

Variants in *LSM7* impair LSM complexes assembly, neurodevelopment in zebrafish and may be associated with an ultra-rare neurological disease

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Summary

Leukodystrophies, genetic neurodevelopmental and/or neurodegenerative disorders of cerebral white matter, result from impaired myelin homeostasis and metabolism. Numerous genes have been implicated in these heterogeneous disorders; however, many individuals remain without a molecular diagnosis. Using whole-exome sequencing, biallelic variants in *LSM7* were uncovered in two unrelated individuals, one with a leukodystrophy and the other who died *in utero*. *LSM7* is part of the two principle LSM protein complexes in eukaryotes, namely LSM1-7 and LSM2-8. Here, we investigate the molecular and functional outcomes of these *LSM7* biallelic variants *in vitro* and *in vivo*. Affinity purification-mass spectrometry of the *LSM7* variants showed defects in the assembly of both LSM complexes. *Lsm7* knockdown in zebrafish led to central nervous system defects, including impaired oligodendrocyte development and motor behavior. Our findings demonstrate that variants in *LSM7* cause misassembly of the LSM complexes, impair neurodevelopment of the zebrafish, and may be implicated in human disease. The identification of more affected individuals is needed before the molecular mechanisms of mRNA decay and splicing regulation are added to the categories of biological dysfunctions implicated in leukodystrophies, neurodevelopmental and/or neurodegenerative diseases.

Leukodystrophies encompass a group of rare genetic neurodevelopmental and neurodegenerative disorders affecting cerebral white matter. Recent advances in genetics and neuroimaging have led to the discovery of many subtypes that are clinically and genetically heterogeneous.^{1–3} These diseases can be classified based on specific brain magnetic resonance imaging (MRI) patterns as either hypomyelinating or non-hypomyelinating. Hypomyelinating leukodystrophies are associated with abnormal myelin formation during development, whereas non-hypomyelinating leukodystrophies are associated with abnormal myelin homeostasis.^{4,5} These heterogeneous diseases collectively affect as many as 1 in 7,600 individuals, with a broad range of onset spanning from prenatal life to late adulthood.^{6–8} Although the era of next-generation sequencing (NGS) has facilitated rare disease diagnosis, 20%–30% of individuals with leukodystrophies remain genetically undiagnosed.^{2,9,10} These individuals and their families often undergo extensive diagnostic odysseys, which adversely impacts their wellbeing and healthcare.^{11,12}

Leukodystrophies were initially identified and classified predominantly as disorders of myelin production or turn-

over, for example Pelizaeus-Merzbacher disease (MIM: 312080) and metachromatic leukodystrophy (MIM: 250100).⁸ Recent advances in research have revealed that pathogenic variants in many transcription- and translation-related genes are implicated in white matter pathologies. For example, it is well-established that pathogenic variants in the genes encoding RNA polymerase III subunits (i.e., *POLR3A* [MIM: 614258], *POLR3B* [MIM: 614366], *POLR1C* [MIM: 610060], and *POLR3K* [MIM: 606007]) as well as tRNA synthetase genes (i.e., *EPRS1* [MIM: 138295], *RARS1* [MIM: 107820], *VARS1* [MIM: 192150], etc.) are associated with white matter disorders.^{13–19}

LSM proteins (“like Sm”) are a family of proteins that form multi-subunit complexes that interact directly with RNA.^{20–24} The 8 main LSM proteins (LSM1-8) form two heteroheptameric ring-shaped complexes composed of different subunits. More specifically, the LSM1 through

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LSM7 proteins form the LSM1-7 complex, and the LSM2 through LSM8 proteins form the LSM2-8 complex. The LSM1-7 complex interacts with the mRNA processing and degradation factor protein PATL1, localizing to the cytoplasmic processing bodies (P-bodies), and plays a role in mRNA decay.^{20,21,24} Eukaryotic cytosolic mRNA decay, a well-characterized and highly conserved process, occurs via one of two pathways, both beginning with the shortening of the poly(A) tail and followed by degradation in either the 3′–5′ or 5′–3′ direction.^{21,23,24} Studies in yeast revealed that the LSM1-7 complex likely acts as an enhancer of decapping in the 5′–3′ pathway, as cells with variants in Lsm1p through Lsm7p block mRNA decay at the decapping step.^{21,25,26} Conversely, the LSM2-8 complex is localized to the nucleus, where it interacts with U6 small nuclear RNA (snRNA), which is the catalytic component of the primary spliceosome.^{20,22,23} Splicing is a multi-step reaction involving the interaction and subsequent rearrangements of the 5 primary spliceosomal snRNAs (U1, U2, U4, U5, and U6) and their protein interactors.^{25–29} The LSM2-8 complex acts as a chaperone for U6 snRNA, supporting its rearrangements during splicing and its recycling following the spliceosome reaction.^{30,31} Thus, the LSM1-7 and LSM2-8 complexes play critical roles in the highly regulated cellular mechanisms of mRNA decay and splicing, respectively.²⁰

Here, we report two individuals with biallelic variants in *LSM7* (MIM: 607287), one with a clinical diagnosis of leukodystrophy and the other who died *in utero*. These *LSM7* variants were investigated on a molecular and functional level to evaluate their impact on the LSM complexes. A zebrafish model was also created to study the functional impact of wild-type (WT) and mutant *LSM7* on the development of the central nervous system (CNS).

