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ARTICLE

LARP1 haploinsufficiency is associated with an autosomal dominant neurodevelopmental disorder

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Summary

Autism spectrum disorder (ASD) is a neurodevelopmental disorder (NDD) that affects approximately 4% of males and 1% of females in the United States. While causes of ASD are multi-factorial, single rare genetic variants contribute to around 20% of cases. Here, we report a case series of seven unrelated probands (6 males, 1 female) with ASD or another variable NDD phenotype attributed to *de novo* heterozygous loss of function or missense variants in the gene *LARP1* (La ribonucleoprotein 1). *LARP1* encodes an RNA-binding protein that post-transcriptionally regulates the stability and translation of thousands of mRNAs, including those regulating cellular metabolism and metabolic plasticity. Using lymphocytes collected and immortalized from an index proband who carries a truncating variant in one allele of *LARP1*, we demonstrated that lower cellular levels of LARP1 protein cause reduced rates of aerobic respiration and glycolysis. As expression of *LARP1* increases during neurodevelopment, with higher levels in neurons and astrocytes, we propose that *LARP1* haploinsufficiency contributes to ASD or related NDDs through attenuated metabolic activity in the developing fetal brain.

Introduction

Neurodevelopmental disorders (NDDs) are defined in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) as early-onset developmental deficits (e.g., failure to achieve cognitive, motor, or social milestones) that lead to long-term impairments. Such deficits may be limited (e.g., specific learning disorders) or generalized (e.g., global developmental delay). Many are highly heritable; where specific genetic loci have been identified, they often overlap between multiple NDDs.[1] Autism spectrum disorder (ASD) is diagnosed in 2.8% of children and is an NDD defined by impairments in social communication and the presence of restricted or repetitive behaviors or interests.[2] Although common genetic variants observed in over 1% of the population account for the majority of variance in the ASD trait, single rare genetic variants contribute to about 20% of individuals affected by ASD.[3] These are often rare *de novo* variants, frequently occurring in genes with roles in transcriptional or messenger RNA (mRNA) regulation or neuronal communication,[4] although some biallelic variants affect genes with roles in metabolism.[5,6] It is estimated that many more genes associated with NDDs and ASD remain to be discovered.[4]

LARP1 (La ribonucleoprotein 1; translational regulator; ENSG00000155506; HGNC:29531), located on chromosome 5 (at 5q33.2), encodes the LARP1 (UniProt: Q6PKG0) RNA-binding protein (RBP)[7] and is highly conserved across evolution. Large-scale human population reference databases reveal dramatically fewer than expected protein-truncating variants (PTVs) in *LARP1*, suggesting strong selective pressure with the loss of one functioning copy (haploinsufficiency, pLI = 1.0, LEOUF = 0.17 [top decile], gnomAD v.2) and from missense variation (gnomAD v.2 missense *Z* score = 3.07).[8] Cohorts of individuals with developmental delays and ASD have identified two *de novo* PTVs and four *de novo* missense variants, which did not meet genome-wide significance in either

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cohort.[4,9] However, a combined analysis suggested that *LARP1* might be associated with NDDs (false discovery rate [FDR] = 0.047). A single *de novo* missense variant was also reported in a cohort of 232 individuals affected by congenital hydrocephalus.[10]

Levels of LARP1 protein are aberrantly elevated in cancer cells, where many of its physiological functions were identified.[11–13] Its activation is dependent on phosphorylation at multiple sites by upstream kinases, including mTOR, CDK1, and Akt/S6K1, whereupon LARP1 selectively binds target mRNAs via its La module and DM15 domain to alter their stability and, hence, their translation efficiency.[14–16] In this way, LARP1 acts at the point of convergence of afferent signaling cascades to post-transcriptionally regulate specific genes, notably those required for ribosomal biogenesis, cellular metabolism, and survival.[17,18] Of its metabolic targets, LARP1 binds mRNAs encoding enzymes within the glycolytic, pentose phosphate, lipogenesis, and mitochondrial Krebs and oxidative phosphorylation (OXPHOS) pathways and coordinates switching between glycolytic and mitochondrial metabolism.[15,17–19] This metabolic plasticity enables cancer cells to survive in conditions of fluctuating nutrient or oxygen availability but is also required for normal cells during embryonic development, cellular stress, or tissue regeneration.[12,13] Metabolic plasticity is particularly important during embryonic neurogenesis, as brain precursor cells switch between mitochondrial metabolism and glycolysis during key developmental stages.[20]

Here, we report a case series of seven unrelated probands with heterozygous loss of function or missense *LARP1* variants who present with a variable neurodevelopmental phenotype that includes intellectual disability, hypotonia, motor delay, and/or ASD. The *LARP1* variants were shown to be *de novo* in all seven probands. Using immortalized lymphoblasts obtained from one affected proband (proband 1), we conducted *ex vivo* studies to assess the functional impact of pathogenic *LARP1* alteration. Based on our findings, we propose that by reducing metabolic activity, *LARP1* haploinsufficiency contributes to a newly described autosomal dominant NDD.

Material and methods

Proband cohort

The proband cohort was established using the web-based tool GeneMatcher.[21] Clinical details were obtained from medical records and provided by their respective institutions. Gene variants were identified via standard laboratory procedures and whole-exome sequencing (WES) or whole-genome sequencing (WGS) performed in diagnostic laboratories. The MANE v.1.2 reference sequence for *LARP1* (ENSG00000155506.19) uses the NM_033551.3/ENST00000518297.6 transcript.

