absent	Nervous system
Generalized hypotonia	Present	Musculature
Hypertonia	Present	Musculature
Seizure	Present	Nervous system
Ataxia	n.a.	Nervous system
Choreoathetosis	Absent	Nervous system
Global developmental delay	n.d.	Nervous system
Autosomal recessive inheritance	Present	Inheritance
Infantile muscular hypotonia	Present	Musculature
Decreased urinary sulfate	n.d.	Metabolism/laboratory abnormality
Fine hair	Absent	Skin, hair, and nails
Increased urinary sulfite	n.d.	Metabolism/laboratory abnormality
Ectopia lentis	n.d.	Eye
Hemiplegia	Absent	Nervous system
Sulfite oxidase deficiency	n.d.	Metabolism/laboratory abnormality
Delayed eruption of teeth	n.a.	Head and neck
Agitation	Absent	Nervous system
Eczema	Absent	Skin, hair, and nails

(n.d.) Not determined, (n.a.) not applicable.

Her mother also reported at least one late fetal demise. A diagnosis was not found for either of the patient's siblings.

On day of life (DOL) 2, she was noted to have generalized tonic–clonic movements. These were confirmed to be seizures on electroencephalogram (EEG; Table 1). They continued despite treatment with several antiepileptic medications. She had one episode of blood-tinged stool. She continued to be tachypneic, had increasing oxygen requirements, and was intubated on DOL 3. She also required feeding via nasogastric tube to maintain nutritional requirements (Table 1). At this time, WGS was discussed with the parents, but declined as they felt "genetic testing" had not helped their older daughter who died at age 22 mo.

An ultrasound of the head on DOL 2 was within normal limits and showed no evidence of hemorrhage or fluid collections. Brain magnetic resonance imaging (MRI) on DOL 5, however, demonstrated restricted diffusion involving the cerebral cortex and subcortical white matter bilaterally, with relative sparing of the frontal cortex (Fig. 2C,D), suggestive of hypoxic ischemic injury or an inborn error of metabolism. It also demonstrated intraventricular, subdural, and subarachnoid hemorrhage with developing hydrocephalus and an enlarged posterior fossa filled with cerebrospinal fluid (CSF)-intensity fluid (Fig. 2B).

She remained intubated until DOL 13. Upon extubation, she continued to be unable to clear secretions and required frequent suctioning. Further investigation was declined by parents. On DOL 15, she developed persistent emesis despite continuous nasogastric feeding, after which feeding was discontinued. That day, she also had an acute increase in plasma lactate from 4.0 mmol/L to 6.1 mmol/L. Laboratory testing demonstrated that she had

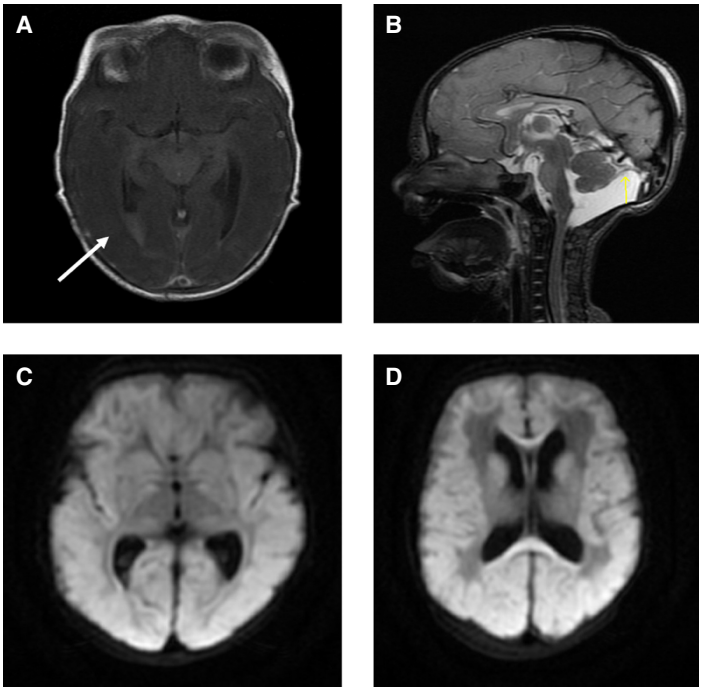

Figure 2. Magnetic resonance imaging (MRI) findings on day of life 5. (*A*) T1-weighted image demonstrating hemorrhage layering in occipital horn of the right lateral ventricle, (*B*) enlarged posterior fossa filled with cerebrospinal fluid (CSF) intensity fluid, and (*C*,*D*) diffusion-weighted imaging (DWI) demonstrating near-symmetric restricted diffusion of the bilateral cerebral cortex with relative sparing of frontal cortex.

