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# *PPP2R5E*: new gene potentially involved in specific learning disorders and myopathy

**Antonino Musumeci[‡a], Mirella Vinci[‡a], Iris Verbinnen[b], Simone Treccarichi[a], Eleonora Nigliato[b], Valeria Chiavetta[a], Donatella Greco[a], Girolamo Aurelio Vitello[a], Concetta Federico[c], Veerle Janssens[b], Salvatore Saccone[c]*, and Francesco Calì[a]**

[a] *Oasi Research Institute-IRCCS, via Conte Ruggero 73, 94018 Troina (EN), Italy.*

[b] *Laboratory of Protein Phosphorylation & Proteomics, Department of Cellular & Molecular Medicine,University of Leuven (KU Leuven), Herestraat 49, PO-box 901, B-3000 Leuven, Belgium.*

[c] *Department of Biological, Geological and Environmental Sciences, University of Catania, Via Androne 81, 95124 Catania, Italy.*

[‡]These authors contributed equally to this work.

* Correspondence: salvatore.saccone@unict.it

## Abstract

Protein phosphatase 2A (PP2A) is a family of multifunctional enzymatic complexes crucial for cellular signalling, playing a pivotal role in brain function and development. Mutations in specific genes encoding PP2A complexes have been associated with neurodevelopmental disorders with hypotonia and high risk of seizures. In the current work, we present an individual with specific learning problems, motor coordination disorders, hypotonia and behavioural issues. Although whole exome sequencing (WES) did not unveil pathogenic variants in known genes related to these symptoms, a *de novo* heterozygous variant Glu191Lys was identified within *PPP2R5E*, encoding the PP2A regulatory subunit B56ε. The novel variant was not observed in the four healthy brothers and was not detected as parental somatic mosaicism. The mutation predicted a change of charge of the mutated amino acid within a conserved LFDSEDPRER motif common to all PPP2R5 B-subunits. Biochemical assays demonstrated a decreased interaction with the PP2A A and C subunits, leading to disturbances in holoenzyme formation, and thus likely, function. For the first time, we report a potential causal link between the observed variant within the *PPP2R5E* gene and the symptoms manifested in the subject, spanning specific learning problems and motor coordination disorders potentially associated with myopathy.

**Keywords**: PP2A; PPP2R5E; B56ε; hypotonia; neurodevelopmental delay; myopathy.

## 1. Introduction

Protein phosphorylation is a vital mechanism in eukaryotic organisms, regulating diverse signaling pathways crucial for cell function. This post-translational modification, involving kinases and phosphatases, impacts metabolism, transcription, cell cycle, differentiation, cytoskeleton organization, apoptosis, and intercellular communication, including neuronal and immunological functions (**Johnson et al., 2009; Floyd et al., 2021; Bilbrough et al., 2022**).

Among these modifying enzymes, Protein Phosphatase type 2A (PP2A) is noteworthy, existing as a heterotrimer composed of catalytic (PP2A-C), structural scaffold (PP2A-A), and regulatory (PP2A-B) subunits. PP2A's role extends to neuronal functions, facilitated by its partially nuclear localization (**Glatter et al., 2009; Leonard et al., 2020; Verbinnen et al., 2021; Sandal et al., 2021**). Furthermore, PP2A complexes actively regulate muscle development, contraction, and metabolism (**Terry et al., 2006; Park et al., 2013; Nardi et al., 2014; Labuzan, 2019**), influencing processes such as myogenesis, muscle cell differentiation, and the regulation of proteins involved in muscle function. Dysregulation of PP2A has been implicated in muscle-related disorders and diseases, including (cardio)myopathy (**Nemazanyy et al., 2013; Lubbers and Mohler, 2016; Reynhout and Janssens, 2019**). Moreover, *de novo* mutations of *PPP2CA*, *PPP2R1A* and *PPP2R5D* genes can be causally associated to a broad range of neurologic diseases related to brain neurobiology and development (**Houge et al., 2015**; **Reynhout et al., 2019; Verbinnen et al., 2021**). Specifically, PPP2R5D-related disorder is characterized by early childhood onset, presenting with intellectual disability, hypotonia, autism spectrum disorder (ASD), macrocephaly, seizures and dysmorphic features (**Houge et al., 2015**; **Shang et al., 2016; Biswas et al., 2020; Papke et al., 2021; Oyama et al., 2023**).