This project was approved by the Research Institute of the McGill University Health Centre Research Ethics Board (PED-11-105, 2019-4972), and informed consent was obtained from all participants in this study. The first affected individual was referred at the age of 3 years for neurological assessment at the McGill University Health Center. He presented with spastic quadriplegia, white matter anomalies, developmental delay, progressive sensorineural hearing loss, hypospadias, and feeding difficulties. He is currently 7 years old and wheelchair bound. His MRIs showed cerebellar hypoplasia and progressive white matter signal abnormalities between 17 and 35 months, indicative of a leukodystrophy (Figure 1). To determine the genetic cause, massive parallel (NextGen) sequencing on an Illumina system was performed by GeneDx as previously described,³² using DNA isolated from saliva samples of the affected individual and his parents. The quality threshold of the exome sequencing for this individual was 98.4%, with a mean depth of coverage of 88 reads. Analysis was completed using the general assertion criteria for variant classification and is publicly available on the GeneDx ClinVar submission page. A second analysis of the sequence variants was conducted in-house at the Research Institute

of the McGill University Health Centre. Sequence variants were prioritized using the standards and guidelines for interpretation set out by the American College of Medical Genetics (ACMG).³³ These analyses revealed a homozygous variant in *LSM7* (GenBank: NM_016199.2; GRCh37/hg19) at position c.121G>A (p.Asp41Asn) (chr19: 2324172C>T) as a strong candidate for pathogenicity (Figures 2A and 2B). Targeted Sanger sequencing confirmed the presence of the variant as homozygous in the affected individual, with both parents being carriers (Figure 2D). Subsequently, a second individual was found through GeneMatcher.^{34,35} This family was referred for genetic investigations following recurrent fetal deaths. The proband died *in utero* at 32 weeks of gestation and was found to have thick nuchal translucency and encephalocele. Karyotyping was performed in 3 of the pregnancies that ended in fetal death and revealed no chromosomal abnormalities. Duo exome sequencing of the consanguineous parents was conducted as previously described³⁶ and revealed that both parents were carriers of the same variant in *LSM7* at position c.206G>C (p.Arg69Pro) (chr19: 2321785C>G). The parents were not found to both be carriers for any known recessive diseases, and no other gene candidate was identified. Their chromosomal analyses were also normal. The multiple fetal deaths are thereby presumed to be caused by the homozygous variant in *LSM7*. Unfortunately, DNA was not available from the fetuses or the 3 healthy children of this couple for sequencing.

Both *LSM7* variants are present in highly conserved amino acid residues (Figure 2C) and are classified as rare. The p.Asp41Asn variant was observed only 9 times as heterozygous in 232,800 alleles in large population cohorts³⁷ (minor allele frequency 3.87e–5), while the p.Arg69Pro variant has never been reported in large population control databases. *In silico* genetic analysis predict both variants to be deleterious on protein structure and function (p.Asp41Asn: Mutation Taster 0.99, CADD 28.8, Provean –4.61, SIFT 0.001; p.Arg69Pro: Mutation Taster 0.99, CADD 24.9, Provean –7.00, SIFT 0.00) (Table S1).^{38–42}

To examine these variants at a molecular level, we performed RT-qPCR and immunoblot using RNA and protein extracts (n = 6) from fibroblasts of the first affected individual as well as two age- and sex-matched healthy control individuals. RT-qPCR primers were designed and efficiency tested in accordance with the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines.^{43,44} All data were normalized to *SDHA* (MIM: 600857) or *RPL30* (MIM: 180467) using the $\Delta\Delta C_t$ method, which revealed reduced levels of *LSM7* mRNA (p < 0.01) in affected individual 1 compared to control individuals (Figure 3A). Immunoblots were performed on protein extracts using antibodies targeting *LSM7* (Abcam 241656, dilution 1:10,000) and beta-tubulin (Abcam 6046, dilution 1:20,000), followed by incubation with a polyclonal goat anti-rabbit IgG (H+L) (Novus Biologicals, dilution 1:5,000) antibody and visualization using

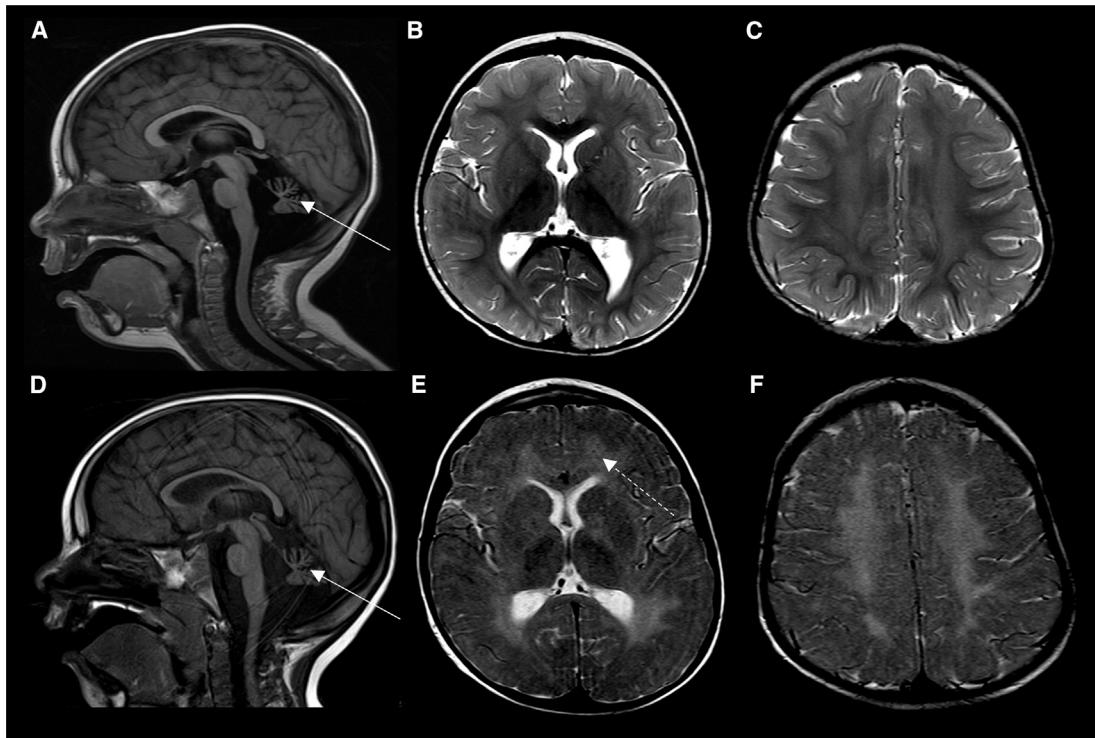


Figure 1. MRI characteristics of individual 1

Brain MRI of individual 1 at 17 months (A–C) and 35 months of age (D–F).