Lymphoblast immortalization

After consent was obtained, lymphoblasts were collected from index proband 1 (see below) and immortalized using a standard Epstein Barr virus [EBV]-transformation technique. Samples were also collected from proband 1's mother and father and similarly immortalized for comparison. *LARP1* was sequenced in all cell lines. Lymphoblasts were maintained in RPMI-1640 (Sigma R8758) with 15% FBS and 1% antibiotic/antimycotic (Sigma A5955) at 37°C (5% CO_2).

CRISPR-Cas9 knockout of LARP1 in HEK293T cells

LARP1 knockout generation in HEK293T cells was performed using Edit-R CRISPR-Cas9 Gene Engineering by transducing with All-in-One Lentiviral sgRNA particles (Dharmacon, source clone IDs: VSGHSOH_28505935, VSGHSOH_28704059, and VSGHSOH_28730675). After lentiviral transduction, cells were selected by growth in puromycin (10 μg/mL), and individuals clones were expanded and validated for successful knockout of *LARP1* by western blotting. HEK293T cells were maintained in RPMI (Thermo Scientific, 11875093) supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin/streptomycin (15140122) at 37°C (5% CO_2).

LARP1 expression vector transfection

The *LARP1* expression vector was prepared by cloning full length *LARP1* (encoding the 1,096 amino acid isoform, UniProtKB/Swiss-Prot: Q6PKG0.2) was cloned into the expression vector pcDNA4/HisMax A (Thermo Scientific, V86420). Site-directed mutagenesis was performed to generate a LARP1 expression construct with c.2164dupA (chr5:154,803,344:A:AA, GRCh38), confirmed by sequencing. Transfection was performed with Fugene6 (Promega) using a 3:1 ratio of Fugene6 to plasmid and 2 μg plasmid per well of a 6-well plate. Successful expression of the wild-type and genetic variant constructs were validated by western blotting.

mRNA and protein quantitation

mRNA levels of individual genes were measured by quantitative reverse transcription polymerase chain reaction (RT-PCR) and normalized to the levels of *ACTB* mRNA. For quantitative RT-PCR, mRNA was extracted from cells using the GenElute mammalian Total RNA Miniprep Extraction Kit (Merck, RTN70-1KT) and reverse transcribed to synthesize cDNA using the High Capacity cDNA Reverse Transcription Kit (Thermo Scientific, 4368814). mRNA concentrations were equalized by nanodrop prior to reverse transcription. Quantitative PCR was performed using Fast SYBR green master mix (Thermo Scientific, 4385610) on a StepOnePlus Real-Time PCR system. Primer sequences were as follows: LARP1 N-terminal: (F) GAACCCATTTTGACTACCAG, (R) TTGATGTAGT CTTTGAGCAG; LARP1 C-terminal: (F) CGACACTCAGTGGTAG CAGG, (R) ATTTGGCATCTTCCCGGACA; and ACTB: (F) CACCAT TGGCAATGAGCGGTTC, (R) AGGTCTTTGCGGATGTCCACGT. Western blots were imaged using an Odyssey scanner (Licor), with densitometry analysis performed using ImageStudio software (Licor). The antibodies used in western blotting were LARP1 N terminus (Abcam, ab86359), LARP1 C terminus (13708-1-AP), and B-actin (Santa Cruz, sc-47778).

Metabolic and viability assays

Cells were plated onto a Seahorse XFe96 FluxPak plate (Agilent, 102416-100) at a density of 5×10^4 cells per well (lymphoblasts) or 2×10^4 cells per well (HEK293T) for 24 h. On the day of the assay, the medium was replaced with Seahorse XF DMEM medium (pH 7.4) with 5 mM HEPES and supplemented with 5 mM glucose, 5 mM pyruvate, and 4 mM L-glutamine. Oxygen consumption and extracellular acidification rates were measured by Seahorse

XF analyzer and normalized using the Cyquant NF (Thermo Scientific, C35006) cell viability assay to account for differences in cell number.

Results

LARP1 isoform usage in human brain tissue

The long (full length 1,096 amino acid) isoform is generally used for clinical interpretation (MANE transcript NM_033551.3, ENST00000518297.6). To determine whether this is the best transcript for assessing brain-related phenotypes, we assessed exon-level *LARP1* expression in the developing human brain using BrainVar bulk tissue RNA sequencing (RNA-seq) data from 176 postmortem samples of the dorsolateral prefrontal cortex (Figure 1A).[22] The long isoform was substantially more highly expressed than the short isoform (NM_001367719.1, ENST00000524248.5), demonstrated by the high levels of expression at the transcriptional start site of the long isoform but not the short isoform (Figure 1B). Dominance of the long isoform in brain tissue was maintained across development, from the early fetal stage through to 6 years and older (Figure 1C).

Proband characteristics

*Proband 1 (PTV: p.Thr722Asnfs*5)*

Proband 1 is a male aged 18 at the time of evaluation. His history included a diagnosis of ASD, language delay, anxiety, and sensory processing disorder and symptoms of muscle cramps after exercise. He did not have intellectual disability (Full-Scale Intelligence Quotient score = 88) or a history of significant motor delays. At the time of his most recent examination, his growth parameter *Z* scores were height +0.93 and weight +2.11. Other findings include recurring headaches.

