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Missense variants in *DPYSL5* cause a neurodevelopmental disorder with corpus callosum agenesis and cerebellar abnormalities

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Summary

The collapsin response mediator protein (CRMP) family proteins are intracellular mediators of neurotrophic factors regulating neurite structure/spine formation and are essential for dendrite patterning and directional axonal pathfinding during brain developmental processes. Among this family, CRMP5/DPYSL5 plays a significant role in neuronal migration, axonal guidance, dendrite outgrowth, and synapse formation by interacting with microtubules. Here, we report the identification of missense mutations in *DPYSL5* in nine individuals with brain malformations, including corpus callosum agenesis and/or posterior fossa abnormalities, associated with variable degrees of intellectual disability. A recurrent *de novo* p.Glu41Lys variant was found in eight unrelated patients, and a p.Gly47Arg variant was identified in one individual from the first family reported with Ritscher-Schinzel syndrome. Functional analyses of the two missense mutations revealed impaired dendritic outgrowth processes in young developing hippocampal primary neuronal cultures. We further demonstrated that these mutations, both located in the same loop on the surface of DPYSL5 monomers and oligomers, reduced the interaction of DPYSL5 with neuronal cytoskeleton-associated proteins MAP2 and βIII-tubulin. Our findings collectively indicate that the p.Glu41Lys and p.Gly47Arg variants impair DPYSL5 function on dendritic outgrowth regulation by preventing the formation of the ternary complex with MAP2 and βIII-tubulin, ultimately leading to abnormal brain development. This study adds *DPYSL5* to the list of genes implicated in brain malformation and in neurodevelopmental disorders.

Intellectual disabilities (IDs) define a group of frequently associated neurodevelopmental disorders (NDDs), which affect at least 1% of the general population and consequently represent a major public health issue.[1] NDDs are characterized by compromised brain and cognitive functions and impaired social behaviors. With the recent development of next-generation DNA sequencing in the past decades, it has been well established that more than 900

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genetic factors are implicated.[2] The integration of molecular and neurobiological data as well as animal and neuronal models has highlighted the critical role of impairments targeting synapse structure and function, chromatin remodeling, and the interplay between the mechanisms regulating gene expression and synaptic function.[3,4] Brain malformations, such as agenesis of the corpus callosum (ACC) or cerebellar dysplasia, are congenital abnormalities sometimes associated with NDDs. In particular, ACC is present in around 1%–3% of individuals with impaired neurodevelopment.[5,6] The etiologies of ACC are diverse and there has been a growing list of genetic causes identified in the last years: more than 25 genes are involved in either isolated or syndromic forms of ACC.[5] Indeed, whole-exome sequencing (WES) approaches allowed for the identification of novel candidate genes and pathogenic variants leading to ACC through abnormal vascular development, Semaphorin-mediated axonal guidance, and neuronal migration and specification.[7,8]

In the frame of our research program on the genetic causes of isolated or syndromic severe ID using trio-based WES (HUGODIMS), we initially identified a *de novo* heterozygous missense mutation in *DPYSL5* (*Dihydropyrimidinase like 5*, [MIM: 608383] [GenBank: NM_020134.4]) in a simplex male individual with severe ID and complete ACC. This mutation (Chr2[GRCh37]: 27121488 G>A, c.121G>A [p.Glu41Lys] [GenBank: NM_020134]) located in the second exon of *DPYSL5* leads to a non-conservative valine to lysine substitution at position 41 of DPYSL5 (Figure 1). We subsequently collected seven other unrelated individuals carrying the same *de novo* heterozygous variant as well as one individual whose father was unavailable for genetic analysis (Figures S1–S3). In each participating center, written informed consent was obtained from the individual's parents or legal representatives before blood sampling. The study received approval from the relevant local ethical committees. Clinical information was obtained by review of medical records and examination of affected individuals. When available, the brain MRI scans were reviewed by an expert (W.B. Dobyns), and the majority of them included axial, coronal, and sagittal T1 and T2 sequences at a minimum. Routine clinical genetic and metabolic screenings performed during initial workup were negative in each case, which warranted further investigation on a research basis. All these individuals had severely delayed development with hypotonia (8/8) and severe ID (7/7) and often with absent speech (6/8) and behavioral problems, mainly aggressiveness (5/7). In addition, all individuals had neurological problems consisting of abnormal movements (5/8), ataxia (3/6), and strabismus (5/8) and seizures were observed in three individuals. Head circumference was below the normal range in three individuals (around −3 SD). ACC was observed in all individuals and associated with anomalies of the posterior fossa, consisting mostly of cerebellar hypoplasia (6/7) or Dandy-Walker malformation with encephalocele (1/7). When a careful review of the MRI was possible, hippocampal dysplasia was also noticed in three individuals (Figure 1, Figure S4). No consistent dysmorphic features seem to be recognizable in this series of individuals (Table 1 and supplemental notes).