(A) and (D) Sagittal T1-weighted images at the midline showing cerebellar hypoplasia (thin arrows).

(B, C, E, and F) Axial T2-weighted images at the level of the basal ganglia (B and E) and *centrum semiovale* (C and F) showing progressive diffuse demyelination (dotted arrow).

Amersham ECL Western Blot Detecting Reagent according to manufacturer's protocol (GE Life Sciences). Band intensity was quantified using ImageJ software, and statistical significance was determined using a parametric, unpaired t test (two tailed). A decreased amount of LSM7 protein ($p < 0.001$) was seen in the affected individual as compared to control individuals (Figures 3B and 3C). Samples from the fetuses described in the second family (p.Arg69Pro) were not available for analysis.

In order to evaluate the impact of these variants on the formation of the LSM complexes, we generated structural models of wild-type and mutant yeast Lsm1-7 (PDB: 4M75)⁴⁵ and Lsm2-8 complexes (PDB: 4M77)⁴⁵ using PyMOL Molecular Graphics System, version 2.0 Schrödinger (Figures 2E and 2F). All the LSM proteins (LSM1 through LSM8) are highly conserved from yeast to humans. Based on their homology, we mapped the human variants at positions Asp41 and Arg69 to their corresponding yeast residues at Asp56 and Arg87, respectively. Using PyMOL, we modeled the effects of the predicted deleterious variants. The first variant, p.Asp56Asn in yeast, is predicted to result in the loss of polar contacts with other Lsm7 residues in both the Lsm1-7 and Lsm2-8 complexes (Figures S1A–S1D). Meanwhile, the second variant, p.Arg87Pro in yeast, is predicted to result in the loss of polar contacts with Lsm7 residues and a residue on the neighboring Lsm5 protein in both the Lsm1-7 and Lsm2-8 complexes (Figures S1E–

S1H). Based on this modeling, we predicted that each of these substitutions would lead to a compromised ability of Lsm7 to interact with the other Lsm subunits, and consequently, the assembly of the Lsm1-7 and Lsm2-8 complexes would be significantly impaired.

To determine the potential pathogenic role of these variants, we assessed their impact on the assembly of the LSM1-7 and LSM2-8 complexes in human cells. A FLAG-tagged version of the wild-type LSM7, as well as that containing either the p.Asp41Asn or p.Arg69Pro variant, were produced transiently in HEK293 cells. Anti-FLAG affinity purification was conducted on cell extracts according to standard procedures in 4 independent replicate experiments.^{46,47} The protein extracts were digested with trypsin, and the resulting tryptic peptides were purified and identified using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with a high-performance liquid chromatography (HPLC) system coupled to Orbitrap fusion mass spectrometer (Thermo Scientific) through a Nanospray Flex Ion Source. Protein database searching was performed using MaxQuant version 1.6.10.43^{48–53} against the SwissProt human protein database downloaded on April 4, 2019. Known affinity purification mass spectrometry (AP-MS) protein contaminants including keratins were excluded, and protein intensities were analyzed using Perseus version 1.6.10.43.^{54–57} Proteins not present in at least 3 out of 4 replicates were excluded. Wild-type,

