REPORT

De Novo Truncating Variants in *SON* Cause Intellectual Disability, Congenital Malformations, and Failure to Thrive

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SON is a key component of the spliceosomal complex and a critical mediator of constitutive and alternative splicing. Additionally, SON has been shown to influence cell-cycle progression, genomic integrity, and maintenance of pluripotency in stem cell populations. The clear functional relevance of SON in coordinating essential cellular processes and its presence in diverse human tissues suggests that intact SON might be crucial for normal growth and development. However, the phenotypic effects of deleterious germline variants in *SON* have not been clearly defined. Herein, we describe seven unrelated individuals with de novo variants in *SON* and propose that deleterious variants in *SON* are associated with a severe multisystem disorder characterized by developmental delay, persistent feeding difficulties, and congenital malformations, including brain anomalies.

Whole-exome sequencing (WES) is an essential tool in the diagnostic evaluation of individuals with suspected genetic disorders for which a genetic etiology has not been established by conventional approaches. Studies of large cohorts of individuals have demonstrated a diagnostic yield of 25%–30% when WES is applied to otherwise perplexing cases.[1–3] The additional benefit of the unbiased sequencing approach of WES is the ability to ascertain genes in which variants have not been previously reported to cause disease. In our clinical WES cohort of over 6,000 unrelated individuals—the majority of whom have neurologic manifestations and are of pediatric age—we identified six individuals (subjects 1–6) with truncating variants in *SON* (SON DNA binding protein [MIM: 182465]) and overlapping clinical features. We analyzed parental samples by Sanger sequencing or WES and confirmed de novo status of all six variants. Subsequently, we ascertained one additional individual (subject 7) with two de novo missense variants in *SON* and similar features. Herein, we comprehensively phenotype all seven individuals and propose that deleterious variants in *SON* are associated with severe developmental outcomes.

This study was performed in accordance with a protocol that was prospectively reviewed and approved by the Baylor College of Medicine Institutional Review Board. Written informed consent was obtained from all study participants. The key clinical features of our cohort are summarized in Table 1. Detailed clinical summaries for all subjects are provided in the Supplemental Data, and photographs are included in Figure 1. All subjects had dysmorphic features including, for example, mild midface retrusion with apparently deep-set eyes (n = 6), frontal bossing and bitemporal narrowing (n = 2), downslanting palpebral fissures (n = 5), and epicanthal folds (n = 3). All subjects had either a smooth or short philtrum (n = 7), and a subset had thin lips (n = 5) and/or a short mouth (n = 3). All subjects exhibited developmental delay, which appeared to progress with age into moderate to severe intellectual disability. All but one individual had additional neurological features including regression (n = 3), epilepsy or other electroencephalography (EEG) abnormalities (n = 4), autism spectrum disorder (n = 3), and hyper- or hypotonia (n = 5). Additionally, five of six subjects evaluated had abnormalities detected on brain imaging; features suggestive of volume loss specifically were seen in all five. Five subjects had congenital malformations. An atrial septal defect, ventricular septal defect, patent ductus arteriosus, left lung agenesis, single kidney, dysplastic kidney, and agenesis of the gallbladder were each seen in a single individual; several subjects had more than one malformation. All subjects had a history of feeding difficulties, which were evident as early as the

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Table 1. Clinical Features of Subjects with De Novo *SON* Variants

