Congenital lethal motor neuron disease with a novel defect in ribosome biogenesis

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ABSTRACT

Objective: We describe a novel congenital motor neuron disease with early demise due to respiratory insufficiency with clinical overlap with spinal muscular atrophy with respiratory distress (SMARD) type 1 but lacking a mutation in the *IGHMBP2* gene.

Methods: Exome sequencing was used to identify a de novo mutation in the *LAS1L* gene in the proband. Pathogenicity of the mutation was validated using a zebrafish model by morpholino-mediated knockdown of *las1l*.

Results: We identified a de novo mutation in the X-linked *LAS1L* gene in the proband (p.S477N). The mutation is in a highly conserved region of the *LAS1L* gene predicted to be deleterious by bioinformatic analysis. Morpholino-based knockdown of *las1l*, the orthologous gene in zebrafish, results in early lethality and disruption of muscle and peripheral nerve architecture. Coinjection of wild-type but not mutant human RNA results in partial rescue of the phenotype.

Conclusion: We report a patient with a SMARD phenotype due to a mutation in *LAS1L*, a gene important in coordinating processing of the 45S pre-rRNA and maturation of the large 60S ribosomal subunit. Similarly, the *IGHMB2* gene associated with SMARD type 1 has been suggested to have an important role in ribosomal biogenesis from its role in processing the 45S pre-rRNA. We propose that disruption of ribosomal maturation may be a common pathogenic mechanism linking SMARD phenotypes caused by both *IGHMBP2* and *LAS1L*. **Neurology®**

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GLOSSARY

cDNA = complementary DNA; **DSHB** = Developmental Studies Hybridoma Bank; **hpf** = hours postfertilization; **LAS1L** = LAS1-like (*S cerevisiae*); **NHLBI** = NIH Heart, Lung, and Blood Institute; **OMIM** = Online Mendelian Inheritance in Man; **PBS** = phosphate-buffered saline; **rRNA** = ribosomal RNA; **SMARD** = spinal muscular atrophy with respiratory distress; **SNP** = single nucleotide polymorphism; **UTR** = untranslated region.

Spinal muscular atrophy with respiratory distress (SMARD) is a rare autosomal recessive disorder of neonatal weakness and early respiratory failure (Online Mendelian Inheritance in Man [OMIM] #604320).¹ SMARD was first described in 1974 as a variant of Werdnig-Hoffmann disease (spinal muscular atrophy type I) but is distinguished by the prominence of early respiratory failure and distal muscle weakness or joint contractures.² Since discovery of the *IGHMBP2* gene as a cause for SMARD,³ appreciation of the clinical and genetic heterogeneity has been increasing.^{2,4,5} *IGHMBP2* is a ubiquitously expressed helicase that colocalizes with factors controlling RNA splicing in the cytosol and nucleus.⁶ A role for *IGHMBP2* in translation has been proposed based on colocalization in the cytoplasm with ribosomal proteins and ribosomal RNA (rRNA).^{6,7} As in many other disorders with motor neuron involvement, it is unclear why mutations in *IGHMBP2* have a disproportionate effect on motor neurons.⁸

Infants presenting with a SMARD phenotype but lacking mutations in *IGHMBP2* are common, accounting for up to two-thirds of reported patients.^{4,9} We describe an infant who presented with distal weakness and primary respiratory failure associated with diaphragm paralysis but lacking a

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mutation in the *IGHMBP2* gene. We identified a de novo mutation in the *LAS1*-like (*S cerevisiae*) gene (*LASIL*) by diagnostic exome sequencing. *LASIL* is an X-linked gene that has a critical role in ribosomal biogenesis.¹⁰ We provide evidence of pathogenicity using a zebrafish (*Danio rerio*) model and propose that disruption of ribosomal maturation may be a common pathogenic mechanism linking SMARD phenotypes caused by mutations in both *IGHMBP2* and *LASIL*.