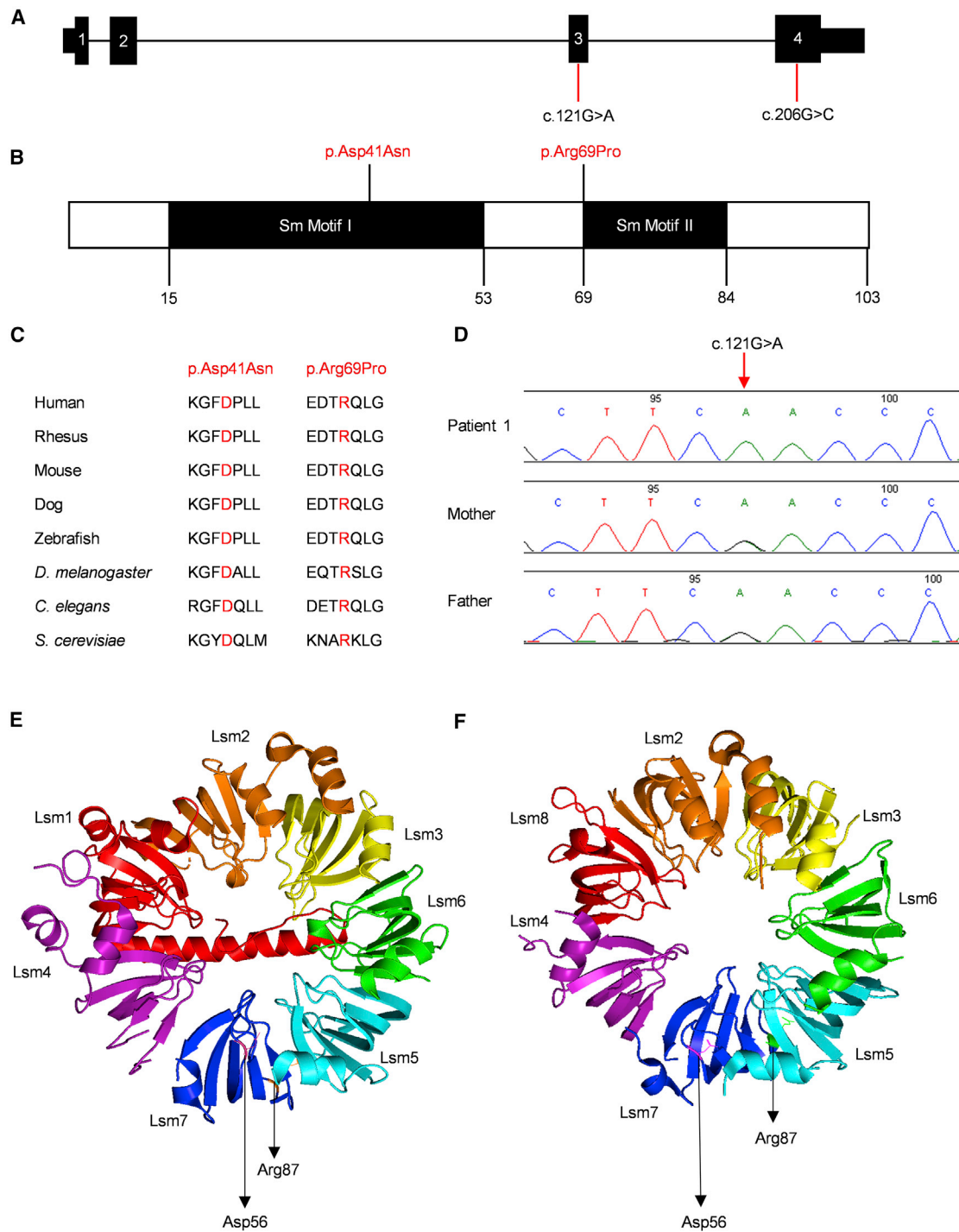


Figure 2. Biallelic variants in *LSM7*

(A) Genomic organization of *LSM7* in humans (UCSC Genome Browser hg19) with the position of the pathogenic variants within the *LSM7* gDNA indicated.

(B) Major motifs of the 103 amino acid *LSM7* protein with position of variants indicated.

(C) *LSM7* variants in affected individuals are at conserved amino acid residues.

(D) *LSM7* gene sequencing of affected individual 1 and parents.

(E and F) 3D representations (created with PyMOL) of yeast (E) Lsm1-7 and (F) Lsm2-8 complexes displaying location of variants.

p.Asp41Asn, and p.Arg69Pro proteins were compared against FLAG empty vector control samples and were labeled as high-confidence interactors when their adjusted p values were under 0.05 and their intensity ratios over 2.

The p values were adjusted for multiple hypothesis testing with a permutation-based approach using an s0 correction factor of 0.1 with 10,000 iterations.⁵⁸ A Student's t test was performed between p.Asp41Asn and p.Arg69Pro against

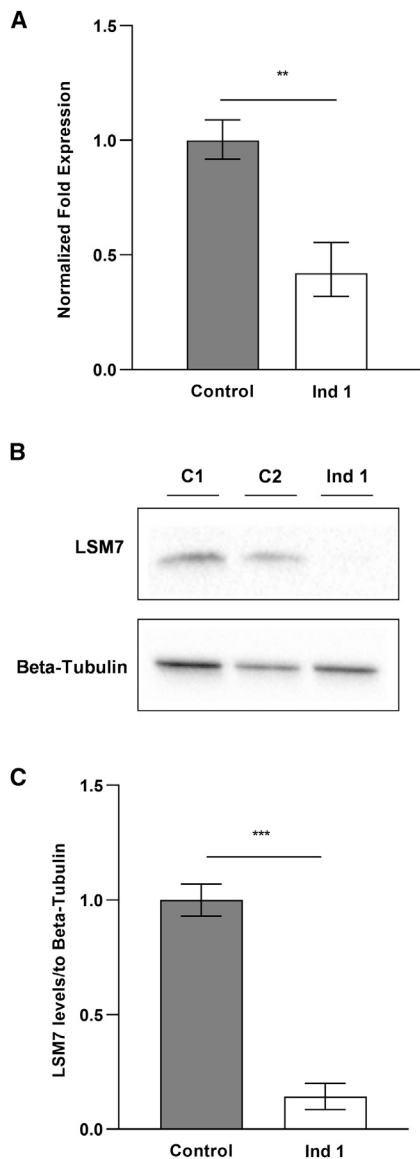


Figure 3. LSM7 mRNA and protein levels are decreased in individual 1 compared to control individuals

(A) RT-qPCR analysis of *LSM7* mRNA extracted from individual 1's fibroblasts and two age- and sex-matched control individuals ($n = 6$). The results are represented in terms of fold change after normalizing to *RPL30* and *SDHA* mRNAs. Each value represents the mean \pm SEM (unpaired t test [two tailed] $**p < 0.01$).

(B) Total protein lysates extracted from individual 1 and age- and sex-matched control individual fibroblasts were immunoblotted with anti-LSM7 and anti-beta-tubulin.