Additionally, we found a heterozygous missense variant (Chr2[GRCh37]: 27121506 G>A, c.139G>A [p.Gly47Arg] [GenBank: NM_020134]) in the family from the original description of the Ritscher-Schinzel syndrome (RSS [MIM: 220210]), which includes two affected sisters (Figure 1, Table 1).[8,9] One individual was deceased and no genetic material was available for testing. The variant occurred *de novo* in the other affected sister as it was not detected in the parental DNAs, suggesting a likely gonadal mosaicism. Such an inheritance mechanism has been previously proposed for other families with RSS.[10] These two sisters, born to unrelated parents, both presented posterior fossa abnormalities (consisting of Dandy-Walker malformation in one), an atrioventricular canal, and craniofacial dysmorphic features with a large bulging skull.

Both p.Glu41Lys and p.Gly47Arg variants were absent in the control population database Genome Aggregation Databases (gnomAD v.2.1.1, v.3.1),[11] and were predicted to be pathogenic by several *in silico* prediction programs (Table 2). Gene constraint metrics (gnomAD v.2.1.1, 141,456 samples) indicate that *DPYSL5* is intolerant to both protein-truncating variation (pLI = 1) and missense variation (missense Z score = 3.08). Four nonsense or frameshift variants are described in the "non-neuro" v.2.1.1 and v.3.1 gnomAD datasets.

DPYSL5 is strongly expressed in the developing brain and encodes a protein, initially described as an antigen in autoimmune neurological syndromes, belonging to the collapsin response mediator proteins (CRMPs) family.[12–14] These proteins are involved in the regulation of neuronal migration, axonal guidance, dendritic outgrowth, and synapse formation and plasticity during brain development.[14–16] DPYSL5 (or CRMP5) is a cytosolic protein (GenBank: NP_064519.2) relaying semaphorin3A signaling, one of the molecular cues conducting axon and dendrite growth and guidance.[16,17] In adults, DPYSL5 is particularly detected in the midbrain, spinal cord, dorsal root ganglia, Purkinje cells, and in neurogenesis areas of the brain.[17,18] DPYSL5 was also identified as a component of the postsynaptic density proteome,[19] and it was recently described to modulate the surface trafficking and endocytosis of the AMPA receptor subunit GluA2 via the phosphorylation of GluA2 at Serine 880, suggesting a specific function at the glutamatergic synapse.[20]

DPYSL5 can form homo- as well as hetero-tetramers with DPYSL2, 3, and 4 to participate in signal transduction, cytoskeleton rearrangements, and endocytosis.[21] Protein structural modeling analyses using the crystal structure of human DPYSL5 indicated that glutamate 41 and glycine 47 are both located in the same loop on the surface of DPYSL5 monomers and oligomers (Figure 1C). No significant difference in calculated free energy (ΔG) between the wild-type (WT) and variant forms of DPYSL5 was