Proband 2 (missense: p.Asp423His)

Proband 2 is a female aged 19 at the time of evaluation. Prenatally, she was noted to have mild intrauterine growth retardation. She subsequently had delayed motor milestones and did not walk until 19 months of age. An evaluation revealed an intellectual disability, attention-deficit/hyperactivity disorder (ADHD), and hallucinations. Her language development was also significantly delayed, and she was 4 years of age before she spoke using full sentences. Physical examination findings included joint hyperlaxity, high arched palate, exaggerated cubitus valgus, and 2,3 toe cutaneous syndactyly. She also has had recurrent otitis media. At the time of her most recent clinical evaluation, her growth parameters were a height of 155 cm (10^{th} percentile, $Z = -1.4$ SD), a weight of 58 kg (60^{th} percentile, $Z = +2$ SD), and head circumference of 53 cm (10^{th} percentile, $Z = -1$ SD).

Proband 3 (missense: p.Ala460Pro)

Proband 3 is a male aged 22 at the time of evaluation. He had a significant history of motor and language delays and hypotonia and began walking at 18–24 months; his first clear words were spoken at 4–5 years of age. Developmental testing revealed intellectual disability and ASD. There were also concerns for seizure-like activity that were not captured on electroencephalogram (EEG). At the time of his most recent evaluation, his growth parameters were a height of 175.5 cm (43rd percentile, $Z = -0.2$ SD), a weight of 66.7 kg (37th percentile, $Z = -0.34$ SD), and a head circumference of 60 cm ($>99^{th}$ percentile, $Z = +2.1$ SD). No dysmorphic features were noted.

Proband 4 (missense: p.Glu707Lys)

Proband 4 is a male aged 11 at the time of evaluation. He had a significant history of delayed motor development and intellectual disability and had been diagnosed with ASD. Neurologic testing revealed ataxia and cerebellar hypoplasia without hypotonia or seizures. At the time of his most recent evaluation, his growth parameters were a height of 131 cm (3^{rd} percentile, $Z = -1.9$ SD), a weight of 26.8 kg (3^{rd} percentile, $Z = -1.9$ SD), and a head circumference of 53 cm (44^{th} percentile, $Z = -0.2$ SD).

Proband 5 (missense: p.Ile763Phe)

Proband 5 is a male aged 3 at the time of evaluation. He was referred to genetic counseling due to intellectual disability (developmental quotient [DQ] = 54) and ASD. He also had episodes of febrile and, latterly, non-febrile seizures. At the time of his most recent evaluation, his height was 100 cm (90^{th} percentile, $Z = 1.3$ SD), weight was 15 kg (90^{th} percentile, $Z = 1.3$ SD), and head circumference was 50 cm (60^{th} percentile, $Z = 0.5$ SD). Physical findings included thick eyebrows and long eyelashes.

Proband 6 (2× missense: p.Asp793Glu and p.Lys819Arg)

Proband 6 is a male aged 4 at the time of evaluation for global developmental delay. He presented with mild hypotonia and delayed motor milestones. An evaluation also revealed delayed language development. The proband's behavior was described as anxious, without evidence of ASD. The proband has not undergone cognitive testing. Dysmorphic features include small palpebral fissures, protruding columella, and large ears. At the time of most recent evaluation, he had a significant growth delay, with a height of 96.5 cm (2.5th percentile, $Z = -2$ SD), a weight of 12 kg (0.1st percentile, $Z = -3$ SD), and a head circumference of 48 cm (0.8th percentile, $Z = -2.5$ SD).

Proband 7 (missense: p.Ile558Thr)

Proband 7 is a male aged 3 with developmental delay at the time of evaluation. He walked at 3 years of age and continues to have significant language delay. Dysmorphic features include bushy eyebrows and prominent ears. Growth parameters showed a weight of 16.4 kg (29th percentile, $Z = -0.6$ SD) and a height of 103 cm (27th percentile, $Z = -0.6$ SD). His fronto-occipital circumference at the age of 18 months was assessed to be 52 cm (93rd percentile, $Z = 1.5$ SD). Additionally, he was noted to have mild hydronephrosis.

Summary of *LARP1* variants

All seven probands were identified based on novel heterozygous variants in the *LARP1* gene detected by genetic