METHODS **Patients.** The family presented here includes the male proband, his 2 unaffected brothers, and unrelated parents. Eleven male patients from the University of Utah Pediatric Motor Disorders Research Program with severe neonatal-onset motor neuron disease and normal *SMN1* deletion testing were screened for mutations in *LASIL*. In addition, we screened 103 deidentified samples from male *SMN1* deletion-negative infants younger than 7 months from the Ohio State Molecular Diagnostic Laboratory for mutations in *LASIL* exon 11.

Standard protocol approvals, registrations, and patient consents. Written informed consent or parental consent for the proband and family members and the 11 Utah patients screened for *LASIL* was obtained under an approved protocol of the University of Utah Institutional Review Board. Consent for deidentified samples from 103 patients from the Ohio State Molecular Diagnostic Laboratory was obtained under a separate Institutional Review Board-approved protocol.

Exome sequencing and variant analysis. DNA from the proband and his parents was referred for diagnostic exome sequencing to Ambry Genetics (Aliso Viejo, CA). Genomic DNA was prepared for whole exome sequencing using the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA) according to the manufacturer's protocol.¹¹ Briefly, each DNA sample was sheared, blunt-end repaired, and tagged using indexed adapters. Coding exons and flanking intronic sequences were enriched using solution-based hybridization with oligonucleotide probes. The enriched exome libraries were then applied to the solid surface flow cell for clonal amplification and sequencing using paired-end, 100-cycle chemistry on the Illumina HiSeq 2000 (Illumina, San Diego, CA).

Initial data processing and base calling, including extraction of cluster intensities, was done using RTA 1.12.4 (HiSeq Control Software 1.4.5). Sequence quality filtering was executed with the Illumina CASAVA software (version 1.8.2; Illumina, Hayward, CA). Sequence fragments were aligned to the reference human genome (GRCh37) and variant calls were generated using CASAVA. Exons plus at least 2 bases into the 5' and 3' ends of all the introns were analyzed. Analysis of variants focused on nonsense variants, small insertions and deletions, canonical splice variants, and nonsynonymous missense variants. Online databases, including the Human Gene Mutation Database,¹² dbSNP (Single Nucleotide Polymorphism Database),¹³ 1000 Genomes,¹⁴ HapMap,¹⁵ and online search engines (e.g., PubMed, OMIM), were used to search for known mutations and polymorphisms. Filtering included the sequential removal of common single nucleotide polymorphisms (SNPs), 3'/5' UTR (untranslated region) variants, nonsplice-related intronic variants, and synonymous variants. Annotated variants associated with known disorders in the Human Gene

Mutation Database or OMIM database were protected in the filtering pipeline. Remaining variants were filtered based on family history and inheritance models. Variants were annotated with the Ambry Variant Analyzer to assess nucleotide and amino acid conservation (NIH Heart, Lung, and Blood Institute [NHLBI] Exome Sequencing Project and 1000 Genomes databases),^{16–20} and predicted functional impact (PolyPhen²¹ and SIFT^{22,23}). Assemblies and sequence alignments were viewed using IGV (Integrative Genomics Viewer software).²⁴ Candidate variants were confirmed using automated fluorescence dideoxy sequencing. Cosegregation analysis was performed using the trio and the 2 unaffected brothers (figure e-1A on the *Neurology*[®] Web site at Neurology.org). Amplification primers were designed using PrimerZ.²⁵ PCR primers were tagged with established sequencing primers on the 5' end. Sequencing was performed on an ABI3730 (Life Technologies, Carlsbad, CA) using standard procedures.

***LASIL* sequencing in the screening cohort.** Coding regions from genomic DNA from 11 male subjects from the University of Utah with neonatal onset of a severe motor neuron disorder and normal *SMN1* deletion testing were sequenced for all 14 exons of *LASIL* by Sanger sequencing. Primer sequences for amplification of genomic DNA are included in table e-1. Sequencing was performed using ABI Prism BigDye Terminators and cycle sequencing with Taq FS DNA Polymerase (PE Applied Biosystems Division, Foster City, CA). DNA sequence was collected and analyzed on an ABI 3730xl capillary sequencer (PE Applied Biosystems Division) according to standard methods. Sequence comparisons between the patients and the reference sequence (NM_031206.4) were made using Sequencher software (GeneCodes Corp., Ann Arbor, MI). DNA from an additional 103 deidentified male subjects was sequenced for exon 11 of the *LASIL* gene at the Ohio State Molecular Diagnostic Laboratory (table e-1).

