COLD SPRING HARBOR
Molecular Case Studies

RAPID COMMUNICATION

Maternal mosaicism for a missense variant in the *SMS* gene that causes Snyder–Robinson syndrome

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Abstract There is increasing recognition for the contribution of genetic mosaicism to human disease, particularly as high-throughput sequencing has enabled detection of sequence variants at very low allele frequencies. Here, we describe an infant male who presented at 9 mo of age with hypotonia, dysmorphic features, congenital heart disease, hyperinsulinemic hypoglycemia, hypothyroidism, and bilateral sensorineural hearing loss. Whole-genome sequencing of the proband and the parents uncovered an apparent de novo mutation in the X-linked *SMS* gene. *SMS* encodes spermine synthase, which catalyzes the production of spermine from spermidine. Inactivation of the *SMS* gene disrupts the spermidine/spermine ratio, resulting in Snyder–Robinson syndrome. The variant in our patient is absent from the gnomAD and ExAC databases and causes a missense change (p.Arg130Cys) predicted to be damaging by most in silico tools. Although Sanger sequencing confirmed the de novo status in our proband, polymerase chain reaction (PCR) and deep targeted resequencing to ~84,000×–175,000× depth revealed that the variant is present in blood from the unaffected mother at ~3% variant allele frequency. Our findings thus provided a long-sought diagnosis for the family while highlighting the role of parental mosaicism in severe genetic disorders.

[Supplemental material is available for this article.]

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Ontology terms: bilateral sensorineural hearing impairment; congenital hypothyroidism; defect in the atrial septum; hyperinsulinemic hypoglycemia; severe muscular hypotonia

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CASE PRESENTATION

Here we report an infant male who presented at 9 mo of age with hypotonia, dysmorphic features, congenital heart disease, hyperinsulinemic hypoglycemia, hypothyroidism, and bilateral sensorineural hearing loss. The patient was born at 36 + 6 wk gestation weighing 2096 grams. Pregnancy was complicated by maternal rheumatoid arthritis treated with hydroxychloroquine and oligohydramnios. There was no maternal diabetes. Apgars were 3 at 1 min and 6 at 5 min. He required continuous positive airway pressure (CPAP) initially but weaned to room air after 36 h. He had a history of hypotonia, developmental delay, and shallow breathing (considered secondary to hypotonia). His growth—including weight, length, and head circumference—started small but gradually drifted upward above the 3rd percentile at ~6 mo of age. He also had congenital heart disease including an atrial septal

defect and pulmonary valve stenosis. His dysmorphic features on exam included coarse facial features, intermittent esotropia, myopathic tented mouth, micrognathia, sacral dimple/gluteal cleft, and hirsutism of the face, back, and extremities. A magnetic resonance image (MRI) of the brain at 1 mo showed T2 hyperintense signal in the periventricular white matter, most prominently in the bifrontal and bilateral periatrial regions. On a subsequent study, signal intensities were felt to have resolved. On follow-up study at 10 mo of age, the corpus callosum was identified as hypoplastic but intact (Fig. 1D). He also developed infantile spasms and presented to the intensive care unit (ICU) with an increase in seizure activity (electroencephalogram [EEG] was severely abnormal with severe infantile epileptic encephalopathy/West syndrome). The patient passed away during hospital admission at 10 mo of age as a result of respiratory distress and neurologic dysfunction (see Table 1 for detailed clinical features). All growth parameters were in the normal range at the time of death.

TECHNICAL ANALYSIS AND METHODS

DNA libraries were generated using an NEBNext Ultra II library prep kit, and sequencing was performed using an Illumina NovaSeq 6000 instrument. Reads were mapped to the GRCh38 reference sequence, and data analysis was performed using Churchill (Kelly et al. 2015) followed by variant annotation and prioritization as described previously (Koboldt et al. 2018). Deep targeted sequencing was performed on DNA samples of the proband, his parents, and a human reference DNA sample (GM24143) obtained from Genome in a Bottle Consortium as the negative control. More details on the sequencing and analysis can be found in the Supplemental Analysis and Methods section. Sequencing metrics are provided in Supplemental Table 1. The variant diagram in Figure 1A was generated with Lollipops v1.5.1 (Jay and Brouwer 2016) using information from the ClinVar (Landrum et al. 2018) database (accessed January 2, 2021).