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7
Current age	6 years	23 years	9 years	3 years	15 years	9 years	3 years
Sex	female	male	female	female	female	female	female
Pregnancy	IUGR, placenta previa	maternal hypertension	IUGR	IUGR, maternal borderline diabetes, factor V deficiency	maternal hypertension	IUGR, oligohydramnios, pre-eclampsia, fetal anomalies	IUGR, fetal anomalies
Age at birth	32 weeks	full term	full term	33 weeks	35 weeks	36 weeks	36 weeks
Delivery	C-section for fetal distress	C-section for fetal distress	wrapped cord, variable heart rate, failure to progress	C-section for fetal distress	C-section for maternal hypertension	vaginal delivery	C-section for fetal distress
Postnatal course	respiratory failure, feeding difficulties	feeding difficulties	feeding difficulties, hypoglycemia	respiratory failure, feeding difficulties	feeding difficulties, respiratory issues	feeding difficulties, respiratory issues	respiratory distress, feeding difficulties
Height	2nd percentile	40th percentile	25th percentile	75th percentile	3rd percentile	−3 (*Z* score)	1st percentile
Weight	3rd percentile	1st percentile	−2.29 (*Z* score)	85th percentile	12th percentile	2nd percentile	−3 (*Z* score)
OFC	2nd percentile	50th percentile	−4 (*Z* score)	60th percentile	72nd percentile	12th percentile	−2.5 (*Z* score)
Distinctive features	frontal bossing, bitemporal narrowing, epicanthal folds, thin lip, smooth philtrum	downslanting palpebral fissures, bifid uvula, submucous cleft palate, short philtrum	downslanting palpebral fissures, downturned mouth, short philtrum, thin lip, thin limbs	submucous and laryngeal cleft, frontal bossing, bitemporal narrowing, epicanthal folds, thin lip, smooth philtrum	downslanting palpebral fissures, laterally flared eyebrows, short philtrum	downslanting palpebral fissures, long face, full cheeks, short philtrum, thin lips	downslanting palpebral fissures, epicanthal folds, smooth philtrum, thin lips
Developmental delay	yes	yes	yes	yes	yes	yes	yes
Regression	yes	yes	no	yes	no	no	no
ASD	yes	yes	yes	NA	NA	no	no
Seizures	yes	yes	no (abnormal EEG)	staring spells	NA	no (abnormal EEG)	no
Tone	hypotonia	NA	hypotonia and spasticity	hypotonia	normal	hypotonia	hypotonia
Brain imaging	global volume loss, thin corpus callosum, mild periventricular gliosis	progressive ventricular and subarachnoid space dilatation, arachnoid cyst	unremarkable	periventricular leukomalacia with mild dilation of the lateral ventricle	prominent extra-axial spaces, dysgenesis of corpus callosum	evidence of prior MCA stroke, prominent ventricles	not done
Congenital malformation	atrial septal defect (resolved)	NA	NA	abnormal placement of carotid arteries in the neck	single kidney	dysplastic kidney, congenital lobar emphysema	VSD, PDA, agenesis of the left lung, gallbladder agenesis
Vision	exotropia, nystagmus	progressive vision loss, myopia, exotropia	NA	esotropia, CVI, blue sclera, segmental optic nerve hypoplasia	history of bilateral eye surgery	strabismus	no concerns

(*Continued on next page*)

Table 1. *Continued*

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7
Hearing	PE tubes	auditory hallucination	PE tubes	PE tubes	NA	inconclusive hearing assessment	no concerns
Gastro-intestinal features	delayed gastric emptying, feeding difficulties	pancreatic lipase insufficiency, dysphagia	failure to thrive, chronic diarrhea, feeding difficulties	failure to thrive, G-tube feeding, diarrhea, reflux, gastric dysmotility	feeding difficulties	dysphagia, G-tube feeding	failure to thrive, G-tube recommended
Musculo-skeletal features	joint laxity	scoliosis, arachnodactyly, dolichostenomelia	joint laxity, cervical rib	joint laxity, cervical ribs, mild syndactyly	exaggerated lumbar lordosis	none	hemivertebrae, rib fusion, thumb agenesis, syndactyly
Hematologic features	DVT	NA	IgG and IgA deficiency, recurrent infection	IgA deficiency, recurrent infection	borderline IgG levels	prior MCA infarct, multiple TIAs	NA