(C) The pixel densities of immunoblot bands from at least 3 independent replicates were quantified using ImageJ software, normalized over beta-tubulin immunoblot and represented as mean \pm SEM (unpaired t test [two tailed], $***p < 0.001$).

the wild-type samples. These results revealed that cells producing the wild-type LSM7 protein resulted in the co-precipitation of all the LSM1-7 and LSM2-8 complex subunits, along with multiple other known interactors of these complexes (Figures 4A and 4B). However, pull-downs from lysates of HEK293 cells producing the p.Asp41Asn or p.Arg69Pro variants revealed dramatically reduced associa-

tion of the mutated LSM7 protein with the other LSM proteins constituting the LSM1-7 and LSM2-8 complexes. Consistent with this defect, most of the known interactors of these complexes were also not pulled down in the mutant strains (Figure 4C). The LSM1-7 and LSM2-8 complexes are known to interact with many mRNA decay and splicing factors, respectively. Relative to the FLAG-tagged wild-type LSM7, the p.Asp41Asn and p.Arg69Pro mutants showed reduced interaction with the mRNA decay factors PATL1 and EDC4 as well as with triple small nuclear ribonucleoprotein (tri-snRNP) factors including PRPF3, PRPF4, SART3, and SNRNP200. These splicing factors are required for the formation of the U4/U6/U5 tri-snRNP complex, an important precursor in the major spliceosome reaction.^{59–61} Similarly, PATL1 is a highly conserved interacting partner of the LSM1-7 complex that plays a key role in mRNA decay.⁶² Thus, these findings suggest that both variants lead to defects in the assembly of the LSM1-7 and LSM2-8 complexes and result in the subsequent loss of key complex interactors.

Since the levels of LSM7 protein were found to be decreased in affected individual 1 compared to control individuals (Figure 3C), we predicted that the defect in LSM1-7 and LSM2-8 complex assembly could be further exacerbated by reduced starting levels of the mutant LSM7 protein. In our LC-MS/MS experiments, when the levels of FLAG-tagged mutant LSM7 were normalized to that of the FLAG-tagged wild-type, we found that both mutated subunits pulled down relatively lower amounts of the other LSM complex subunits. Therefore, reduced protein levels of mutant LSM7, as seen in affected individual 1, could potentially intensify the defects we observed in our LC-MS/MS experiments. We also saw reduced levels of *LSM7* mRNA in this individual compared to control individuals (Figure 3A). Since the levels of the LSM2-8 complex are expected to be lower in this individual (Figure 4C), reduced *LSM7* transcript levels could result from inefficient splicing of *LSM7* pre-mRNA. Several other studies have shown that both missense and synonymous variants can impact RNA structure, stability, folding, microRNA (miRNA) binding, splicing regulatory sites, and/or translation efficiency.^{63–65} Therefore, we hypothesize that this missense variant could alter the *LSM7* pre-mRNA, further targeting it for degradation and thereby reducing transcript levels.

To demonstrate the functional impact of wild-type and mutant *LSM7*, a zebrafish model was created and used to study the impact of LSM7 hypofunction on CNS development. The zebrafish experiments were performed in strict accordance with relevant institutional and national guidelines and regulations. Adult fish were bred according to standard methods, and embryos were raised at 28.5°C in E3 embryo medium and staged by time and morphology. The zebrafish ortholog *lsm7* (ENS DART00000081188.7) and human *LSM7* share significant homology. The zebrafish *lsm7* protein has 95% amino acid identity with human *LSM7*, including conservation of the two amino

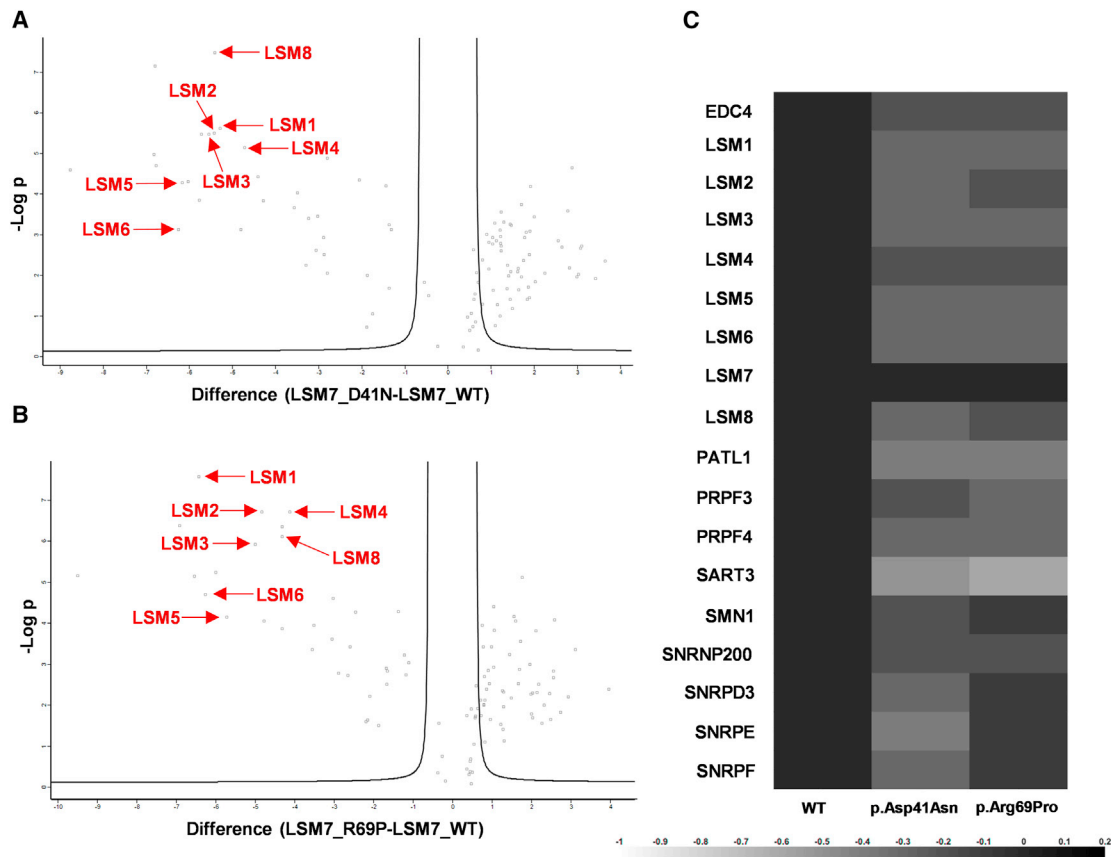


Figure 4. Impact of LSM7 variants on LSM1-7 and LSM2-8 complex assembly

(A and B) Volcano plots of the \log_2 -transformed MaxQuant ratios of the p.Asp41Asn or p.Arg69Pro/wild-type (x axis) and the $-\log_{10}$ -transformed p values (adjusted with permutation-based false discovery rate) resulting from the two-tailed two-sample Student's t tests. (C) Heatmap contains the MaxQuant ratios for the subunits of the LSM complexes and known important complex interactors.