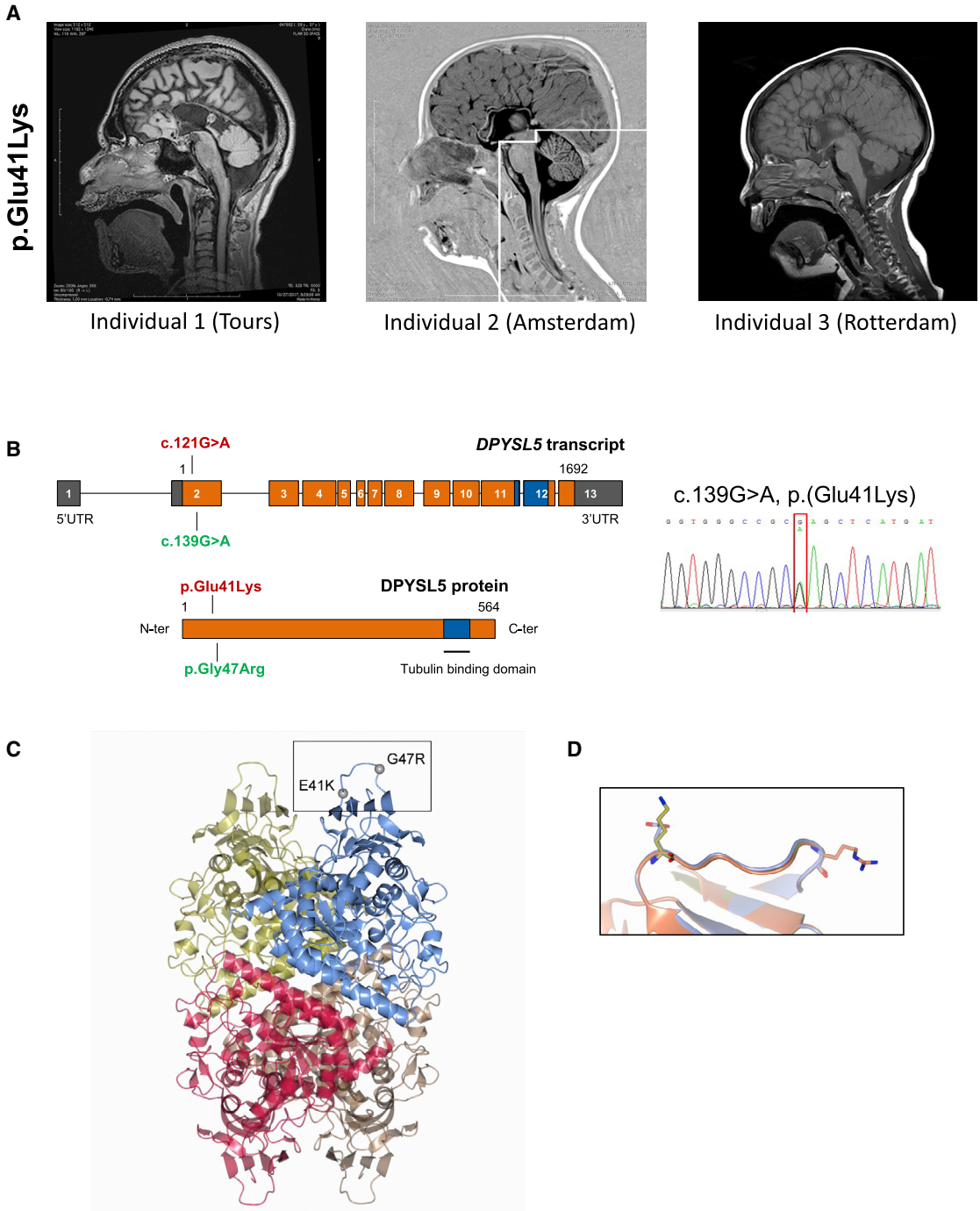

Figure 1. Neuro-anatomical and molecular analyses of *DPYSL5* mutations

(A) Midsagittal brain MRIs of three individuals carrying a *de novo* p.Glu41Lys heterozygous variant of *DPYSL5* revealing complete agenesis of corpus callosum (ACC) and mild cerebellar hypoplasia (individual 1), total ACC with normal cerebellum size but with mild mega cisterna magna (individual 2), and total ACC and mild hypoplasia of the inferior cerebellar vermis (individual 3).

(B) Schematic representation of *DPYSL5* transcript and protein and localization of the two variants p.Glu41Lys (E41K) and p.Gly47Arg (G47R).

(C) Homo-tetramer model of human DPYSL5. The four subunits are shown in ribbon representation and colored differently (green, blue, brown, and red). In one chain (blue), the location of the mutation sites is labeled and indicated by gray spheres.

(D) Close-up view of the mutation site. Mutated residues are shown in stick representation. The crystal structure of human DPYSL5 (4b91) is shown in blue. The modeled structures of E41K and G47R are shown in gold and coral, respectively.

found. The loop is a part of the β sandwich domain and is not involved in interactions between the subunits of the DPYSL5 tetramer (Figures 1C and 1D). The modeled structures are very similar to the WT protein with minimal differences observed in the loop region (Figure 1D). Based on this, the p.Glu41Lys and p.Gly47Arg variants are unlikely

Table 1. Clinical findings of the individuals carrying missense mutations in *DPYSL5*