Abbreviations are as follows: ASD, autism spectrum disorder; C-section, cesarean section; CVI, cortical visual impairment; DVT, deep-vein thrombosis; EEG, electroencephalography; G-tube, gastrostomy tube; IgA, immunoglobulin A; IgG, immunoglobulin G; IUGR, intrauterine growth restriction; MCA, middle cerebral artery; NA, not ascertained; OFC, occipitofrontal circumference; PDA, patent ductus arteriosus; PE tubes, pressure-equalizing tubes; TIA, transient ischemic attack; and VSD, ventricular septal defect.

neonatal period and associated with growth failure in most cases. Several subjects required a gastrostomy feeding tube. Most subjects also had ophthalmologic concerns including strabismus (n = 4) and vision loss (n = 2). Six subjects had skeletal abnormalities including joint laxity (n = 3), cervical ribs (n = 2), scoliosis (n = 1), and thumb agenesis (n = 1). Pregnancy and delivery complications were common in the cohort. Five of the seven subjects had intrauterine growth restriction, at least four had significant fetal distress requiring delivery via cesarean section, and five were born prematurely. Three subjects had a history of borderline low or frankly deficient immunoglobulin levels, and two subjects had episodes of suspected abnormal clotting, including unprovoked deep-vein thrombosis in subject 1 and a history of a right middle cerebral artery infarct and multiple transient ischemic attacks in subject 6.

Sequencing results are summarized in Table 2. Variant nomenclature is consistent with *SON* transcript GenBank: NM_138927.2 (UCSC Genome Browser hg19). All truncating and missense variants were confirmed by Sanger sequencing and found to be de novo by parental testing. Subjects 1–6 had truncating variants including one premature stop variant in exon 3 (c.286C>T [p.Gln96*]), three frameshift variants in exon 3 (c.3073dupA [p.Met1025Asnfs*6], c.3852_3856delGGTAT [p.Met1284Ilefs*2], and c.5753_5756delTTAG [p.Val1918Glufs*87]), and one frameshift variant in exon 4 (c.6233delC [p.Pro2078Hisfs*4]) (Figure 2). Of note, the c.5753_5756delTTAG (p.Val1918Glufs*87) variant was observed in two unrelated subjects. Subject 7 had two de novo missense changes in *cis* configuration in exon 3 (Figure S1). SIFT and PolyPhen-2 predicted the c.4909A>T (p.Thr1637Ser) missense variant to be deleterious and benign, respectively, and the c.5528C>A (p.Ser1843Tyr) variant to be deleterious and damaging, respectively. It is unclear whether this is a complex allele or whether an individual variant contributes to the disease phenotype.

SON is located in human chromosomal region 21q22.11 and consists of 12 exons.[5] A striking feature of the gene's structure is the size of exon 3, which accounts for 82% of the entire coding region (GenBank: NM_138927.2). According to the Exome Aggregation Consortium (ExAC) Browser, *SON* is predicted to be intolerant to loss-of-function mutations given that 49.1 loss-of-function variants are expected but only one loss-of-function variant is observed (pLI = 1.00).[6] *SON* does not appear to be intolerant to missense variation,[6] however, suggesting that cautious interpretation of the missense changes detected in subject 7 is warranted.

The canonical SON isoform (GenBank: NP_620305.2, isoform F) encoded by GenBank: NM_138927.2 is a 2,426 amino acid protein that is ubiquitously present in human tissues and highly conserved[7,8] and has an estimated 84% sequence homology between human *SON* and mouse *Son*.[9] SON contains several recognizable domains implicating it as a modulator of RNA processing; these include an