acid residues of the variants in the affected individuals (p.Asp41Asn and p.Arg69Pro) (Figure 5A). We evaluated *lsm7* gene expression using an antisense digoxigenin-uridine triphosphate (UTP) labeled riboprobe, which was synthesized according to the manufacturer's instructions (Roche). *In situ* hybridizations were performed as previously described⁶⁶ from 1 day post-fertilization (dpf) to 5 days post-fertilization, and the color reaction was carried out using NBT/BCIP substrate (Roche). At 1 day post-fertilization and 2 days post-fertilization, the *lsm7* transcript was detected in the developing brain, spinal cord, and eye (Figure 5B). By 3 days post-fertilization and persisting through 5 days post-fertilization, expression was present in the olfactory bulb, cerebellum, and hindbrain (Figure 5B).

To investigate the physiological function of Lsm7 in an animal model of neurodevelopment, we used CRISPR-Cas9 gene editing to develop Lsm7-Crispant zebrafish lacking functional Lsm7 protein. Single guide RNA (sgRNA) target sites for the zebrafish *lsm7* gene (Ensembl Zv11: ENS-DART00000081188.7) were designed and off-target effects checked. Co-injection of *lsm7* exon 3 sgRNA (460 pg) and Cas9 protein (460 pg, PNA BIO) was done on one-cell-stage embryos and efficiently induced insertions and deletions

as determined by high-resolution melting analysis (HRMA) PCR (Figure S2).

Embryos injected with *lsm7* sgRNA and Cas9 protein showed impaired nervous system development and normal lifespan (1.6% lethality, $n = 128$). We categorized the surviving embryos into two phenotypes according to the severity: a mild phenotype who displayed normal trunk morphology but brain and eye size reduction, and a moderate phenotype exhibiting a bent trunk, edema in the pericardial sac, and decreased eye and brain size (Figures 5C–5E). At 5 days post-fertilization the fish larvae were fixed, and one eye was dissected out, mounted laterally, and imaged. The eye size was measured along the horizontal axis using ImageJ software (Figure 5E, dashed line) in Lsm7-Crispant (injected with *lsm7* sgRNA and Cas9 protein). Eye diameter was found to be significantly reduced in Lsm7-Crispant compared to controls ($p < 0.001$) (Figures 5E and 5F). We also evaluated motor behavior of Lsm7-Crispant by analyzing the response to light stimuli at 7 days post-fertilization in 96-well square bottom plates (Krackeler Scientific) using video analysis software (Noldus EthoVision). We found that larvae response to light stimuli was significantly reduced in the Lsm7-Crispant ($p < 0.001$) (Figure 5G).