Individuals	1	2	3	4	5	6	7	8	9 (Ritscher-Schinzel)	10 (sister of 9)
DPYSL5 cDNA	c.121G>A	c.121G>A	c.121G>A	c.121G>A	c.121G>A	c.121G>A	c.121G>A	c.121G>A	c.139G>A	deceased (not tested) N/A
DPYSL5 protein	p.Glu41Lys	p.Glu41Lys	p.Glu41Lys	p.Glu41Lys	p.Glu41Lys	p.Glu41Lys	p.Glu41Lys	p.Glu41Lys	p.Gly47Arg	
Inheritance	htz, *de novo*	htz, *de novo*	htz, *de novo*	htz, *de novo*	htz, *de novo*	htz, *de novo*	htz, *de novo*	htz, *de novo*	htz, gonadal mosaicism suspected	
Gender	M	M	M	F	F	F	F	F	F	F
Age on last examination	33 years	2.5 years	8.5 years	22 years	22 years	4.5 years	17.5 years	5 years	3.5 years	6 years
Prenatal findings	weak fetal movements	N/A	N/A	N/A	N/A	dilated ventricles at 36 weeks	weak fetal movements	None	N/A	N/A
Birth parameters										
Term (weeks)	38	42	42	40	39	39	39	40	37	37 + 4
Birth length/ centile	50 cm/67th	unknown	unknown	unknown	unknown	unknown	49 cm/42nd	53.3 cm/ 90th	51 cm/90th	48 cm/50th
Birth weight/ centile	3,360 g/68th	3,040 g/7th	4,600 g/97th	3,800 g/75th–90th	3,685 g/75th	3,969 g/95th	2,600 g/6th	3,200 g/33rd	3,100 g/75th	3,110 g/50th
Birth HC/ centile	Unknown	unknown	unknown	unknown	unknown	39.5cm/>97th	unknown	unknown	34.8 cm/90th	34.5 cm/75th
Neurodevelopment										
OFC	−2.5 SD	N/A	−0.3 SD	10th centile	−2.2 SD	−0.7 SD	−3.7 SD	26th centile	+3.9 SD hydrocephaly	+1.8 SD
Delayed development	+	+	+	+	+	+	+	+	+	+
Hypotonia	+	+	+	+	+	+	+	+	+	+
ID	+	+	+	+	+	+	+	+	N/A	+
Degree	Severe	severe	severe	severe	severe	N/A	severe	severe	N/A	N/A
Absent speech	+	+	+	−	+	−	+	+	−	−
Sitting	3.5 years	1.5 years	−	−	20 months	11 months	12 months	18 months	N/A	N/A
Walking	7 years	4 years	−	−	−	3 years	5.5 years	5 years (in progress)	N/A	N/A
Toilet trained	−	−	−	−	−	−	−	−	N/A	N/A

(*Continued on next page*)

Table 1. ***Continued***

Individuals	1	2	3	4	5	6	7	8	9 (Ritscher-Schinzel)	10 (sister of 9)
Swallowing difficulties	+	–	+	–	+	+	+	+	–	–
ASD	–	–	–	–	–	+	–	–	N/A	–
Behavioral problems	aggressiveness	–	N/A	sensory processing disorder	aggressiveness	aggressiveness	aggressiveness	impulse control	N/A	–
Abnormal movements	Stereotypic	–	choreatic	–	athetoid and stereotypic	arm extension	–	involuntary movements	N/A	–
Ataxia	N/A	–	–	+	+	+/– (wide gait)	–	N/A	N/A	–
Strabismus	+	–	+	–	+	–	+	+	N/A	–
Seizures	–	–	+ (generalized tonic-clonic)	–	–	+/– (abnormal movements treated with levetiracetam/ normal EEG)	+ (focal seizures)	–	–	–
Brain imaging										
Corpus callosum agenesis	+	+	+	+	+	+	+	+	–	–
Hippocampal dysplasia	+	+	N/A	N/A	N/A	N/A	–	N/A	N/A	N/A
Cerebellar hypoplasia	+	+/–	+	+	+	N/A	–	+	+	+
Hydrocephaly	–	–	–	–	–	N/A	–	–	+	–
Other	–	–	–	–	–	abnormal myelination	DW, encephalocele with intracranial hypertension	–	–	–
Other features										
Short stature	–	–	–	–	+	–	+	+	+	+
Scoliosis	+	–	+	+	+	–	+	–	–	+
Other orthopedic problems	feet malposition, limited extension of the knees	–	premature closure of anterior fontanel	hip dysplasia requiring surgery	hip dislocation	hypermobile joints	declination of the body to the left side	–	11 pairs of ribs, foramina parietalia, large fontanelles	foramina parietalia, large fontanelles
Heart defect	–	–	–	–	–	–	–	–	atrio-ventricular canal	atrial septal defect and cleft of the mitral valve
Abnormal genitalia	micropenis	–	micropenis, cryptorchidism	not examined	–	–	–	–	slightly gaping vulva	slightly gaping vulva