Validation of pathogenicity of *las1l* mutation in zebrafish. All zebrafish experiments were performed in strict accordance of guidelines from the University of Utah Institutional Animal Care and Use Committee, regulated under federal law (the Animal Welfare Act and Public Health Services Regulation Act) by the US Department of Agriculture and the Office of Laboratory Animal Welfare at the NIH, and accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Adult fish were bred according to standard methods. Embryos were raised at 28.5°C in E3 embryo medium and staged by time and morphology.²⁶

Morpholino and RNA injections. One-cell stage embryos were injected with translation-blocking or splice-blocking morpholinos (GeneTools, LLC, Philomath, OR) as previously described.²⁷ Morpholinos were targeted against zebrafish *las1l*, *LOC561321* (NM_001126400.1). Sequence of the translation-blocking morpholino was 5'-CCCCACCGTTTCTTCTTCATCTTC-3'; sequence of the splice-blocking morpholino against exon 10 was 5'-CTGCCACACAATGACAGAGAGGGCT-3'. For rescue experiments, wild-type embryos were injected at the one-cell stage with 2 ng splice-blocking morpholino, 2 ng splice-blocking morpholino and 5 pg wild-type human *LASIL* RNA (NM_031206.4), 2 ng splice-blocking morpholino and 5 pg mutant human *LASIL* RNA (c.1430G>A), or 5 pg wild-type human *LASIL* RNA. More than 50 embryos were scored for each condition at 24 hours postfertilization (hpf).

In situ hybridization and immunohistochemistry. Whole-mount immunohistochemistry was performed as described.²⁸ Embryos at 24 hpf were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) overnight at 4°C, washed briefly in PBS, dehydrated stepwise from PBS to 100%

Table 1 Variant filtering based on inheritance model, clinical, and bioinformatics analysis

	Model filtering	Manual filtering ^a	Notable candidate genes ^b
Autosomal dominant	30 (31) ^c	6 (6)	0 (0)
Autosomal recessive	14 (39)	3 (5)	0 (0)
X-linked recessive	3 (3)	2 (2)	1 (1)
X-linked dominant	1 (1)	1 (1)	1 (1)
Y-linked	0 (0)	0 (0)	0 (0)
Total	48 (74)	12 (14)	2 (2)

^aManual filtering includes the removal of variants in genes with characterized phenotypes unrelated to the patient's evaluated phenotype and alterations considered benign by bioinformatics analysis.

^bNotable candidate gene: variants selected for cosegregation analysis.

^cNumbers indicate the number of genes with variant passing that level of filtering. Number in parentheses is the total number of variants passing filtering.

MeOH, and stored in 100% MeOH at -20°C . Antibodies and dilutions included goat anti-mouse Alexa 488 1:500 (Invitrogen, Carlsbad, CA); mouse anti-SV2 1:300 (Developmental Studies Hybridoma Bank [DSHB]); mouse f59 1:5 (DSHB); mouse f310 1:20 (DSHB); and Cy-3 anti-rabbit 1:400. Apoptotic cells were stained using TUNEL (Millipore, Billerica, MA) as described.¹⁹ Image acquisition and analysis were performed as described previously.²⁹ Embryos were processed and placed in a solution of 80% glycerol/20% PBST, and mounted on a glass slide with a #0 coverslip. NIH ImageJ software was used to merge slices to create maximal intensity z-stack projections.

***las1l* reverse transcription PCR.** Total RNA from 24 hpf embryos was extracted using Trizol (Invitrogen), purified (Qiagen RNeasy mini-columns), and reverse-transcribed (SuperScript III First-Strand Invitrogen kit). Primers for *las1l* were used to amplify the region spanning exon 10; forward primer in exon 9: 5'-TACATCGCCTCCTGGATCTC-3'; reverse primer in exon 13: 5'-TCAGATCCACTGGCTCCTCT-3'.