VARIANT INTERPRETATION

Whole-genome sequencing (WGS) initially revealed an apparent de novo mutation in the X-linked *SMS* gene (PS2) (Table 2 and Supplemental Table 2). The c.388C > T variant in our patient is absent from large population cohorts including gnomAD and ExAC (Lek et al. 2016; Karczewski et al. 2020) (PM2) and causes a missense change (p.Arg130Cys) predicted to be damaging by 20/22 in silico tools (Kopanos et al. 2019) (PP3). The p.Arg130Cys is located within the spermidine synthase tetramerization domain (Fig. 1A) and is believed to reduce SMS dimer stability by disrupting the structure of the adjacent spermine binding site (Abela et al. 2016) (PS3). Further, this amino acid change has been described in one family with twin boys presenting similar features, which provided further supporting evidence (Abela et al. 2016) (PS1). We interpreted the variant as pathogenic (Table 2) under standard guidelines set forth by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) (Richards et al. 2015). This variant was submitted to the ClinVar database in January 2021 (SCV001468506.1). No other pathogenic or likely pathogenic variant was observed in the proband genome that could explain his phenotype (Supplemental Table 3).

SUMMARY

The *SMS* gene encodes spermine synthase that catalyzes the production of spermine from spermidine. Inactivation of the *SMS* gene disrupts the spermidine/spermine ratio, resulting