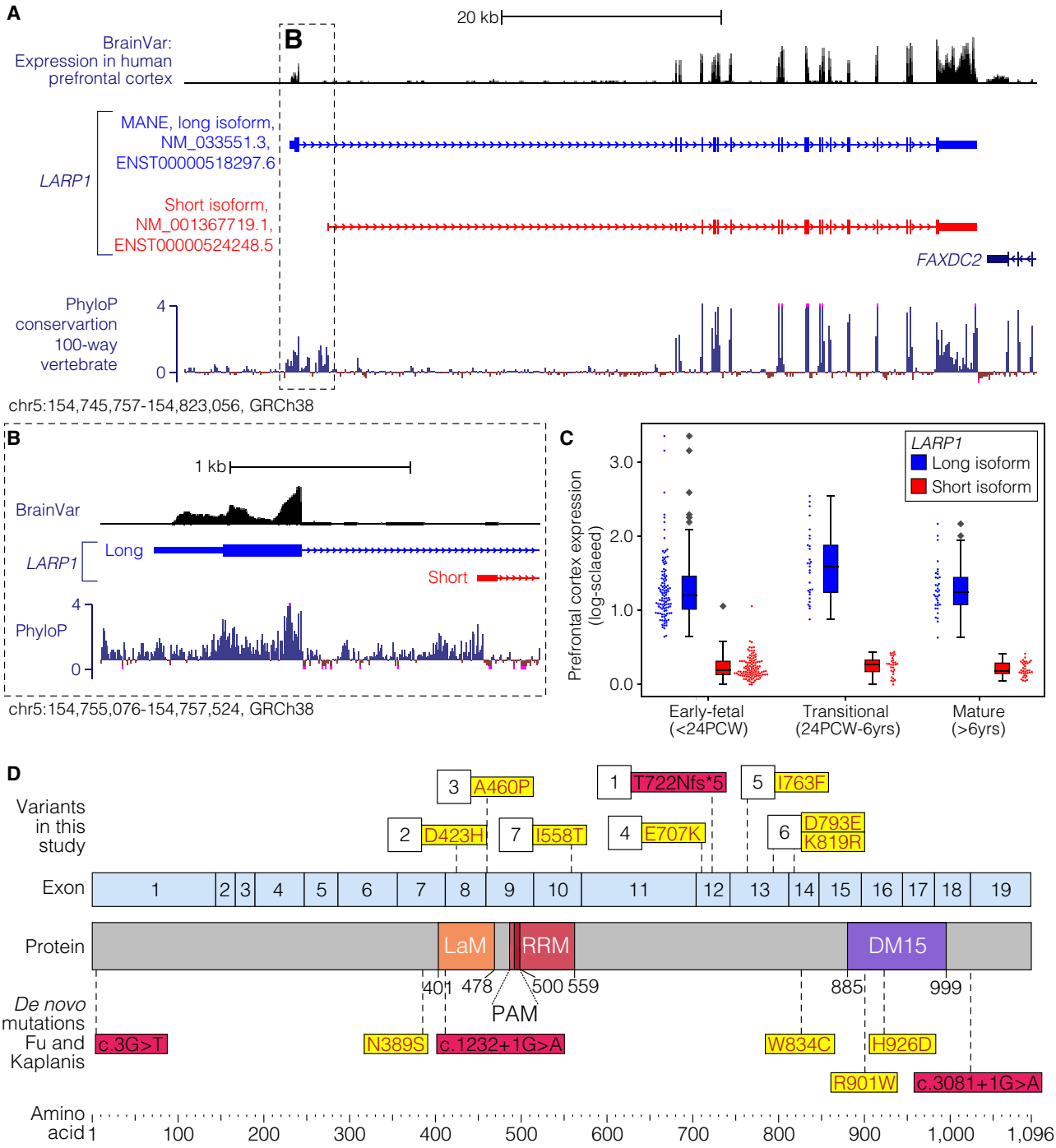

Figure 1. LARP1 isoforms and pathogenic variants in probands

(A) *LARP1* is a positive strand gene on chromosome 5 with two major isoforms: long/full length (blue), in which the first three exons encode amino acids, and short (red), in which the first three exons are non-coding. Mean expression across 176 postmortem human prefrontal cortex samples is shown at the top (BrainVar[22]), and 100-way conservation across species (PhyloP) is shown at the bottom.
(B) Zoomed-in view of the differing transcription start sites from (A).
(C) Expression of the first exon from the long and short isoforms of *LARP1* are shown for each of the 176 samples split into three developmental stages (PCW, post-conceptual weeks).
(D) Schematic representation of LARP1 protein and the variation by proband (numbered above) described in this study and *de novo* genetic variants in developmental delay and ASD cohorts.[4,9] LaM, La motif; RRM, RNA recognition motif; PAM, PABP-interacting motif; DM15, LARP1-specific HEAT-like tandem repeat region.

sequencing (Table 1). Six had missense changes in coding regions of the gene. The remaining subject (proband 1) had a PTV due to a single-nucleotide insertion leading to a frameshift and premature stop codon (Figure 1D). Probands 2, 3, and 7 had missense variants in the region encoding the highly conserved La module, required for RNA

Table 1. Summary of pathogenic *LARP1* variants and clinical features of the seven probands in this study