***LASIL* site-directed mutagenesis.** *LASIL* human complementary DNA (cDNA) in pDNR-LIB was obtained from Open Biosystems (clone ID 4079376). The QuikChangeII site-directed mutagenesis kit (Agilent) was used to change the cDNA position 1430 in from G to A, using forward primer 5'-CTCACCTTGCTGGGCCAACCCCAACT-3' and reverse primer 5'-AGTTGGGGTTGGCCAGCAAGGTGAG-3'. The resultant clone was Sanger-sequenced in both directions to validate the sequence change.

Electronic-database information. Accession numbers and URLs for data in this article are as follows:

- ESP: Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (<http://evs.gs.washington.edu/EVS/>) (accessed March 2013).
- OMIM: McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD (<http://omim.org/>) (accessed March 2013).
- RefSeq: The NCBI Handbook. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; 2002 Oct. Chapter 18, The Reference Sequence (RefSeq) Project (see ref guidelines: <http://www.ncbi.nlm.nih.gov/books/NBK50679/>).

RESULTS Clinical. We evaluated the proband in the newborn intensive care unit at 34 days of age after

several unsuccessful attempts to extubate him. He was born at 38^{6/7} weeks by elective cesarean delivery due to Coombs positivity for anti-C and anti-E antibodies. Shortly after birth, he developed respiratory distress and was intubated. He was extubated on day of life 6 to high-flow nasal cannula and remained successfully extubated until day of life 19 when he developed recurrent apnea and bradycardia episodes. Over the next 3 weeks, repeated attempts to extubate him proved unsuccessful. He was moderately hypotonic with normal muscle bulk and spontaneous antigravity movements of all limbs. He had mild flexion contractures of the toes and modestly reduced finger extension. Tongue fasciculations were present. He had no dysmorphic features. When positive pressure ventilation was reduced, he demonstrated a breathing pattern consistent with diaphragm weakness. Deep tendon reflexes were preserved. Creatinine kinase level was <20 U/L. Nerve conduction studies revealed normal median and ulnar sensory responses. Compound muscle action potentials for ulnar (0.9 mV, normal >4.0 mV) and tibial (0.1 mV, normal >5.0 mV) motor responses were severely diminished, with the legs more severely affected. EMG revealed reduced recruitment of motor units in intrinsic muscles of hands and feet consistent with chronic distally predominant denervation. Weakness in the diaphragm and the limbs progressed over the next 3 weeks. Fluoroscopy confirmed paralysis of the diaphragm. Parents elected to withdraw support after they were counseled regarding the poor prognosis for successful extubation. Overall, the clinical picture was characteristic of a SMARD-like motor neuronopathy. Brain MRI, EEG, and routine CSF studies were unremarkable, as were serum lactate, urine organic acids, and plasma amino acids and acylcarnitines, suggesting that mitochondrial or metabolic disorders were unlikely. Initial genetic

Figure 1 Morpholino-mediated knockdown of *las1l* in zebrafish results in dysmorphic embryos with early lethality

(A) Exon structure of zebrafish *las1l* gene; arrow indicates the orthologous position to the p.S477N seen in the proband; blue and green boxes indicate locations of translation-blocking (TL) and splice-blocking (spl) morpholino. (B) Injection of 2 ng of TL or spl morpholino results in significant early lethality of embryos at 24 hours postfertilization (hpf). (C) Bright-field microscopy of spl morphant compared with wild-type (wt) embryo at 24 hpf shows dysmorphic phenotype including small malformed brain and shortened, necrotic tail (arrowheads). (D) Reverse transcription (RT)-PCR demonstrates that injection of 2 ng of spl morpholino at the one-cell stage leads to loss of normal transcript at 24 hpf (arrow). (E) Spl morpholino concentrations of ≥ 2 ng result in $>75\%$ dysmorphic embryos at 24 hpf.

testing included normal findings on *SMN1* deletion testing and cytogenomic SNP array (Cytogenetics Whole-Genome 2.7M Array; Affymetrix, Santa Clara, CA). Sanger sequencing excluded pathogenic mutations in *IGHMBP2* (SMARD type 1), *PHOX2B*, *MPZ*, *PMP22*, *EGR2*, and *PRX*. Tissue was not made available for muscle or nerve biopsy.