Escherichia coli bacteremia. Seizures were controlled on multiple medications, and EEG showed persistent burst suppression. On the morning of DOL 16, she was pale, mottled, and bradycardic with a heart rate between 80 and 90 beats per minute, which dropped to 60–70 beats per minute. The infant's mother was informed of her status and requested that she not be reintubated. She died shortly thereafter. Postmortem laboratory testing showed low plasma halfcystine and increased urinary S-sulfocysteine (Table 2) confirming a defect in metabolism of sulfur-containing amino acids.

The older sibling of the proband who died at 22 mo presented similarly at 6 wk of age in 2006 with status epilepticus and feeding difficulties with severe dysphagia and aspiration. Birth history was unremarkable—she was born at term and went home within 24 h of delivery. A gastrostomy tube was placed. An extensive metabolic workup during that admission, including urine organic acids, plasma amino acids, and CSF neurotransmitter metabolites, was negative. Of note, urine S-sulfocysteine was not measured. Plasma halfcystine was measured at 2 micromol/L and homocysteine was 0 micromol/L. Although sulfite determination

Table 2. Lab values

Substance (units, source)	Value	Reference range
Serum uric acid (mg/dL, plasma)	2.3	1.9–7.9
Homocysteine (micromol/L, plasma)	<3.0	<10.4
S-Sulfocysteine (micromol/g creatinine, urine)	452	≤80
Halfcystine (micromol/L, plasma)	3	34–196

in urine is a rapid way to identify sulfite oxidase deficiency, this test was not performed. Workup for infectious etiologies including cytomegalovirus (CMV), toxoplasmosis, syphilis, rubella, and herpes viruses was also negative. MRI demonstrated microcephaly and overall decreased brain volume. Extensive abnormal signal was identified within the left temporal, parietal, and occipital lobes as well as marked ventriculomegaly and a hypoplastic corpus callosum. She had multiple subsequent hospitalizations for status epilepticus and remained severely developmentally impaired. She was admitted at age 22 mo after developing respiratory distress with episodes of apnea. On day 13 of that hospitalization she became hemodynamically unstable with a blood pressure of 45/25 and profound bradycardia and died shortly thereafter. A definitive etiology for her seizure disorder and progressive respiratory failure was not identified at the time.

GENOMIC ANALYSES

rWGS was performed following the infant's death. Genome coverage was 38-fold and 89.4% of the coding variants had a quality score of >40 (Table 3). The percent of the genome encompassed by regions of homozygosity was 12.1% (Fig. 1B). A homozygous frameshift indel variant (c.1390_1391del, p.Leu464fs) in the sulfite oxidase (*SUOX*) gene (OMIM: #606887) was identified. This variant has not been reported in the literature or ClinVar database to our knowledge; however, loss-of-function variation in SUOX is a known mechanism of disease. The variant was scored as pathogenic on the basis of PM2 (absent from controls including gnomAD, Exome Sequencing Project, 1000 Genomes Project, and ExAC) and PVS1 (null variant in a gene in which loss-of function is an established mechanism of disease). No other diagnostic variants were found in other genes. *SUOX* maps to Chromosome 12q13.2. The variant lay within a 21.5-Mb region of homozygosity (Fig. 1B). *SUOX* encodes sulfite oxidase (EC 1.8.3.1, Fig. 1C), which catalyzes the conversion of sulfite to sulfate, an essential step in the metabolism of sulfur-containing amino acids, including homocysteine and cysteine (Fig. 1D). Isolated sulfite oxidase deficiency (ISOD, OMIM #272300) can be either classic early-onset or late-onset. In the early-onset form, intractable seizures begin within the first few days of life and progress to encephalopathy and early death (Bindu et al. 2017). Other clinical features of ISOD include ectopia lentis and increased urinary excretion of thiosulfate, sulfite, and S-sulfocysteine (Edwards et al. 1999). In a recent review of 47 cases, MRI abnormalities like herein were identified in all cases (Eichler et al. 2006; Bindu et al. 2017; Claerhout et al. 2018; Misko et al. 2020). There is currently no effective treatment for ISOD. The enzymatic activity of sulfite oxidase is dependent on a molybdenum cofactor and therefore some of the hallmarks of molybdenum cofactor deficiency caused by variants in the MOCS1 gene are shared with sulfite oxidase deficiency (Kingsmore et al. 2020).