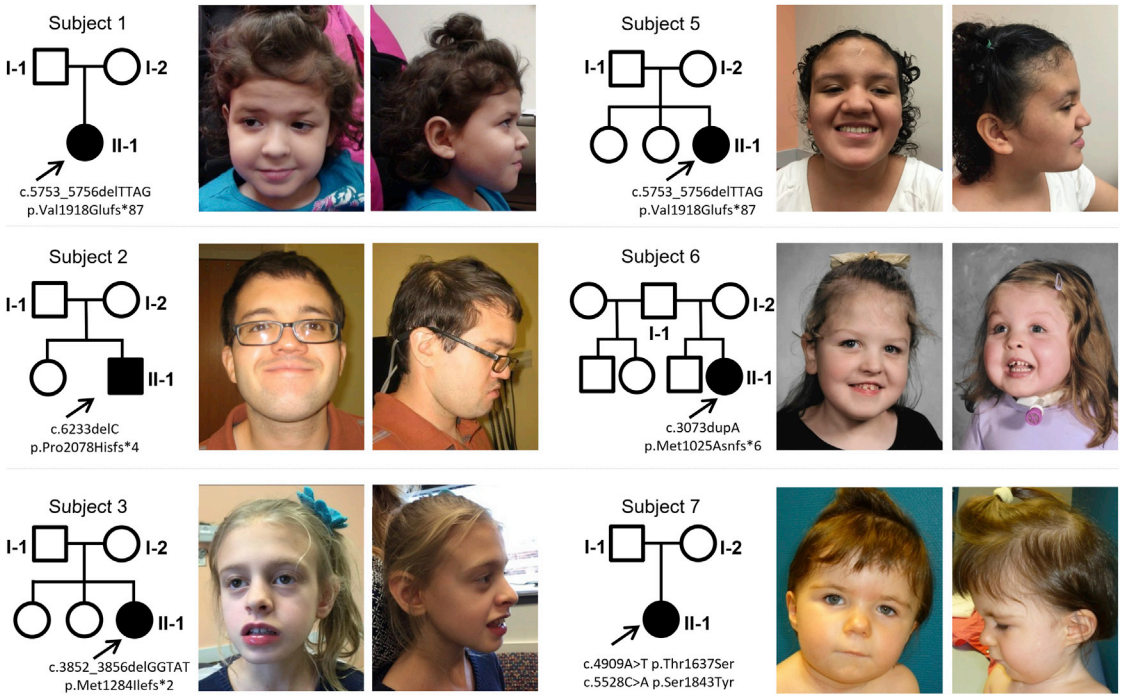

Figure 1. Photographs and Pedigrees of Subjects with *SON* Variants
Photographs show subjects reported in this article, and pedigrees illustrate the de novo status of all detected *SON* variants. Shaded symbols represent affected individuals.

arginine/serine (RS)-rich domain, a G-patch domain, and a double-stranded RNA-binding motif (Figure 2).[4,9,10] The RS domain is involved in protein-protein interactions and RNA processing.[11–14] Interestingly, the c.5528C>A substitution affects the serine at amino acid 1,843 within the RS region (Figure 2), thus altering the composition of a crucial functional domain of SON.

Previous analyses of murine and human cells have shown nuclear staining of SON in a stippled pattern consistent with localization to the nuclear speckle.[9,10,15,16] The nuclear speckle is a subcellular intranuclear compartment that is enriched with pre-mRNA splicing factors, including small nuclear ribonucleoprotein particles[17] and SR protein family members known to be involved in RNA splicing.[4] SON's functional domains and its localization in the nuclear speckle suggest that it plays a role in pre-mRNA processing. Functional studies have confirmed that SON is an important mediator of both constitutive and alternative splicing[4,18] and that it is specifically involved in splicing short introns with suboptimal or weak splice sites.[8,19] Known targets of SON-mediated splicing include cell-cycle and microtubule genes, as well as genes involved in DNA repair.[4,19] Indeed, depletion of SON by RNAi leads to an array of adverse cellular consequences, including mitotic arrest, disordered spindle architecture with abnormal chromosomal alignment, aneuploidy in cells that continue to divide,[16,19] and loss of genomic integrity, as evidenced by increased double-stranded DNA breaks and micronuclei formation in cells lacking functional SON.[19] In addition, the regulatory effect of SON on splicing has been shown to be essential for the maintenance of pluripotency and self-renewal in human embryonic stem cells.[8]