(*Continued on next page*)

Table 1. *Continued*

Individuals	1	2	3	4	5	6	7	8	9 (Ritscher-Schinzel)	10 (sister of 9)
Dysmorphic features	brachycephaly, deep-set eyes, short philtrum, full lips, macrostomia, ulnar deviation of the hands, narrow hands	–	prominent eyes and upper lip, large ears	deep set eyes, hypotelorism, high and narrow palate	plagiocephaly, hypotelorism, deep set eyes, high and narrow palate, tapering fingers	curly hair, hypertelorism, downslanting palpebral fissures, broad nasal root	not specific	N/A	ocular hypertelorism, downslanting palpebral fissures, depressed nasal bridge, apparently low-set ears, narrow palate	ocular hypertelorism, downslanting palpebral fissures, depressed nasal bridge, apparently low-set ears, narrow palate

Abbreviations: M, male; F, female; N/A, not available; htz, heterozygous; SD, standard deviation; ID, intellectual disability; HC, head circumference; OFC, occipitofrontal circumference; DW, Dandy-Walker malformation; ASD, autism spectrum disorder.

to affect either protein stability or oligomeric assembly. However, both variants will alter potential interaction areas of DPYSL5 with other proteins in various ways. The p.Glu41Lys variant changes the charge and hence the electrostatic surface so that potential interaction may not be possible anymore. The p.Gly47Arg again introduces a positive charge thus altering the surface potential. Additionally, the flexibility of the loop will be restricted and a larger side chain is introduced, giving potentially steric problems for interactions as well.

Accumulating evidence indicates that DPYSL5 is an inhibitory regulator of both neurite outgrowth and axonal guidance contributing to neuronal polarity, as well as dendrite development at early developmental stages.[17,22,23] We thus evaluated the impact of both p.Glu41Lys and p.Gly47Arg variants on axonal and dendritic outgrowth of young developing hippocampal primary neuronal cultures transfected (days *in vitro* 4) with plasmids containing EGFP-DPYSL5 WT or variant forms (Figure 2A). We used a pEGFP-C1 plasmid construct containing the full-length human *DPYSL5* cDNA, allowing the production of GFP-DPYSL5 WT fusion proteins.[22] The candidate mutations were then generated via site-directed mutagenesis (see supplemental material and methods). All constructs were initially tested in HEK293T cells and displayed similar DPYSL5 protein levels, indicating that both mutant proteins are stable as expected from the structural calculations (Figure S5). When DPYSL5 WT and variants were transfected in primary neuronal cultures, the total axonal length per neuron was similar between GFP (control), EGFP-DPYSL5 WT, and EGFP-DPYSL5 Glu41Lys or Gly47Arg conditions (Figure 2B). As reported before,[22] the total dendritic length was decreased in EGFP-DPYSL5-WT neurons (Figure 2B). However, both DPYSL5 mutant proteins abolished this inhibitory function on dendrite outgrowth, suggesting a loss-of-function mechanism (Figure 2B). Although the p.Glu41Lys and p.Gly47Arg variants are not likely to affect either protein stability or oligomeric assembly, they could alter the interaction of DPYSL5 with other proteins. Both variants add an exposed positive charge to the electrostatic surface of the protein, which may result in disruption of protein-protein interactions.

To elicit its inhibitory function on neurite outgrowth, DPYSL5 counteracts DPYSL2 by creating a ternary complex with MAP2 and βIII-tubulin, and increased levels of DPYSL5 in primary neuronal cultures transfected with GFP-DPYSL5 inhibit tubulin polymerization and neurite outgrowth.[22,23] We hypothesized that the loss of the inhibitory function of DPYSL5 in neurite outgrowth was related to deficient binding to MAP2 and βIII-tubulin. We transfected the DPYSL5 variants in a cathecholamine A-differentiated (CAD) neuronal cell line prior to performing co-immunoprecipitation (Figure 3A). We found that both variants reduced DPYSL5 binding to MAP2 and βIII-tubulin (Figure 3B), and the p.Gly47Arg substitution increased DPYSL5 binding to DPYSL2. These results

Table 2. *In silico* pathogenicity prediction scores of p.Glu41Lys and p.Gly47Arg variants