Table 3. Proband genome sequencing metrics

Metric	Value
Read length	2 × 101 nt
Mean coverage	38-fold
Nucleotide variants identified	5,319,759
Variants with quality scores >40	89.40%
Coding nucleotide variants identified	28,571
Homozygous: heterozygous ratio of coding nucleotide variants	0.66
Transition to transversion ratio of coding nucleotide variants	2.91

The original death certificate for this infant was analyzed for accuracy. The primary cause of death was stated as cardiorespiratory failure, with contributing factors of *Escherichia coli* bacteremia, diffuse brain injury, and intractable seizures. Based on the results presented herein, a revised death certificate would list ISOD as the cause of death.

DISCUSSION

ISOD is a rare, autosomal recessive condition characterized by early onset of severe, intractable seizures and encephalopathy, which usually leads to death (Tan et al. 2005; Bindu et al. 2017; Claerhout et al. 2018). Accumulation of sulfite in neural tissues results in the decreased functioning of multiple crucial enzymes, including glutathione peroxidase, glucose-6-phosphate dehydrogenase, glutathione S-transferase, glutathione reductase, and creatine kinase (Ozturk et al. 2010; Wyse et al. 2019; Glänzel et al. 2020). S-sulfocysteine buildup may also mediate excitotoxic cell death in neurons (Kumar et al. 2017). Unfortunately, there is to date no effective treatment for ISOD. Here we have presented a family of 12 siblings, three of whom died after presenting similarly with intractable seizures and encephalopathy, and one of whom had a confirmed homozygous, pathogenic variant in *SUOX*. None of the three were diagnosed during life. A definitive diagnosis in the first affected child would have enabled provision of precise genetic counseling regarding recurrence risk and made a variety of preconceptual and prenatal options available to the parents.

Prenatal screening currently includes a combination of ultrasound measurements for fetal neural tube defects and major congenital abnormalities, quad screening for trisomies, and cell-free DNA analysis (ACOG Practice Bulletin 2020). It is used to estimate risk for common aneuploidies. Parental carrier screening identifies common, known pathogenic variants in established disease genes and may be performed prior to or during pregnancy. Novel sequence variants, especially those in genes associated with rare conditions, are not detected by standard prenatal and prepregnancy screening.

In contrast, next-generation sequencing can be diagnostic for aneuploidies, copy-number and structural variants, and nucleotide variants. Coupled with a rapid turnaround time, it thus has significant potential as a diagnostic test in pregnancies highly suspected to have a genetic condition in the absence of a familial genetic diagnosis (Lord et al. 2019; Petrovski et al. 2019). Likewise, dried blood spots (DBS) are stored for every newborn in the state of California and present an enormous resource for the diagnosis of previously undetected rare genetic diseases. They can yield high-quality genomic data, even after long-term storage (Bassaganyas et al. 2018). Additionally, although a rapid turnaround time is not needed in postmortem investigations, recent advances in automated sequencing and interpretation pipelines allow for scalable investigation of infant death. The use of archived DBS for investigation of unexplained infant deaths may yield useful information about underlying causes of infant mortality; however, currently, not all states archive DBS, despite the ease of storage and collection.

In the current family, had WGS of the blood spots of an elder sibling been performed, it is likely that the *SUOX* variant would have been identified. It is of note that although the variant identified herein is novel, there is systematic underrepresentation of people of African descent in gnomAD and other population databases. Future studies are needed to evaluate the generalizability of postmortem WGS on dried blood spots when concern for a genetic etiology is increased given consanguinity and recurrent mortality in infancy. Effective, long-term follow-up of families is necessary for these services to be offered. Of note, nondiagnostic genomic sequencing in a previous child does not preclude such testing because WGS technology and knowledge of genetic disorders are advancing rapidly, and periodic reanalysis may reveal previously unestablished gene disease associations (James et al. 2020).