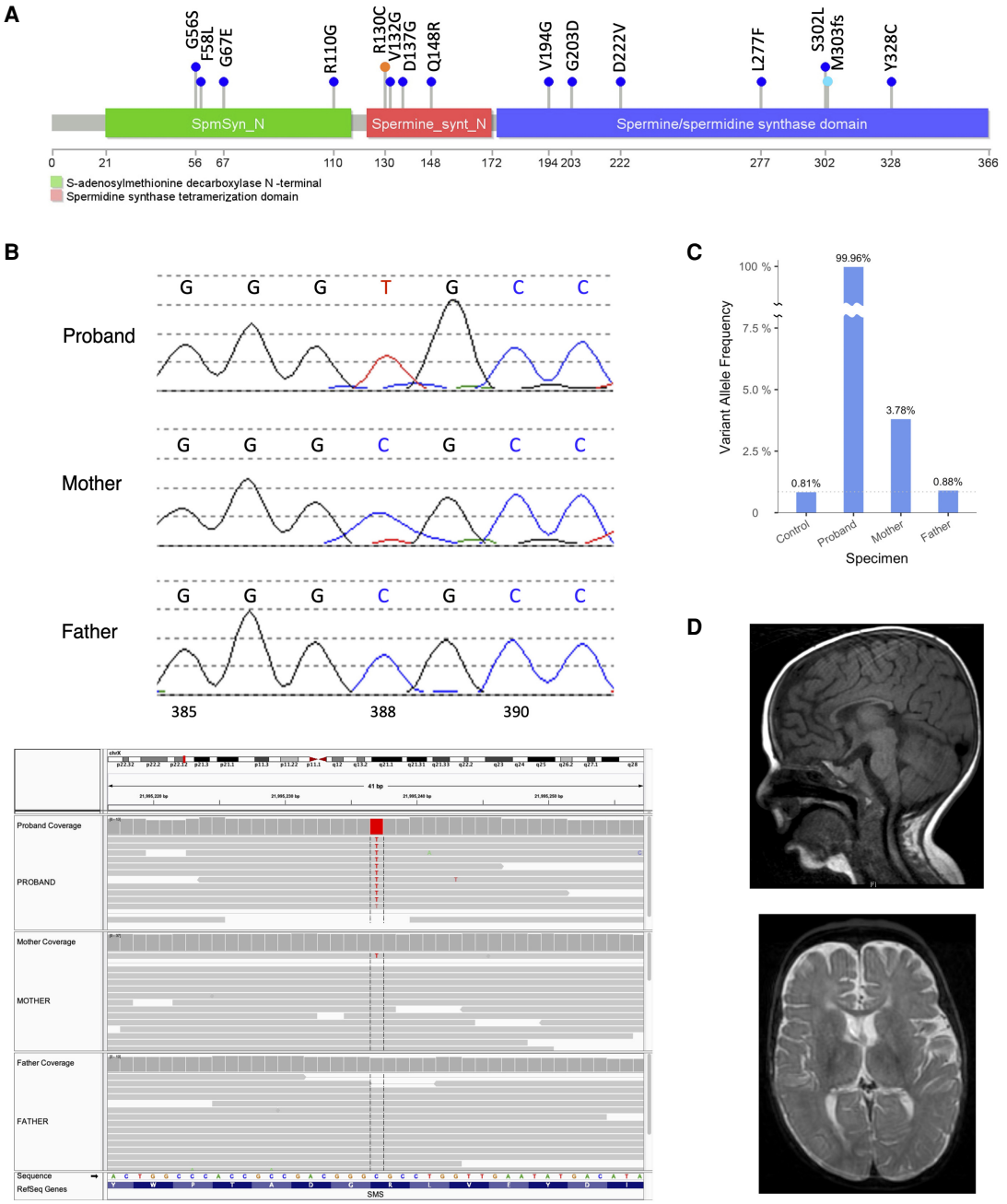

Figure 1. Sanger sequencing confirmed the de novo status of the c.388C > T variant in the proband, whereas deep targeted resequencing revealed that the mother is mosaic for the variant. (*A*) Pathogenic and likely pathogenic variants reported in the ClinVar database as of January 2, 2021. Blue dots represent missense variants. The p.Arg130Cys variant is shown in orange, and the only reported frameshift variant is shown in cyan. (*B*) Sanger sequencing traces (*top*) of the region harboring the p.Arg130Cys variant in the proband and the parents in addition to the Integrative Genomics Viewer (IGV) screenshot (*bottom*) for the same region. Numbers below the trace represent the nucleotide position as in the cDNA of the canonical transcript (NM_004595.5). The residual trace of the variant in the Sanger sequencing trace and a single variant-supporting read in the maternal sample raised the possibility of mosaicism. (*C*) Deep targeted sequencing results revealed the mosaic status of the mother. Shown is the variant allele frequency in the proband, the parents, and a control sample. The dashed line marks the background level of the variant (i.e., baseline sequencing error) in the paternal sample and an unrelated control specimen. (*D*) Proband magnetic resonance imaging (MRI) of the brain with no contrast at 10 mo of age when admitted to pediatric intensive care unit with increased seizure frequency showing (*top*) sagittal T1 imaging: small but intact corpus callosum and (*bottom*) axial T2-weighted imaging: areas of restricted diffusion in the basal ganglia.

Table 1. Clinical features present in Snyder–Robinson syndrome (SRS) patients, this proband, and/or the patient reported by Abela et al. (2016)

Clinical features	Reported in SRS patients	This study	Abela et al. (2016)
Growth			
Tall stature	+	-	N/R
Short stature	+	-	+
Head and neck			
Cleft palate	+	+	N/R
Facial asymmetry	+	+	N/R
Thick lower lip vermilion	+	-	N/R
Abnormality of the pinna	+	+	N/R
Hypertelorism	+	+	N/R
Dental crowding	+	N/A	N/R
Webbed neck	+	-	N/R
High myopia	+	+	N/R
Mandibular prognathia	+	-	N/R
Short philtrum	+	-	N/R
Micrognathia	-	+	N/R
Esotropia	-	+	N/R
Bilateral sensorineural hearing impairment	-	+	N/R
Neurologic			
Broad-based gait	+	N/A	N/R
Hypotonia	+	+	+
Intellectual disability	+	N/A	N/R
EEG abnormality	+	+	+
Seizure	+	+	+
Global developmental delay	+	+	+
Skeletal			
Kyphoscoliosis	+	-	N/R
Long fingers	+	-	N/R
Osteoporosis	+	N/A	N/R
Recurrent fractures	+	+	+
Pectus carinatum	+	-	N/R
Pectus excavatum	+	-	N/R
Cardiovascular			
Atrial septal defect	-	+	N/R
Pulmonary stenosis	+	+	N/R
Endocrine system			
Hyperinsulinemic hypoglycemia	+	+	N/R
Congenital hypothyroidism	-	+	N/R
Musculature			
Decreased muscle mass	+	+	+
Nasal speech	+	N/A	N/R

(*Continued on next page.*)

Table 1. (*Continued*)

Clinical features	Reported in SRS patients	This study	Abela et al. (2016)
Cryptorchidism	+	+	N/R
Wide intermamillary distance	+	+	N/R
Renal anomalies	–	+	N/R
Other			
Hirsutism	–	+	N/R
Sacral dimple	–	+	N/R