In spite of extensive work showing a critical functional role for SON in coordinating splicing and evidence that aberrant splicing contributes to human disease,[20,21] mutations in *SON* have not yet been definitively linked to a phenotype in humans. The first de novo truncating variant in *SON* was identified in a large cohort of individuals with severe intellectual disability.[22] Zhu et al. later described another individual with a de novo truncating variant in *SON*.[23] This individual had developmental delay, epilepsy, minor dysmorphic features, macrocephaly, brain white-matter abnormalities, intestinal atresia, and a ventricular septal defect. However, this individual also had a de novo missense change in a second candidate gene, *C5AR1* (MIM: 113995), confounding the clinical relevance of the *SON* change. This published individual and the seven subjects in our cohort exhibit many of the same features, suggesting that deleterious variants in *SON* cause a consistent phenotype. Moreover, two of our subjects (1 and 5) share the same frameshift variant as the individual described by Zhu et al., indicating that this is a recurrent pathogenic change.

Variants in genes encoding other components of the spliceosomal machinery have been implicated in several developmental disorders, including Guion-Almeida type mandibulofacial dysostosis (MFDGA [MIM: 610536]) and Nager syndrome (MIM: 154400), among others (recently

Table 2. Putative Pathogenic Variants in *SON*

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7[a]	Subject 7[a]
DNA variant	c.5753_5756delTTAG	c.6233delC	c.3852_3856delGGTAT	c.286C>T	c.5753_5756delTTAG	c.3073dupA	c.4909A>T	c.5528C>A
Protein change	p.Val1918Glufs*87	p.Pro2078Hisfs*4	p.Met1284Ilefs*2	p.Gln96*	p.Val1918Glufs*87	p.Met1025Asnfs*6	p.Thr1637Ser	p.Ser1843Tyr
Inheritance	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo
ExAC Browser	novel	novel	novel	novel	novel	novel	novel	novel
SIFT	–	–	–	–	–	–	deleterious	deleterious
PolyPhen-2	–	–	–	–	–	–	benign	damaging
CADD	–	–	–	–	–	–	15.19	15.67

Variant nomenclature is based on GenBank: NM_138927.2.
[a]These variants are in *cis* configuration, and both are confirmed de novo changes.

reviewed by Lehalle et al.[24]). MFDGA is caused by mutations in *EFTUD2* (MIM: 603892), which encodes a highly conserved spliceosomal GTPase.[24] The phenotype associated with MFDGA mirrors both in breadth and severity the features common to our cohort, including psychomotor delay, growth retardation, musculoskeletal anomalies, and cardiac, brain, and visceral malformations.[24] Nager syndrome, which is caused by mutations in *SF3B4* (MIM: 605593), is characterized by midface retrusion, downslanting palpebral fissures, and thumb anomalies.[25] All of these features were present in one or more of our subjects with *SON* variants. This phenotypic overlap with established spliceosomal disorders confers plausibility to the hypothesis that defects in *SON* cause the features seen in our cohort.

Orthogonal evidence of the potential clinical relevance of *SON* haploinsufficiency derives from reports of individuals with copy-number variants (CNVs) involving this gene. Non-recurrent microdeletions encompassing 21q22.11, the locus harboring *SON*, have been extensively described in the literature.[26–36] Roberson et al. performed genotype-phenotype correlations for 46 individuals with partial 21q monosomy and, consistent with other reports,[27,36] found that individuals with deletions encompassing the 21q22.11 locus manifest severe phenotypes.[35] Lindstrand et al. compared 26 individuals who had partial 21q monosomy and for whom reliable molecular data were available.[34] Alignment of the deleted regions and comparison of phenotypes showed a narrow 159 kb region of overlap in 21q22.11 among individuals with