Disease detection may present the opportunity for early, targeted rapid precision medicine. Although there is no current treatment available for isolated sulfite oxidase deficiency, several candidates are currently under investigation. A recently identified mitochondrial-targeted antioxidant, JP4-039, has been shown to preserve antioxidant enzymatic activity and creatine kinase in mouse neural tissue (Glänzel et al. 2020), and bezafibrate has been shown to prevent mitochondrial dysfunction and neuronal damage caused by sulfite in rats (Grings et al. 2017). Additionally, effective treatment is available for the closely related disorder molybdenum cofactor deficiency (Kingsmore et al. 2020). Although established therapies for ISOD in humans may be some years in the future, these results highlight the importance of an accurate molecular diagnosis for targeting of novel therapies as they emerge.

Causes of infant mortality have been extensively studied for the past 50 years. Understanding of causes of infant death inform public health interventions, prioritization of medical research funding, and governmental policy. In the current case, the underlying cause of death was not discovered during the life of the infant nor her affected siblings. As a result, the recorded death certificate was inaccurate. It would have been different based on the molecular diagnosis from rWGS presented here. ISOD is often misdiagnosed, and because of rapid progression, diagnosis is often made after death (Claerhout et al. 2018; Mhanni et al. 2020). Previous studies have demonstrated that 33%–50% of death certificates are inaccurate (Mieno et al. 2016; McGivern et al. 2017). This case demonstrates the need for large-scale, unbiased studies of cohorts of infant deaths utilizing rWGS in order to accurately assess the incidence of genetic etiologies in infant mortality.

METHODS

rWGS was performed on a dried blood spot that had been collected under a research protocol and stored in a biorepository at −20°C. Sequencing was performed in a laboratory that is California-licensed, Clinical Laboratory Improvement Amendments (CLIA)-certified, and College of American Pathologists (CAP)-accredited. The dried blood spot was lysed, DNA was extracted, and sequencing libraries were prepared using the DNA PCR-Free, Tagmentation Library Prep kit (Illumina). Paired-end sequencing was performed on a NovaSeq 6000 and S2 flow cell (Illumina; Table 4). Alignment and variant calling were performed using the DRAGEN v.3.7 pipeline (Illumina). The proband genome had multiple regions of homozygosity, consistent with parental consanguinity. Variant analysis and interpretation were performed using supervised artificial intelligence systems (MOON [InVitae] and GEM [Fabric Genomics]). Phenotypic terms used were seizures, encephalopathy, EEG with burst suppression, respiratory failure, feeding difficulties in infancy, bloody stool, hydrocephalus, abnormal EEG, abnormal corpus callosum morphology, hypoglycemia, hypernatremia, lactic acidosis, *E. coli* infections, status epilepticus, and infantile encephalopathy. Variants were filtered to retain those at <0.5% frequency in population databases and classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines.

Table 4. Variant information

Gene	Chromosomal variant coordinate (build 37.1)	HGVS DNA reference	HGVS protein reference	Variant type	Predicted effect	ACMG classification	Zygosity
SUOX (ENST00000394109)	Chr 12:56398559	c.1390_1391del	p.Leu464GlyfsTer10	Del	Frameshift	Pathogenic	Homozygous

ADDITIONAL INFORMATION

Data Deposition and Access

Consent was not given to make data publicly available. For access to original data, please contact Dr. Stephen Kingsmore, skingsmore@rchsd.org. The SUOX variant was deposited in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and can be found under accession number SCV000641281.

Ethics Statement

rWGS was performed as part of the SOMI Infant Death Project. The Institutional Review Board of Rady Children's Hospital, San Diego, and University of California, San Diego issued a waiver for this project.

Author Contributions

M.J.O. performed variant interpretation, chart review, phenotypic analysis, and wrote the manuscript. All authors reviewed the final version. J.L. and S.C. confirmed the interpretation findings. S.F.K. acted as the principal investigator on the study, edited the manuscript, and contributed to study design. A.F. and J.G. provided clinical input. Z.B.-O. and Y.D. supervised laboratory operations. K.C. provided bioinformatics support.

Competing Interest Statement

The authors have declared no competing interest.

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