Human Phenotype Ontology (HPO) terms are listed with an indication of whether the feature has been observed in SRS patients, in our proband, and in patients reported by Abela et al. (2016).
(N/A) Not available, (N/R) not reported, (+) present, (–) not present.

in Snyder–Robinson syndrome (SRS). The syndrome was first described in 1969: Clinical details (including hypotonia, wide-based gait, slight facial asymmetry) were reported on three of eight affected males (Snyder and Robinson 1969). In 1996, Arena et al. (1996) reinvestigated the same family and found that males exhibited a characteristic set of clinical features and, hence, reported SRS as an X-linked syndrome.

In 2003, the disease was linked to a splice site variant in *SMS* that results in an aberrant splicing event of exon 4 skipping (Cason et al. 2003). To date, 14 pathogenic missense and one frameshift variants in *SMS* have been reported to the ClinVar database (Fig. 1A). Whereas the frameshift variant is reported as a complete loss of function of the *SMS* gene causing a severe form of the syndrome (Larcher et al. 2020), all other reported pathogenic variants are missense variants in patients with mild disease severity (e.g., Zhang et al. 2013; Abela et al. 2016).

We note that the proband, who presented with symptoms overlapping with SRS, passed away at 10 mo of age, and, hence, some common SRS features that appear in later stages of life (e.g., kyphoscoliosis and short stature) were not observed in the proband. Additionally, several features have been observed in the proband that, to our knowledge, have not been reported in other SRS patients (Table 1). As we are not able to confirm the association of these futures with the SMS variant nor was any other explanatory variant found in the proband, studies of more patients with this rare disease are required to confirm or reject the association.

Our initial analysis revealed that our proband carries a de novo c.388C > T variant in the *SMS* gene. Although Sanger sequencing of the patient and both parents confirmed the de novo status of the c.388C > T variant in our proband (Fig. 1B), the residual trace of the variant in the sequencing trace and a single variant-supporting read in the maternal sample raised the possibility of mosaicism. Following the best clinical practice and because of the Sanger confirmation, we report this as a de novo variant. However, to further investigate the possibility of mosaicism, we performed PCR and deep targeted resequencing and obtained

Table 2. Genomic findings and variant interpretation of p.Arg130Cys variant in the *SMS* gene

Gene	Genomic location	HGVS cDNA	HGVS protein	Zygosity	Origin	Interpretation
SMS	Chr X: 21977119	NM_004595.5: c.388C > T	SMS: p.Arg130Cys	Hemizygous	De novo	Pathogenic (PS1, PS2, PS3, PM2, PP3)

Genomic coordinates reflect build GRCh38. The ACMG evidence codes applied to this variant are listed in the last column.

84,000×–175,000× coverage of the *SMS* variant position (Supplemental Table 4). The deep sequencing results revealed that the variant is present in the maternal blood at ~3% variant allele frequency (Fig. 1C). Parental mosaicism has been observed in ~2% of diagnostic exome cases from our clinical laboratory (Miller et al. 2020) and has been reported in 4%–8% of cases with dominant neurodevelopmental disorders (Campbell et al. 2014; Myers et al. 2018). Counseling families on the risk of recurrence for parental mosaic variants is challenging because the prevalence of the variant in parental germ cells is unknown. In the case of this family, it became a pressing question: When we returned these findings to the family, the mother was pregnant. The genetic counselor advised the family that there was no way to be certain, but we felt the recurrence risk was low. The mother gave birth to a healthy baby boy who did not have features of SRS. To our knowledge, this is the first report of parental mosaicism for an *SMS* mutation associated with SRS.

ADDITIONAL INFORMATION

Data Deposition and Access

The variant and our interpretation have been deposited in ClinVar (https://ncbi.nlm.nih.gov/clinvar/) under accession number SCV001468506.1.

Ethics Statement

Written consent for whole-genome sequencing was obtained at the time of enrolling subjects into a research protocol approved by the Institutional Review Board at Nationwide Children's Hospital (IRB11-00215 Study: Using Genome Sequencing to Identify Causes of Rare Birth Defects and Rare Disorders).

Competing Interest Statement

The authors have declared no competing interest.

Referees

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Author Contributions

All authors contributed to scientific discussion, variant interpretation, and manuscript review.

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