	Proband 1	Proband 2	Proband 3	Proband 4	Proband 5	Proband 6	Proband 7	Totals
Age at time of exome analysis	18	19	22	11	3	5	3	–
Sex	male	female	male	male	male	male	male	6:1 (male: female)
LARP1 variant (NM_033551.3)	c.2164dup (p.Thr722AsnfsTer5)	c.1267G>C (p.Asp423His)	c.1378G>C (p.Ala460Pro)	c.2119G>A (p.Glu707Lys)	c.2287A>T (p.Ile763Phe)	c.2379C>G (p.Asp793Glu); c.2456A>G (p.Lys819Arg)	c.1673T>C (p.Ile558Thr)	–
Genomic coordinates of mutation (GRCh38/hg38)	chr5:154,803,344	chr5:154,795,209	chr5:154,799,591	chr5: 154,803,299	chr5:154,803,593	chr5:154,803,685 and chr5:154,804,217	chr5: 154,799,999	–
Inheritance	*de novo*	*de novo*	*de novo*	*de novo*	*de novo*	*de novo*	*de novo*	7/7 *de novo*
Growth parameters								
Height	183 cm/82%tile ($Z = 0.93$ SD)	155 cm/10%tile ($Z = -1$ SD)	175.5 cm/43%tile (−0.2 SD)	131 cm/3%tile ($Z = -1.9$ SD)	100 cm/90%tile ($Z = 1.3$ SD)	96.5 cm/2.5%tile ($Z = -2$ SD)	103 cm/27 %tile ($Z = -0.6$ SD)	–
Weight	101 kg/98%tile ($Z = 2.1$ SD)	58 kg/60%tile ($Z = +2$ SD)	66.7 kg/37%tile (−0.3 SD)	27 kg/3%tile ($Z = -1.9$ SD)	15 kg/90%tile ($Z = 1.3$ SD)	12 kg/0.1%tile ($Z = -3$ SD)	16 kg/29 %tile ($Z = -0.6$ SD)	–
Head Circumference	57 cm/91%tile ($Z = 1.3$ SD)	53 cm/10%tile ($Z = -1$ SD)	60 cm/>99%tile (+2.1 SD)	53 cm/44%tile ($Z = 0.15$ SD)	50 cm/60%tile ($Z = 0.5$ SD)	48 cm/0.8%tile ($Z = -2.5$)	52 cm/93%tile ($Z = 1.5$ SD)	–
Motor delay	–	+	+	+	–	+	+	5/7
Language delay	+	+	+	–	+	+	+	6/7
Intellectual disability	− (FSIQ = 88)	+	+	+	+	not tested	not tested	4/5
Behavioral abnormality	ASD	ADHD, hallucinations	ASD, anxiety	ASD	ASD	anxiety	–	6/7
Hypotonia	+	–	+	not tested	not tested	+ (mild)	not tested	3/4
Seizure concern	–	–	+ (suspected)	not tested	+ (febrile)	–	–	2/6
Facial features	–	–	–	–	thick eyebrows, long eyelashes	small palpebral fissures, protruding columella, large ears	bushy eyebrows, prominent ears, and underdeveloped inferior crus; features resembled father	3/7
Musculoskeletal	muscle cramps	joint hyperlaxity, 2,3 toe cutaneous syndactyly, cubitus valgus	–	–	–	–	–	2/7
Other	headaches	arched palate, recurrent otitis media	–	ataxia, cerebellar hypoplasia	–	–	hypermobility, long digits, heart murmur, mild hydronephrosis	4/7

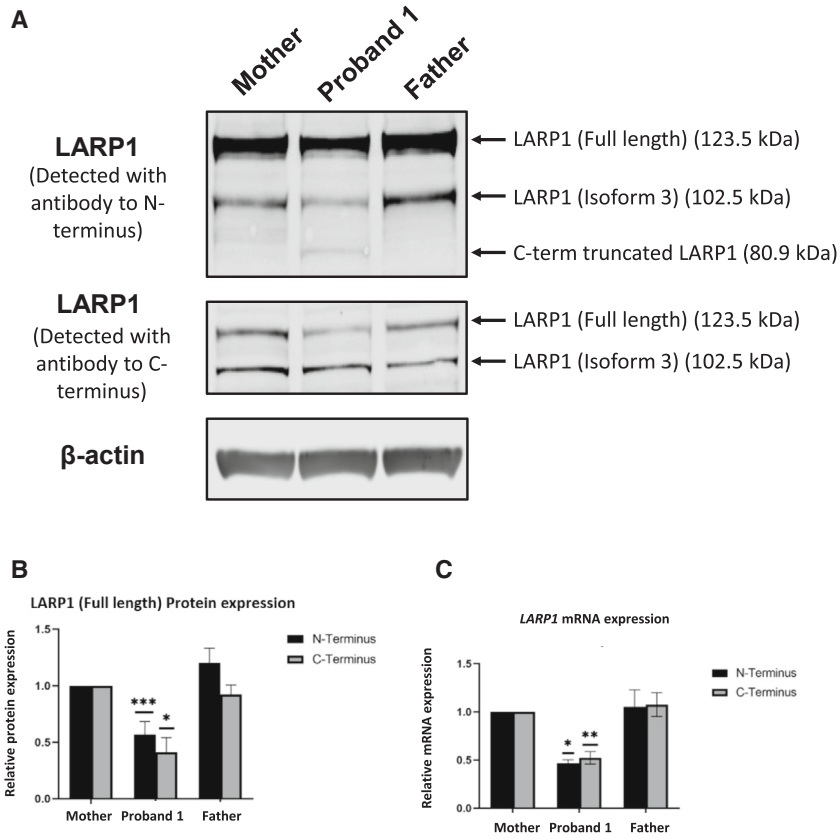

Figure 2. Immortalized lymphoblasts derived from proband 1 express reduced levels of LARP1

(A) Immortalized lymphoblasts derived from proband 1 express ~50% lower levels of full-length LARP1 protein compared with his parents. Immunoblotting was conducted using an antibody raised against human LARP1 amino acids 250–350 (top) and a C-terminal antibody against amino acids 848–1096 (middle). β-Actin was used as a loading control (bottom). A variant protein corresponding to the predicted molecular weight (80.9 kDa) of the C-terminal truncated LARP1 is detected only in proband 1.

(B) The western blot in (A) was quantified by densitometry relative to expression levels in proband 1's mother and confirmed a ~50% decrease in expression levels of LARP1 protein.

(C) *LARP1* mRNA levels were quantified by RT-qPCR using primer pairs corresponding to the N and C termini of *LARP1*. All data were normalized to expression levels in immortalized lymphoblasts from proband 1's mother (LARP1 wild type). $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ ($n = 3$).

interaction.[23] Additionally, proband 6 had two missense variants (phase unknown) upstream of the DM15 RNA-binding region. All probands had confirmed *de novo* variants. All variants were novel and not reported in ClinVar or the Genome Aggregation Database (gnomAD v.2.1).