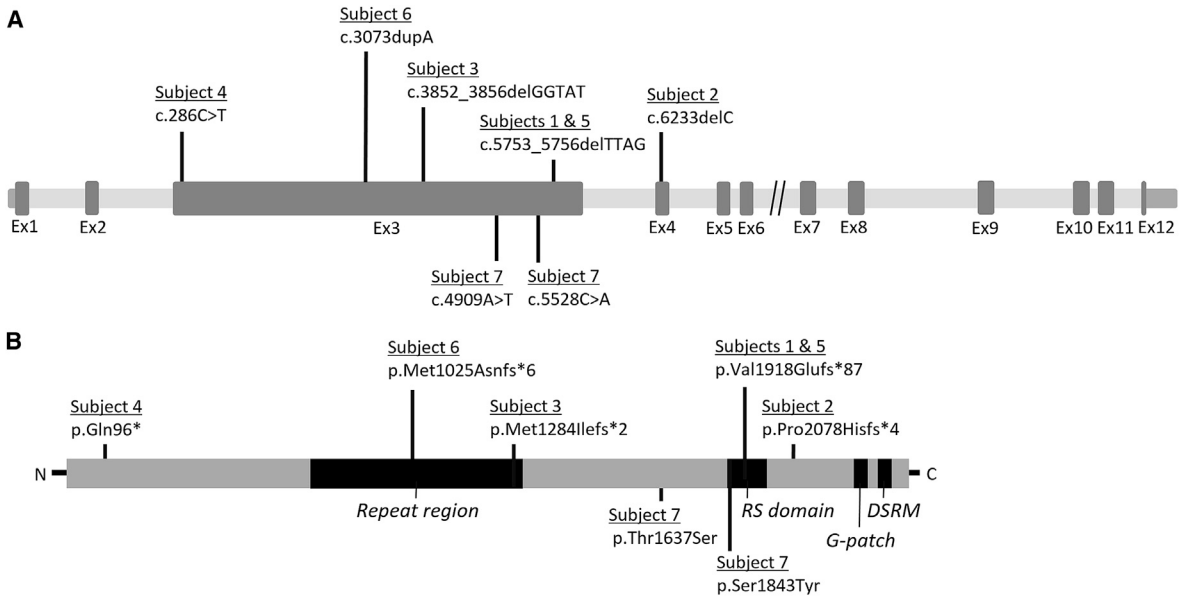

Figure 2. Intragenic Location of *SON* Variants and Key Protein Functional Domains
(A) All but one of the *SON* variants in the described individuals localize to exon 3 of *SON* (GenBank: NM_138927.2).
(B) Approximate location of amino acid changes in relation to SON's key functional domains, which include a unique central highly repetitive region, an RS-rich domain, a G-patch domain, and a double-stranded RNA-binding motif (DSRM). Data were extracted from GenBank: NP_620305.2. This panel was adapted from Hickey et al.[4]

Table 3. *SON*-Associated Clinical Features in Reported Subjects with Deletions Encompassing *SON*

	Subjects 1–7	Izumi et al.[26]	Fukai et al.[27]	Beri-Dexheimer et al.[28] (Patient 2)	Hoyer et al.[29]	Shinawi et al.[30] (Patient 2)	Thevenon et al.[31]	Carrascosa-Romero et al.[32]	Katzaki et al.[33] (Patient 2)	dbVar: nssv577822	Subject 8
Deletion (Mb)	NA	1.9	1.4	3.3	3	1.81	2.97	2.84	2.9	0.341	0.825
Developmental delay	+ (7/7)	NR	+	+	+	+	+	+	+	+	+
IUGR and/or low birth weight	+ (5/6)	+	–	+	NR	+	+	+	+	NR	NR
Short stature	+ (5/7)	+	+	+	+	+	+	+	+	+	+
Respiratory problems	+ (5/7)	+	NR	NR	NR	NR	NR	+	NR	NR	NR
Feeding problems	+ (7/7)	+ (G-tube)	NR	NR	NR	+ (G-tube)	+	+ (G-tube)	NR	NR	–
Seizures	+ (2/6)	NR	+	–	–	–	+	–	+	+	+
Abnormal tone	+ (5/6)	+	+	+	NR	NR	+	+	NR	NR	–
Brain anomalies	+ (5/6)	+	+	+	NR	+	+	+	+	NR	–
Congenital malformations	+ (5/5)	heart	heart	heart	–	NR	heart	renal	–	NR	heart