Exome sequencing. Exome sequencing of the family trio (proband, mother, and father) was performed at Ambry Genetics and resulted in an average of 24 Gb of sequence per sample (figure e-1A, table e-2). Mean coverage of captured regions was $81\times$ per sample, with 89% covered with at least $10\times$ coverage, and an average of 87% base call quality of Q30 or

Figure 2 Splice-blocking knockdown of *las1l* in zebrafish causes abnormal axon and muscle development

(A–H) Whole-mount immunohistochemistry of wild-type (wt) compared with morphant 24-hour postfertilization embryos; scale bar 50 μ m; boxed regions are shown in higher-magnification insets. Confocal microscope maximal-intensity z-projections. (A, B) SV2 antibody labels neurons, axons, and dendrites (green) and reveals gross defects in morphology of primary motor neurons (arrows) and horizontal myoseptum (arrowheads). (C, D) Embryos stained with f310 antibody against myosin light chain (fast-twitch myofibers) show abnormal shortened and nonparallel muscle fibers. (E, F) f59 antibody staining myosin heavy chain (slow-twitch myofibers) reveals shortened and disrupted muscle architecture. (G, H) TUNEL staining displays marked increase in apoptosis in morphants. TUNEL = terminal deoxynucleotidyl transferase dUTP nick-end labeling.

greater. Stepwise filtering to remove common SNPs, intergenic and 3'/5' UTR variants, nonsplice-related intronic variants, and synonymous variants resulted in approximately 12,000 variants per sample (table e-2). Further filtering based on family history and inheritance models for autosomal dominant and recessive, X-linked dominant and recessive, and Y-linked inheritance resulted in 74 variants in 48 different genes (table 1). Manual review of each of these variants to rule out sequencing artifacts and polymorphisms along with medical interpretation to rule out genes lacking clinical overlap with the patient's phenotype resulted in 13 candidate variants in 11 genes (table e-3).

Variants in 2 X-linked genes, *LAS1L* (NM_031206.4; c.1430G>A, p.S477N) and *NAPIL3* (NM_004538.5; c.926A>G, p.K309R), were notable for potential clinical relevance and predicted pathogenicity. The mother was heterozygous for the variant in *NAPIL3* while the proband and both unaffected brothers were hemizygous, eliminating this variant by failure to cosegregate. This variant has been reported in dbSNP135 (rs146057748) with allele frequency of 0.003. The variant in *LAS1L* was not seen in the unaffected parents or siblings. In support of pathogenicity, this allele was not observed in 6,501 alleles studied in the NHLBI Exome Sequencing Project database and has not been reported in public databases. Furthermore, this position is conserved among vertebrate species (figure e-1, B and C). Bioinformatic analyses including SIFT²³ and PolyPhen-2²¹ both predict that this substitution is pathogenic.

Sanger-sequencing confirmation in the proband and screening of similarly affected infants. We screened 11 patients with early-onset severe motor neuronopathy phenotypes and normal *SMN1* testing. Sequencing of the 14 exons of *LAS1L* revealed no variants from the reference sequence. Further, 103 deidentified male infants with presumed motor neuron disease were screened for mutations in exon 11 of *LAS1L*. All patients were negative for the c.1430G>A, p.S477N or other variants in exon 11. Unfortunately, these samples were not made available for additional analysis outside exon 11.