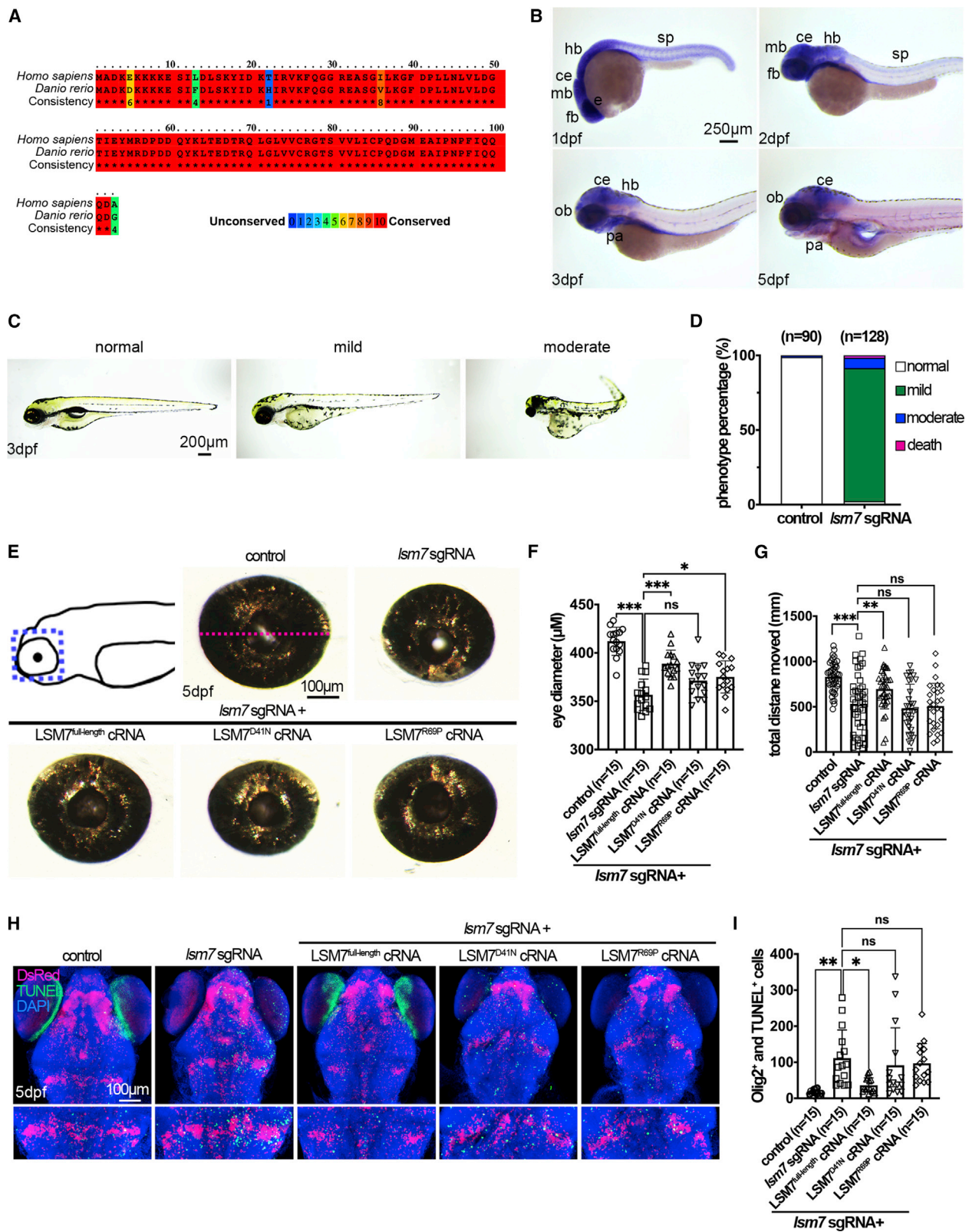


Figure 5. The zebrafish *Lsm7* ortholog is highly conserved with human *LSM7*, is expressed in developing nervous system, and is necessary for embryonic morphology, eye size, motor behavior, and survival of oligodendrocytes

(A) Amino acid alignment of human and zebrafish *LSM7* sequences. Identical residues are marked by red boxes, and percent similarity is shown by different colors.

(B) *In situ* hybridization was performed for detecting *lsm7* expression during zebrafish embryogenesis. Embryo stages are shown in the bottom left of each panel. All panels display the lateral view, with dorsal to the top and rostral to the left.

(C) Phenotypes of *lsm7*-Crispant.

(legend continued on next page)

To explore the effects of the mutant LSM7 proteins, capped RNA (cRNA) encoding the full coding sequence of LSM7^{full-length}, LSM7^{Asp41Asn}, or LSM7^{Arg69Pro} were prepared. The eye phenotype could be rescued by concomitant injection of human LSM7^{full-length} and LSM7^{Arg69Pro} cRNAs, but not by the LSM7^{Asp41Asn} cRNA (Figures 5E and 5F). Since the p.Asp41Asn variant is located on a conserved RNA contacting residue in the Sm motif-I of LSM7, the inability of the LSM7^{Asp41Asn} cRNA to rescue the eye size phenotype of the Lsm7-Crispant implies that RNA processing activity is critical for eye development. Furthermore, the movement deficits observed in the Lsm7-Crispant could only be rescued with the LSM7^{full-length} cRNA but not by either of the mutant cRNAs (LSM7^{Arg69Pro} or LSM7^{Asp41Asn}), indicating that both mutants impact motor behavior (Figure 5G). Overall, these findings demonstrate that *Lsm7* knockdown zebrafish have phenotypic characteristics similar to those observed in the first affected individual.

On a cellular level using our zebrafish model, we explored the role of *LSM7* on the development and survival of oligodendrocytes, the myelin-producing cells of the CNS, which are primarily implicated in leukodystrophies. We used the transgenic line Tg (*olig2:dsRed*) to label oligodendrocytes, oligodendrocyte precursor cells (OPCs), and motor neurons and compared cell counts in larvae injected with wild-type LSM7^{full-length} cRNA to LSM7^{Asp41Asn} or LSM7^{Arg69Pro} cRNAs. The immunohistochemistry was performed as previously described⁶⁷ using rabbit anti-dsRed (Clontech, dilution 1:250), Alexa 555 goat anti-rabbit (ThermoFisher Scientific, dilution 1:400), and 4',6-diamidino-2-phenylindole (DAPI). Additionally, terminal deoxynucleotidyl transferase deoxyuridine triphosphate (dUTP) nick-end labeling (TUNEL) was performed on whole-mount larvae (ApopTag Fluorescein *In Situ* Apoptosis Detection Kit; Millipore) as previously described.⁶⁸ Confocal imaging was used and cells counted using Photoshop's (Adobe) count tool. We found that apoptosis of oligodendrocytes was significantly increased in Lsm7-Crispant ($p < 0.01$) (Figures 5H and 5I) and could be rescued by concomitant injection of LSM7^{full-length} cRNA but not by the LSM7^{Asp41Asn} or LSM7^{Arg69Pro} cRNAs (Figures 5H and 5I). This suggests a role for *LSM7* in oligodendrocyte survival and demonstrates that both variants are associated with an increase in apoptosis of oligodendrocytes. Interestingly, the hypoplastic cerebellum in individual 1 corresponded to the small cerebellar phenotype

observed in Lsm7-Crispant. It is possible that deficiency or dysregulation of *LSM7* in the cerebellum of the first affected individual at key developmental stages could contribute to the observed cerebellar hypoplasia. Overall, this zebrafish model has allowed us to identify a relationship between *LSM7* and CNS development, as well as oligodendrocyte survival, thus providing further evidence of a link to leukodystrophies, genetic neurodevelopmental and/or neurodegenerative disorders.