While clinical features varied among the probands in this cohort, all had delay in at least one developmental domain. Specifically, of the seven individuals studied in this cohort, language delay was observed in six (6/7) and motor delay in five (5/7). Four had intellectual disability (4/7), and six (6/7) had behavioral issues, including ASD, ADHD, or anxiety. Variable phenotypes include hypotonia (3/4), and three (3/7) had seizures. Of note, the majority were male (6/7), which may be consistent with the preponderance of ASD in males, although this study size is currently too small to determine significance.[24]

Immortalized lymphoblasts derived from proband 1 express low levels of LARP1 and exhibit reduced metabolism

To characterize the pathogenic variation and the putative truncated protein produced by proband 1, peripheral blood was collected from him and his parents to generate immortalized lymphoblasts. Sequencing confirmed the previously identified *LARP1* c.2164dupA (p.Thr722Asnfs*5) variant alongside a wild-type *LARP1* sequence, indicating that the variant was carried in one allele and, hence, was heterozygous. Notably, lymphoblasts derived from both parents were shown to be wild type for *LARP1*, indicating that proband 1 carried a *de novo LARP1* variant.

Two major *LARP1* isoforms have been described, the full-length "long" isoform encoding a 1,096 amino acid LARP1 protein (NM_033551.3, ENST00000518297.6) and a second "short" 891 amino acid LARP1 isoform (NM_001367719.1, ENST00000524248.5) with an alternative transcription start site that excludes the first coding exon (Figure 1A).[25] Hence, LARP1 is observable as a duplex band on western blotting (Figure 1A; Table S1). Lymphoblasts derived from proband 1 had approximately 50% levels of full-length LARP1 protein compared to normal (Figures 2A and 2B) and a protein of approximately 80 kDa corresponding to the predicted molecular weight of the truncated LARP1 protein introduced by the c.2164A duplication (725 amino acids). The levels of *LARP1* mRNA were quantified by qPCR using primer pairs corresponding to the regions of mRNA encoding its N terminus or C terminus. Again, mRNA levels of *LARP1* were significantly lower (~50%) in lymphoblasts from proband 1 compared to those from his parents (Figure 2C). Both the 5′ (encoding N terminus) and 3′ (encoding C terminus) regions of *LARP1* mRNA were significantly reduced in proband 1. As the 5′ region lies upstream of the frameshift, this indicates that mRNA transcribed from the variant allele is likely to be unstable or degraded by nonsense-mediated decay, as no difference in the relative ratio of 5′:3′ *LARP1* mRNA was observed (Figure 2C).

We next conducted functional comparisons between lymphoblasts from proband 1 and those derived from his unaffected parents. Although proliferation rates were similar (Figure S1), oxygen consumption (indicative of

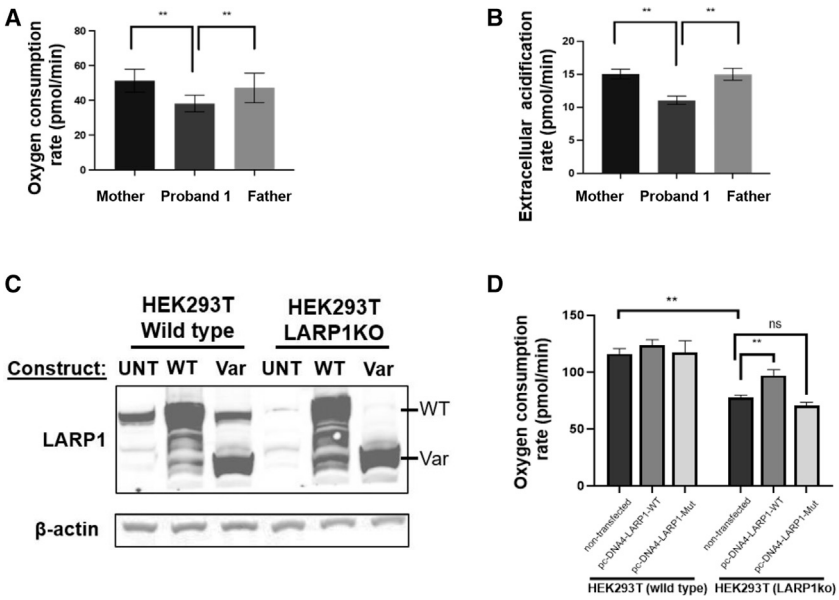

Figure 3. Full-length, but not truncated, *LARP1* promotes cellular metabolism

(A and B) Oxygen consumption rate (A) and extracellular acidification rate (B) of proband 1's lymphoblasts compared to wild-type lymphocytes from his parents. $*p < 0.05$ and $**p < 0.01$ ($n = 3$).

(C) Wild-type or *LARP1* knockout (*LARP1*KO) HEK293T cells were transfected with expression constructs expressing either wild-type *LARP1* or the truncated variant (Var) of *LARP1* found in proband 1. Successful expression of both constructs was confirmed by western blotting.