Zebrafish knockdown of *las1l*. We used a zebrafish model to rapidly test the pathogenicity of the *LAS1L* p.S477N substitution identified in the proband. *LOC561321 (las1l)* on zebrafish chromosome 5 is orthologous to human *LAS1L*. A threonine residue at position 386 in exon 10 of the zebrafish protein (NP_001119872.1) is conserved with the serine residue at position 477 in the human *LAS1L* (NP_112483.1; figure e-1C). To demonstrate the importance of *Las1l* in development of nerve and muscle in the zebrafish, we used a morpholino targeting the

Figure 3 Dysmorphic features induced by *las1l* splice-blocking morpholino are rescued by injection of wild-type human *LAS1L* RNA but not by c.1430G>A mutant RNA

(A) Percent dysmorphic embryos at 24 hours postfertilization (hpf) after injection of splice-blocking morpholino with either wild-type (wt) human RNA or c.1430G>A mutant RNA ($n > 50$ embryos for each condition). Standard error of the proportion is shown (* p value < 0.001 compared with wt; ** p value < 0.001 compared with morpholino alone). (B) Whole-mount bright-field microscopy shows that wt but not mutant human *LAS1L* RNA can partially rescue the morpholino-associated morphologic phenotype.

las1l translation initiation site to block translation of the *las1l* transcript. To demonstrate the importance of the region of the gene orthologous to the p.S477N substitution, we used a splice-blocking morpholino to induce skipping of exon 10. Knockdown of *Las1l* by both translation-blocking or by splice-blocking leads to early lethality and increased apoptosis in the brain, spinal cord, and body musculature (figure 1, A–C). A 2-ng dose of the splice-blocking morpholino led to skipping of exon 10, which contains the critical T386 residue orthologous to the p.S477N mutation seen in

the proband (figure 1, D and E). Morphologically, embryos treated with the splice-blocking morpholino showed disrupted architecture of muscle and peripheral nerve (figure 2, A–F). Both fast and slow twitch muscle fibers were affected (figure 2, D and F). Significantly elevated levels of apoptosis were noted in the morphant embryos (figure 2, G and H), consistent with previous reports that *LAS1L* is necessary for cell-cycle progression.³⁰

To demonstrate sufficiency of human *LAS1L* RNA to rescue the phenotype induced by splice-blocking of exon 10 in the zebrafish and to demonstrate pathogenicity of the p.S477N substitution, we performed rescue experiments. First, we injected full-length human *LAS1L* RNA into zebrafish embryos after first injecting the splice-blocking morpholino to eliminate the native protein. Because the human RNA and zebrafish sequences lack primary sequence conservation, the splice-blocking morpholino had no activity against the human RNA. Second, we injected full-length human RNA carrying the mutation seen in the proband (c.1430G>A) into zebrafish embryos injected with the splice-blocking morpholino. Injection of wild-type but not mutant *LAS1L* RNA partially rescued the dysmorphic effects of the splice-blocking morpholino (figure 3). We conclude that the human p.S477N mutation seen in the proband leads to impaired function or loss of function of *LAS1L*.

DISCUSSION We describe a congenital progressive motor neuron disease associated with a mutation in *LAS1L*, a key component of the ribosome biogenesis pathway. The phenotype overlaps considerably with SMARD type 1, caused by mutations in the *IGHMPB2* gene, and includes distal limb contractures, axial hypotonia with preserved antigravity limb movements, and progressive respiratory failure. Neurophysiologic studies demonstrate a length-dependent motor neuronopathy. Diagnostic exome sequencing identified a de novo mutation in *LAS1L* (c.1430G>A, p.S477N), an X-linked gene important in ribosomal biogenesis. The p.S477N substitution identified in the proband is outside known functional domains, but is highly conserved in vertebrate species. Pathogenicity of this allele is supported by its absence in control populations, high level of conservation, and SIFT and PolyPhen-2 analyses that predict pathogenicity. The early lethality and disruption of muscle and peripheral nerve architecture in zebrafish with morpholino knockdown strategies targeting *las1l* and rescue with human wild-type RNA but not mutant (c.1430G>A) RNA further support its pathogenicity.