Our current data do not allow us to understand why one variant (p.Arg69Pro) led to *in utero* death, while the other variant (p.Asp41Asn) leads to a less severe phenotype. Samples were unavailable from the fetuses described in the second family for mRNA or protein analysis, limiting direct functional studies. However, the p.Arg69Pro variant, but not p.Asp41Asn, is predicted to be located at an exon-intron boundary by MutationTaster,⁴² which could impair or cause dysfunction in pre-mRNA splicing. For this reason, we hypothesize that perhaps the p.Arg69Pro variant causes splice site changes that are detrimental and cause embryonic lethality. Specific splicing variants can cause cell-specific defects, as in the case of individuals presenting with a unique phenotype of basal ganglia abnormalities and the same pathogenic splicing variant in *POLR3A*.^{69–73} This further supports a possible association between distinct splicing defects and phenotypic heterogeneity within individuals with pathogenic variants in the same gene. While a splicing defect could offer support for the difference in phenotypic severity, we would be unable to confidently conclude that it is indeed the only factor at play. There have been several reports in the literature of individuals with the same variant presenting with large discordances in phenotypic severity. One such report describes a sibling pair with the same compound heterozygous variants in *POLR1C*, which led to neonatal death in one sibling, while the other remains alive and is much less affected.⁷⁴ Another example is presented in an inbred family where the same variant in *SLC17A5* results in either a mild or severe phenotype.⁷⁵

In summary, this study identified *LSM7*, a gene involved in the processes of 5'–3' mRNA decay and splicing,²⁰ as playing a role in the development of the CNS and in oligodendrocyte survival. Before a definite association between *LSM7* variants and leukodystrophy, genetic neurodevelopmental and/or neurodegenerative disease can be made, more affected individuals with biallelic variants in *LSM7* need to be identified. Our findings, however, add this

(D) Quantification of control and *Lsm7*-Crispant by phenotype severity.

(E) Top left panel shows a cartoon representation of the dissected region of larvae (blue dashed line rectangle). Images of eyes of control, *Lsm7*-Crispant, LSM7^{full-length} cRNA-, LSM7^{R69P} cRNA-, and LSM7^{D41N} cRNA-rescued larvae are shown. The eye diameter was measured as indicated by the magenta dashed line shown on control eye.

(F) Quantification of eye size.

(G) Motor swimming behavior of the control, *Lsm7*-Crispant, LSM7^{full-length} cRNA-, LSM7^{R69P} cRNA-, and LSM7^{D41N} cRNA-rescued larvae at 7 dpf.

(H) Confocal images of dorsal view of the control, *Lsm7*-Crispant, LSM7^{full-length} cRNA-, LSM7^{R69P} cRNA-, and LSM7^{D41N} cRNA-rescued larvae with rostral to the top. Lower panels are enlarged region from upper panels for apoptotic cell quantification.

(I) Quantification of the number of TUNEL and DsRed double-positive cells of (H). Abbreviations: ce, cerebellum; dpf, days post-fertilization; fb, forebrain; hb, hindbrain; mb, midbrain; ob, olfactory bulb; pa, pharyngeal arches; sp, spinal cord. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s., not significant.

gene as a strong gene candidate for these diseases. In recent years, gene discovery has shifted from identifying pathogenic variants in genes for rare diseases to ultra-rare entities with only a handful of affected individuals. Indeed, this is supported by several recently uncovered leukodystrophy genes, in which only a few individuals were identified and published, including *EPRS1*, *POLR3K*, *RARS1*, *TMEM106B* (MIM: 613413), *CNP* (MIM:123830), and *NKX6-2* (MIM: 605955).^{13,19,76–79} *LSM7*, which encodes a subunit of both the LSM1-7 and LSM2-8 complexes, has not previously been associated with human disease. However, other proteins involved in the processes of mRNA decay and splicing, the primary functions of the LSM1-7 and LSM2-8 complexes, respectively, have been implicated in a variety of diseases.^{21,23,30,80–96} Cellular decay pathways play a critical role in regulating mRNA levels, and alterations in these pathways could be the principle factor underlying many diseases,^{85–95,97,98} including neurological disorders.^{83,84} Similarly, variants in genes encoding proteins involved in the various steps of the splicing reaction have also been linked to numerous neurodevelopmental and neurodegenerative disorders.^{59,99–104} The identification of other individuals with biallelic variants in *LSM7* is needed to add with certainty this gene and the pathophysiological mechanisms of mRNA decay and splicing regulation to genes and categories of cellular dysfunction associated with leukodystrophies, genetic neurodevelopmental and/or neurodegenerative disorders.

Data and code availability

The clinical exomes are not publicly available. The variants generated during this study are available at ClinVar (gene variants; ClinVar: SCV001478394 and SCV001478886).

Supplemental information

Supplemental information can be found online at <https://doi.org/10.1016/j.xhgg.2021.100034>.

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Declaration of interests

Y.S. is an employee of GeneDx, Inc. J.L.B. reports being on the board of directors for wFluidx; stock ownership of Orchard Therapeutics; consulting for Bluebird, Calico, Denali, Enzyvant, Neurogene, and Passage Bio; and royalties from BioFire (spouse). G.B. reports speaker's honoraria from Genzyme (2013) and Actelion Pharmaceuticals (2013); being on the scientific advisory boards of Ionis (2019), Shire (2013), Actelion Pharmaceuticals (2011), and Santhera Pharmaceuticals (2011); research grant from Takeda; being on a site PI for studies sponsored by Takeda/Shire (2020–2021), Bluebird Bio (2019), Shire (2016), and Actelion Pharmaceuticals (2017); and consulting for Passage Bio. All other authors declare no competing interests.

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Web resources

CHOPCHOP, <http://chopchop.cbu.uib.no/>
Compute Canada, <https://www.computecanada.ca/>
GenBank, <https://www.ncbi.nlm.nih.gov/genbank/>
GeneDx ClinVar submission page, <https://www.ncbi.nlm.nih.gov/clinvar/submitters/26957>
OMIM, <https://omim.org/>
PRALINE, <https://www.ibi.vu.nl/programs/pralinewww/>
PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC, <https://www.pymol.org/2/>

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