Variant	*DPYSL5* variant 1	*DPYSL5* variant 2
Mutation data		
Mutation type	missense	Missense
Nucleotide change	c.121G>A	c.139G>A
Predicted amino acid change	p.Glu41Lys	p.Gly47Arg
Exon	2	2
***In silico* predictions**		
SIFT	deleterious (score 0.02)	deleterious (score 0.02)
MutationTaster	disease causing (p value: 1)	disease causing (p value: 1)
PolyPhen-2 (Hum-Div)	benign (score 0.012)	probably damaging (score 1)
CADD_phred	24.5	27.9

suggest that the p.Glu41Lys and p.Gly47Arg variants impair DPYSL5 function by preventing the formation of the ternary complex with MAP2 and βIII-tubulin. This would allow DPYSL2 to keep promoting neurite outgrowth because of loss of inhibition by DPYSL5. Because the residues Glutamate 41 and Glycine 47 of DPYSL5 are not located at the direct interface between monomers of a putative DPYSL5/DPYSL2 heterotetramer, it is unclear how the p.Gly47Arg variant impacts DPYSL5 interaction with DPYSL2 while the p.Glu41Lys variant does not. Recent structural studies suggested that the C-terminal domain of DPYSL2 wraps around the next monomer to stabilize

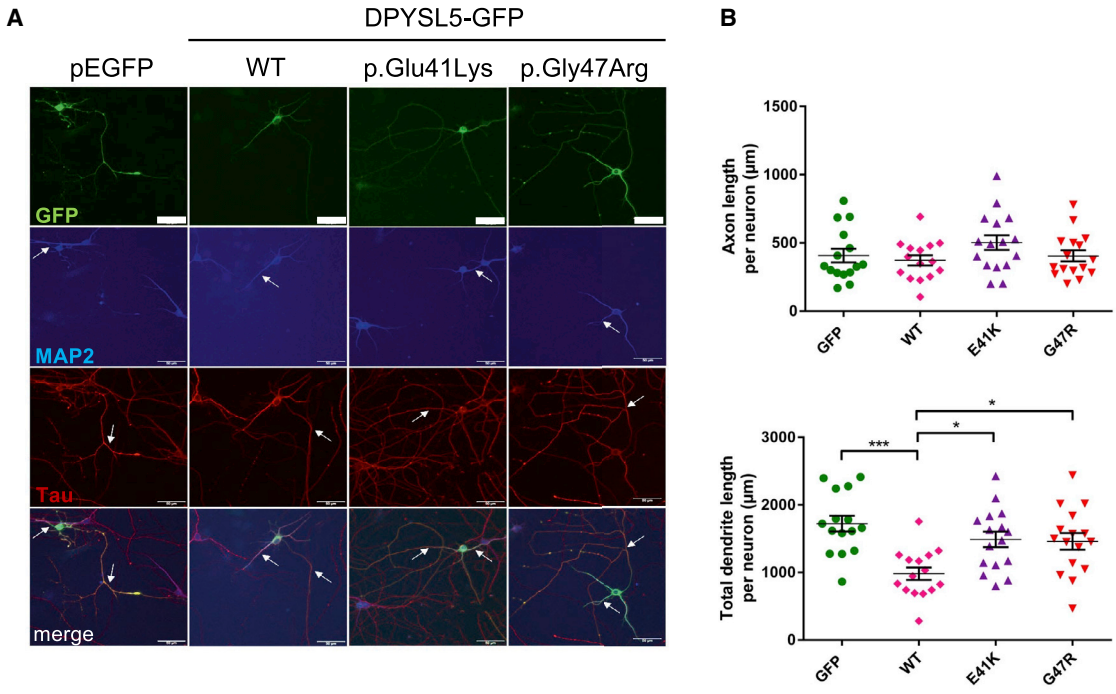

Figure 2. Functional analyses of the impact of DPYSL5 variants on neuronal development

(A) Confocal microscopy images of neurons at 6 days of *in vitro* culture (D.I.V.) transfected at 2 D.I.V. with pEGFP (GFP), pEGFP-DPYSL5 (WT), pEGFP-DPYSL5 p.Glu41Lys (E41K), and pEGFP-DPYSL5 p.Gly47Arg (G47R) and labeled with anti-Tau (axon) and anti-MAP2 (dendrites) antibodies. Scale bar, 50 µm.

(B) Graphical representations of the axon and dendrite length measures in dot plots, each plot corresponding to one neuron for which the axonal length and the total dendritic length were measured. A one-way ANOVA parametric test with multiple comparisons (Tukey's post hoc) was performed and found to be non-significant for the axon length ($p = 0.2272$; $F = 1.488$; R-squared = 0.07148) and significant for total dendritic length ($p = 0.0003$; $F = 7.377$; R-squared = 0.2762). Three independent transfections including 15 neurons "pEGFP" (solid green circle), 15 neurons "EGFP-DPYSL5 WT" (pink diamond), 16 neurons "GFP-DPYSL5 E41K" (purple triangle), and 16 neurons "EGFP-DPYSL5 G47R" (red triangle). *$p = 0.0201$ (WT versus G47R) or $p = 0.0121$ (WT versus E41K); ***$p = 0.0001$ (GFP versus WT). Data are shown as mean ± SEM.