(D) Oxygen consumption rate measured by Seahorse assay in wild-type HEK293T and *LARP1KO* cells transfected with wild-type and truncated *LARP1* expression constructs. The low oxygen consumption rate of HEK293T *LARP1*KO cells was partially rescued by the wild type *LARP1* expression construct but not the truncated *LARP1* expression construct.

aerobic respiration) and extracellular acidification rates (indicative of glycolysis) were both significantly reduced in proband 1's cells (Figures 3A and 3B). We then questioned whether both wild-type and truncated *LARP1* expression vectors could rescue the metabolic phenotype associated with *LARP1* loss. Consistent with other cell lines lacking *LARP1*, *LARP1*-null HEK293T cells display lower oxygen consumption rates than wild-type cells, but this phenotype was partially rescued by the expression of wild-type *LARP1* from the transfected expression vector (Figures 3C and 3D). In contrast, the truncated *LARP1* expression vector was unable to rescue oxygen consumption rates despite stable expression. Of note, both the mRNA and protein expressed from the truncated *LARP1* construct were stable, whereas the endogenous mRNA encoding the truncated variant from proband 1 was rapidly degraded. These findings indicate that hemizygous loss of the functional LARP1 protein from proband 1 was the cause of the reduced metabolism observed in his cells, as the truncated form of LARP1 lacks the functionality of the full-length protein and, furthermore, is only expressed at a very low level.

Expression of *LARP1* in the brain

To explore the role of LARP1 in neuronal development, we examined the expression of *LARP1* in the developing human cortex using bulk RNA-seq data from 176 postmortem samples in BrainVar.[22] *LARP1* is highly expressed in the cortex from the earliest observation in the middle of the first trimester onwards, with slightly higher postnatal expression (Figure 4A). No differences were observed in expression patterns or levels across the brain regions in the BrainSpan dataset (Figure S2).[26] Furthermore, through analysis of a previously published single-cell RNA-seq dataset from the adult human cortex (middle temporal gyrus), *LARP1* was found to be robustly expressed throughout major subclasses of inhibitory and excitatory neurons, with more modest expression in astrocytes, oligodendrocytes, and oligodendrocyte precursor cells.[27] The highest expression of *LARP1* was observed in inhibitory *PAX6*-, *LAMP5*-, *VP*-, and *SST*-expressing neurons, alongside substantial expression in multiple classes of excitatory neurons. The timing and cell specificity of *LARP1* expression in the developing brain indicates the importance of precise regulation and may underpin why the observed variants in *LARP1* are associated with NDDs.

Discussion

LARP1 is a highly conserved RBP that directly binds and stabilizes essential ribosomal biogenesis, cell survival, and metabolism mRNAs.[14–16] Although the functional role of LARP1 in neuronal cells has yet to be established, in cancer cells, the loss of LARP1 attenuates cell respiration and metabolic plasticity, specifically the ratio of glycolytic to mitochondrial metabolism in response to environmental alterations, such as nutrient depletion.[28,29]

Here, we describe the first case series of heterozygous variants in *LARP1*, identifying seven unrelated probands with overlapping neurodevelopmental phenotypes. It is noteworthy that an additional *de novo LARP1* missense variant (c.2743C>T [p.L915F], CADD score: 26.5) has been reported in a proband with congenital hydrocephalus, raising the possibility of a wider phenotypic spectrum.[10] Testing lymphoblasts obtained from one index proband, we observed a truncated, non-functioning form of LARP1 protein with normal expression from the remaining wild-type allele. This halving of the total LARP1 protein dose corresponded to reduced cellular glycolysis and mitochondrial respiration, which was restored *in vitro* upon the re-expression of full-length, but not truncated, LARP1, indicating a non-dominant-negative effect.