Abbreviations are as follows: G-tube, gastrostomy tube; NA, not applicable; and NR, not reported.

intellectual disability.[34] This region contains only five genes and encompasses the entirety of all isoforms of *SON*, suggesting that loss of *SON* might contribute specifically to the intellectual disability in these individuals.[34]

To further explore the potential implications of *SON* copy-number loss, we queried our internal clinical database of chromosomal microarrays (n = ~70,000 affected individuals) and identified an individual (subject 8; Table 3) with a ~825 kb deletion encompassing *SON* and ten additional RefSeq genes. This individual was reported to have global developmental delay, seizures, and a congenital heart defect—features also seen in the described subjects with *SON* sequence variants. We then selectively reviewed published reports of phenotypically characterized individuals with <5 Mb 21q22.11 deletions that partially or completely involve *SON* (Figure S2)[26–33] and found substantial phenotypic overlap between individuals with deletions encompassing *SON* and the seven subjects with *SON* variants reported herein (Table 3). The individuals with microdeletions included both male and female probands, which is notable given the clear predominance of female subjects in our cohort. Seven of eight individuals with deletions of *SON* had developmental delay; all eight individuals had growth failure with short stature, seven of eight had brain anomalies, and six of eight had a history of intrauterine growth restriction and/or low birth weight. Four individuals were reported to have feeding difficulties, which required G-tube placement in three. Table 3 also includes a single individual with a small 341 kb de novo deletion reported in ClinVar (ClinVar: SCV000080160.5; dbVar: nssv577822). This individual is reported to have global developmental delay, seizures, and short stature—all features seen in our subjects with *SON* variants. Thus, although we cannot exclude the possibility that other genes in this region (e.g., *GART* [MIM: 138440], *DONSON* [MIM: 611428], *CRYZL1* [MIM: 603920], and *ITSN1* [MIM: 602442]) contribute to the phenotype in individuals with large deletions, the existing CNV data on this well-studied region strengthens the supposition that *SON* haploinsufficiency is in fact pathogenic.

In summary, we have characterized a clinical phenotype associated with pathogenic variants involving *SON*. The similarity in phenotype between subjects with truncating variants and those with CNVs suggests that haploinsufficiency of *SON* could be the underlying disease mechanism. Although additional studies will be necessary to confirm the functional relevance of heterozygous loss of *SON* and to capture the full phenotypic spectrum, the available human data compellingly support the assertion that deleterious variants in *SON* are associated with a severe human phenotype.

Accession Numbers

The accession numbers for the truncating variants reported in this paper are ClinVar: SCV000297718, SCV000297719, SCV000297720, SCV000297721, and SCV000297722.

Supplemental Data

Supplemental Data include a detailed clinical history of each reported subject and two figures and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2016.06.035.

Conflicts of Interest

The Department of Molecular and Human Genetics at the Baylor College of Medicine derives revenue from molecular genetic testing offered at the Baylor Miraca Genetics Laboratories.

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Web Resources

ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/
ExAC Browser, http://exac.broadinstitute.org/
OMIM, http://www.omim.org/
RefSeq, http://www.ncbi.nlm.nih.gov/RefSeq
UCSC Genome Browser, https://genome.ucsc.edu/

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