To date, mutations in *LAS1L* have not been reported with any human phenotype; however, the ortholog in *Saccharomyces cerevisiae*, *LAS1* is essential

Figure 4 Effects of *IGHMBP2* and *LAS1L* on rRNA processing in ribosomal biogenesis

for cell viability and proliferation.³¹ Depletion of *Las1* results in failure of budding and cell-cycle arrest. Recent studies have demonstrated an important role for *LAS1L* in ribosomal biogenesis.^{10,30} Ribosomal biogenesis is a complex process coordinating production of 4 ribosomal RNAs from 2 different RNA templates, 80 core proteins, and numerous associated proteins (figure 4).¹⁶ Loss of *LAS1* in *S cerevisiae* interrupts the production of the large, 60S ribosomal subunit by disruption of the processing of the 47S pre-rRNA at the second intervening sequence (ITS2), which separates the 5.8S and 28S rRNA sequences.^{30,32} Processing of the 28S rRNA is dependent on the five friends of methylated Chtop (5FMC) complex, which includes *LAS1L*, *SENP3*, *WDR18*, *TEX10*, and *PELP1*. Collectively, this complex coordinates the rate of formation of the large ribosomal subunit and controls its distribution from the nucleolus.^{33,34} It is unclear how a deficiency in *LAS1L* results in specific damage to motor neurons.

Similar to *LAS1L*, recent studies on the function of *IGHMBP2* have suggested a role for *IGHMBP2* in ribosomal biogenesis and translation. A close physical association of the *IGHMBP2* protein with ribosomes in the cytoplasm suggests a link with the translation machinery.³⁵ Furthermore, a role in ribosomal biogenesis is suggested by a close physical interaction in the nucleolus between *IGHMBP2*, *ABT1*, and the

U3 snoRNP, which direct the processing of the 5' external transcribed sequence of the 45S pre-rRNA.^{7,36} Of particular interest is the finding that the *Abt1* gene in mice acts as a modifier of severity of the *nmd* mouse, a model for SMARD that harbors a mutation in *Ighmbp2*.⁷

While direct interactions between *LAS1L* and *IGHMBP2* have not been demonstrated, both have roles in ribosomal biogenesis via processing of the 45S pre-rRNA. The convergence of mechanisms associated with ribosome biogenesis from *IGHMBP2* and *LAS1L*, both resulting in SMARD phenotypes, suggests the importance of this pathway in motor neuron development and/or in the maintenance of distal connectivity once initially established. Other genes important in ribosomal biogenesis may be candidates for mutation screening in patients with SMARD phenotypes that lack mutations in *LAS1L* or *IGHMBP2*. While mutations in *LAS1L* appear to be a rare cause of the SMARD phenotype, identification of additional patients with SMARD or other motor neuron disease phenotypes with mutations in *LAS1L* may provide insight into the vulnerability of motor neurons to defects in the ribosomal biogenesis pathway.

AUTHOR CONTRIBUTIONS

Russell Butterfield, MD, PhD: drafting/revising the manuscript for content, analysis or interpretation of data, acquisition of data, obtaining funding. Tamara J. Stevenson, BA, and Lingyan Xing, BS: analysis or

interpretation of data, acquisition of data. Tara M. Newcomb, MS, LCGC: drafting/revising the manuscript for content, acquisition of data, study coordination. Benjamin Nelson, BS, and Wenqi Zeng, PhD: analysis or interpretation of data. Xiang Li, PhD, Hsiao-Mei Lu, PhD, and Hong Lu, PhD: drafting/revising the manuscript for content, analysis or interpretation of data. Kelly D. Farwell Gonzalez, MS, CGC: drafting/revising the manuscript for content, analysis or interpretation of data, acquisition of data. Jia-Perng Wei, MD, PhD, and Elizabeth C. Chao, MD: drafting/revising the manuscript for content, analysis or interpretation of data. Thomas W. Prior, PhD, and Pamela J. Snyder: analysis or interpretation of data, acquisition of data. Joshua L. Bonkowsky, MD, PhD: drafting/revising the manuscript for content, analysis or interpretation of data, acquisition of data, obtaining funding. Kathryn J. Swoboda, MD: drafting/revising the manuscript for content, analysis or interpretation of data, acquisition of data, study supervision, obtaining funding.

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