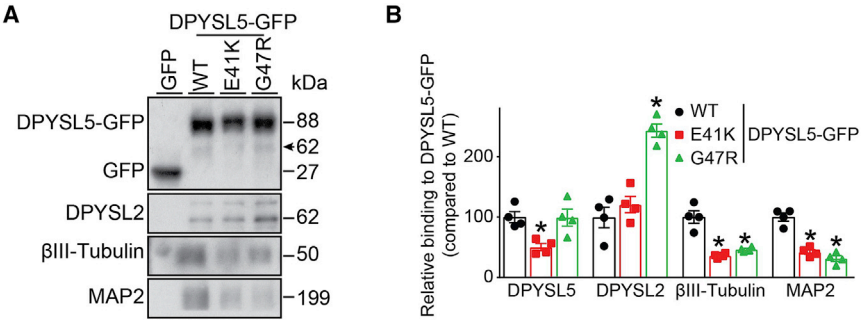

Figure 3. The p.Glu41Lys and p.Gly47Arg variants impair the physical interaction of DPYSL5 with the neuronal cytoskeleton-associated proteins MAP2 and tubulin βIII

(A) Representative immunoblots showing GFP-DPYSL5 or GFP immunoprecipitation and co-immunoprecipitation of DPYSL5-associated proteins DPYSL2, endogenous DPYSL5 (indicated with an arrow), βIII-tubulin, and MAP2 from CAD cells transfected with the indicated DPYSL5 mutants or GFP as a negative control.

(B) Bar graph with scatterplots showing relative binding of the indicated proteins to GFP-DPYSL5. Both E41K and G47R mutations decreased binding to βIII-tubulin and MAP2. DPYSL5-E41K showed decreased binding to endogenous DPYSL5 and DPYSL5-G47R had increased binding to DPYSL2 compared with wild type (WT). *$p < 0.05$, Kruskal-Wallis test with Dunn's post hoc. Data are shown as mean ± SEM ($n = 4$).

this protein complex.[24] We hypothesize that the p.Gly47Arg variant may be located in the vicinity of one of the binding sites on the DPYSL5 monomer for binding to DPYSL2, although no 3D structure for the C-terminal domain of DPYSL2 is available to test this possibility.

In order to identify other *de novo* candidate mutations in *DPYSL5*, we analyzed the denovo-db database (current release v.1.6.1, March 2021 accessed) and we discovered two other missense variants, p.Asp81Asn and p.Val364Ile, previously described in large cohorts of individuals with NDDs, but detailed clinical phenotypes of these individuals are not available.[25,26] However, the p.Asp81Asn variant has been detected in 10 and 13 individuals from the non-neuro gnomAD and from the total datasets of gnomAD, respectively, thus questioning its deleterious impact. The variant c.1090G>A, (p.Val364Ile) is absent from gnomAD and dbSNP databases. It is predicted to be benign by SIFT and PolyPhen-2 *in silico* tools, whereas MutationTaster indicates it would be disease causing. According to the Splice Site Finder-like, MaxEntScan, and GeneSplicer programs, an impact of this variant on splicing could not be excluded. Protein structural modeling analyses revealed that the modeled structure and free energy of the mutant protein is very similar to the WT protein (data not shown).

There is overwhelming evidence about the critical roles of the DPYSL/CRMPs in brain developmental processes such as axonal growth and guidance, dendrite branching, organization and plasticity, and synapse activity and maturation through their interaction with different types of neuronal molecules (cytoskeleton-associated proteins, synaptic receptors, ion channels).[16,27,28] Besides their developmental function (i.e., regulation of axonal growth), the DPYSL/CRMPs proteins have been also detected in synaptic fractions and might play a significant role in synaptic plasticity and activity.[18,29,30]

Furthermore, multi-omics translational approaches indicated that CRMPs expression (RNA, protein) is altered in various human brain disorders, including mental (schizophrenia, mood disorders) and neurological (Alzheimer, prion encephalopathy, epilepsy) conditions.[16] At the genetic level, several missense variants reported in denovo-db have been described in individuals with autism spectrum disorder (ASD) for *DPYSL2* (p.His438Asn and p.Arg601Cys) and *DPYSL3* (p.Val139Ile and p.Ser541-Tyr).[26,31,32]