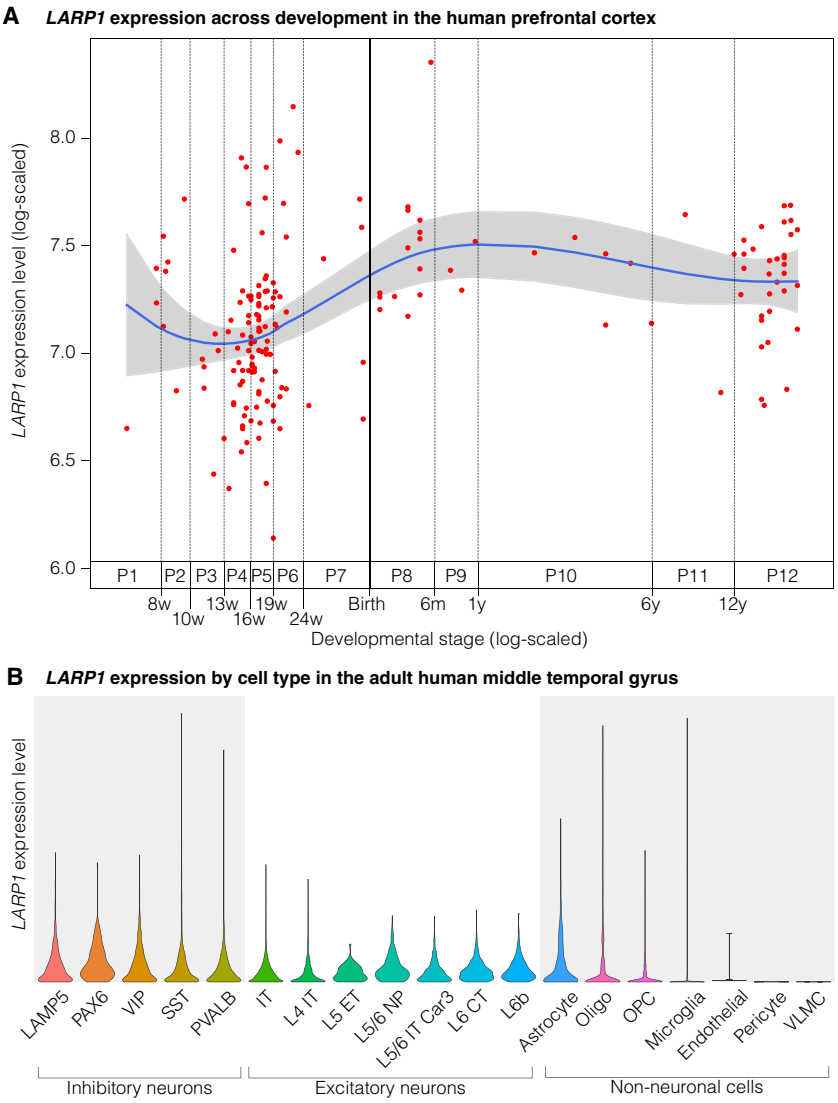

Figure 4. *LARP1* expression across development and by cell type in the human cortex

(A) *LARP1* expression ($\log_2$ counts per million +1) from bulk tissue RNA-seq of the postmortem human prefrontal cortex is shown for 176 samples across brain development (red points) from BrainVar.[22] The line represents the LOESS (locally estimated scatterplot smoothing), with the 95% confidence interval shown by the shaded area.

(B) *LARP1* expression is shown from single-cell RNA-seq data of 8 sample of the adult human middle temporal gyrus.[27] CT, corticothalamic; ET, extratelencephalic projecting; IT, intratelencephalic projecting; L4, L5, and L6, layers 4, 5, and 6 of the cortex; OPC, oligodendrocyte precursor cells; VLMC, vascular and leptomeningeal cells.

Systematic analysis of large cohorts of individuals with NDD, augmented by clinical genetic sequencing, has led to the identification of hundreds of associated genes. In these cohorts, *LARP1* is expressed in a cell-specific and time-dependent manner within inhibitory and excitatory neurons in the developing brain from the fetal stage onwards. Consistent with a role in NDDs via haploinsufficiency, *LARP1* is highly constrained in human population cohorts (gnomAD),[8] highly expressed in the developing brain and in excitatory neurons, and plays a role in mRNA regulation. Combined analysis of these cohort studies supports our smaller cohort observation that variants in *LARP1* can lead to neurodevelopmental delay (FDR = 0.047). This raises the possibility that the onset and location of LARP1 expression in neurons within the developing brain are tightly regulated. Therefore, sequence variants or dysregulated expression of *LARP1* may attenuate cellular metabolism and impact neurological and synaptic development during critical stages of embryogenesis resulting in clinical features of NDDs.

Several other mRNA-associated genes have already been associated with NDDs, including *FMR1* (fragile X), *HNRNPU*, *UPF1*, *ELAVL3*, *DHX30*, *DDX23*, *MSI1*, and *SYNCRIP*.[30–32] Unstable expansion and methylation of CGG trinucleotide repeats within the 5′ UTR of *FMR1* cause its silencing and the clinical features of fragile X syndrome (FXS), an inherited developmental disorder causing up to 3% of all cases of ASD. The silencing of *FMR1* causes loss of the protein product FMRP required for post-transcriptional regulation of mRNAs involved in neuronal synaptic plasticity.[33] Other RBPs, such as Pumilio and HuR (ELAV), have also been shown to contribute to neuronal protein synthesis and synaptic plasticity.[34,35] As synaptic plasticity is increasingly seen as a manifestation of metabolic plasticity and a requirement for normal brain development and neuronal plasticity, it is likely that other, similar RBPs may in the future be linked to NDDs, particularly as the use of exome sequencing becomes more widespread.

The identification of the genetic etiology of NDDs not only has relevance for genetic counseling and patient management but can aid the future identification of pathogenic mechanisms and potential therapeutic approaches. Our findings enable us to conclude that *LARP1* genetic variants are predominantly *de novo*, which, together with the phenotypic patterns, supports the association of *LARP1* haploinsufficiency with an autosomal dominant, monogenic, and highly penetrant NDD.

Data and code availability

This study did not generate datasets or code.

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Author contributions

R.J.L., A.C., R.C.R., B.R.D. and C.S. identified the mutation in proband 1 and R.J.L. collaborated with other centers to identify other probands. J.C. conducted the experimental work, S.J.S. provided in silico data. S.P.B. oversaw the project. S.P.B., S.J.S., J.C. and R.J.C. drafted the manuscript and revisions. All authors contributed to editing and reviewing the manuscript.

Declaration of interests

S.J.S. receives research funding from BioMarin Pharmaceutical. M.L. is an employee and shareholder of Invitae Corp. I.M.W. is an employee of GeneDx, LLC. S.P.B. is a founder and director of RNA Guardian, Ltd.; a patent holder of WO1999062548A9 and WO2016075455A1; has an advisory committee membership to UCB; and has provided consultancy to Simbec Orion, Theolytics, Oxford Drug Discovery, and Ellipses.

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.xhgg.2024.100345.

Web resources

BrainSpan, https://www.brainspan.org/
BrainVar, http://www.brainvar.org/
ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/
gnomAd, https://gnomad.broadinstitute.org/

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