The study of homozygous *Dpysl5* knockout (KO) mouse models (*Dpysl5*$^{-/-}$) revealed abnormal dendritic development of Purkinje cells during the period of dendritic branching at postnatal day 21 (P21) and P28, but not at P14, and altered electrophysiological activity between parallel fibers and Purkinje cells in adult *Dpysl5*$^{-/-}$ mice.[18] Lastly, they display abnormal limb-clasping reflexes that are also consistent with abnormal central synaptic function.[18] These data should however be considered carefully with regard to the missense p.Glu41Lys and p.Gly47Arg variants leading to a possible dominant-negative effect and a different pathophysiological outcome (i.e., brain neuroanatomical defects, ID) because no abnormalities in brain cyto-architecture were described in both heterozygous and full KO mouse models.[18]

Importantly, all individuals with the p.Glu41Lys variant had ACC—a feature that seems to be a hallmark of DPYSL5 deleterious impact—associated, in cases where a careful examination of brain MRI was possible, with infratentorial anomalies such as hypoplasia of vermis and cerebellar hemispheres. Posterior fossa abnormalities are also cardinal features of the Ritscher-Schinzel syndrome, suggesting that *DPYSL5* mutations may cause a spectrum of central nervous system defects, particularly as agenesis of the corpus callosum is not a feature of this condition. On the basis of the cardinal features described in the original family, and further clinical reports published in the following years, the name of cranio-cerebello-cardiac or 3C syndrome was proposed, and additional signs were described. Currently, it is admitted that the clinical criteria of Ritscher-Schinzel syndrome are craniofacial features with a prominent occiput and/or forehead, cerebellar defects, generally associated with a variable degree of ID, congenital heart malformation, and in some cases cleft palate or ocular coloboma (see "GeneReviews" in web resources). However, this condition is very heterogeneous both clinically and genetically.[33] Some cases were proven to be

caused by chromosome anomalies such as 6p terminal microdeletion,[10] and in the last years, bi-allelic pathogenic variants have been reported in *WASHC5* in individuals of First Nations heritage from Canada,[34] hemizygous variants in *CCDC22*,[35] and more recently, bi-allelic variants in *VPS35L*.[33] It is therefore important to note that a mutation in *DPYSL5* is the genetic cause of the condition observed in the original family, thus raising the question of the relevance of considering the Ritscher-Schinzel syndrome as a unique entity.

It is also interesting to note that the affected members from this family had a cardiac defect, but the role of *DPYSL5* in the developing heart is unknown. A recent study (BBI Allen single cell atlases), which reported single-gene expression for several tissues during human fetal development, indicated that *DPYSL5* is expressed at very low levels in the fetal heart (<11 TPM) in contrast to its high expression in brain (362 TPM) and cerebellum (287 TPM).[36] In the adult, *DPYSL5* is exclusively expressed in the brain, according to GTEx portal data. We cannot either exclude the association of two different conditions in this family.

In conclusion, through a multi-center collaboration, we identified two pathogenic missense mutations in *DPYSL5*, including a recurrent *de novo* variant carried by eight individuals. The variants cause a defective inhibitory regulation of neurite outgrowth and dendrite development, and they are responsible for a severe syndromic form of NDD/ID with brain malformations. This study highlights the importance of DPYSL/CRMPs in neuronal development and shows how alterations of their regulatory functions lead to brain dysfunction. The generation and analysis of neuronal (iPSC-derived neurons) and animal models expressing the p.Glu41Lys or p.Gly47Arg variants of DPYSL5 will most likely provide a detailed characterization of their pathophysiological consequences on brain developmental architecture.

Data and code availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.ajhg.2021.04.004.

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Declaration of interests

The authors declare no competing interests.

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Web resources

BBI Allen single cell atlases, https://descartes.brotmanbaty.org/
dbSNP, https://www.ncbi.nlm.nih.gov/snp/
denovo-db, Seattle, WA, http://denovo-db.gs.washington.edu/denovo-db/
GeneReviews, Elliott, A.M., and Chudley, A. (2020). Ritscher-Schinzel syndrome, https://www.ncbi.nlm.nih.gov/books/NBK553049/
gnomAD, https://gnomad.broadinstitute.org
GTEx portal, https://www.gtexportal.org/home/
Online Mendelian Inheritance in Man (OMIM), https://omim.org/
ReqSeq accession number for *DPYSL5*, https://www.ncbi.nlm.nih.gov/gene/56896

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