In the wide array of proteins engaged by the PP2A heterocomplex, *PPP2R5E* stands out as a member within the B subunit family genes, encoding for the regulatory subunit B56ε. *PPP2R5E* has previously been linked to the regulation of embryonic (brain) development and Wnt signaling (**Yang et al., 2003; Jin et al., 2010; Vinyoles et al., 2017**). *Ppp2r5e* knockout mice are viable but show a delay in neonatal growth (**Dyson et al., 2021**). Moreover, downregulation of *PPP2R5E* has been associated with cancers (**Cristobal et al., 2013; Liu et al., 2014**) underscoring its tumor suppressive function in specific tissue contexts. PP2A-B56ε also inhibits nutrient signaling to mTOR through direct dephosphorylation of MAP4K3 (**Yan et al., 2010**). In addition, PPP2R5E phosphatase likely orchestrates microtubule organization by the stabilization of Microtubule Crosslinking Factor 1 (MTCL1) (**Hyodo et al., 2016**) as well as spindle organization by the dephosphorylation of specific kinesins (**Nunes Bastos et al., 2014**). Interestingly, recent studies have also associated mutations within *PPP2R5E* as potentially linked to mild ID and global developmental delay (GDD) (**Murcia Pienkowski et al., 2019; Sandal et al., 2021**).

The aim of the current study is to highlight, for the first time, a potential causative association between an identified novel *de novo* mutation within the *PPP2R5E* gene and specific learning disorders (SLDs) and motor coordination disorders observed in an examined individual.

## 2. Materials and methods

### 2.1. Libraries preparation for WES

Genomic DNA was isolated from peripheral blood leukocytes obtained from both the subject and the healthy parents, as previously described (**Vinci et al., 2023**). Whole exome sequencing (WES) was carried out employing the Ion AmpliSeq™ Exome RDY kits, following the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA, USA). The quality of libraries was assessed using DNA 1000 chips on the Tape Station 4200 (Agilent, Santa Clara, CA, USA) and Qubit dsDNA BR Assay kits (Invitrogen, Waltham, MA, USA). Template preparation, clonal amplification, recovery, and enrichment of template-positive Ion Sphere™ particles and loading of sequencing-ready Ion Torrent semiconductor chips were performed with the Ion Chef™ system (Thermo Fisher Scientific, Waltham, MA, USA). Finally, each loaded Ion 550™ chip on the S5 system (Thermo Fisher Scientific, Waltham, MA, USA) was sequenced. Overall, 98% of regions of interest have a minimum coverage of at least 20X. Data of runs were processed using the Ion Torrent Suite 5.16, Variant Caller 5.16, Coverage Analysis 5.16 (Thermo Fisher Scientific, Waltham, MA, USA), Ion Reporter (Thermo Fisher Scientific, Waltham, MA, USA), and/or wANNOVAR tools (**Chang and Wang, 2012**). DNA sequences were displayed using Integrated Genomics Viewer (IGV) (**Thorvaldsdottir et al., 2013**). Pathogenic variant was confirmed through conventional Sanger sequencing (Applied Biosystems Prism 3130 DNA Analyzer, ThermoFisher Scientific, Waltham, MA, USA), and generated employing the specific National Center for Biotechnology Information (NCBI) web tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). DNA fingerprint analysis was performed from the mother, the child and the father to confirm the *de novo* event, according to a previous protocol (**Calì et al., 2002**). To confirm the detected nucleotide variant, Sanger sequencing was performed using the BigDye Terminator v1.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Foster City, CA, USA), as previously described (**Musumeci et al., 2022**). Primers were: for. 5'-TCCTTTTCTGAAACCGCCTTTG-3', rev. 5'-CTTCTTCCCCCATCACACTCC-3'.

### 2.2. Data analysis

The observed nucleotide variation was classified according to the "American College of Medical Genetics" (ACMG) guidelines (**Richards et al., 2015**) and it was performed with VarSome (**Kopanos et al., 2019**). The list of the criteria employed for classifying the variant were described in the **Supplementary materials (Table S1)**. The models for both the PP2A complex and the PPP2R5E protein were predicted using UCSF ChimeraX (version 1.7, Resource for Biocomputing, Visualization, and Bioinformatics – RBVB,

University of California, San Francisco), as described in a previous study (**Pettersen et al., 2021).** BioEdit software version 7.2 was used for sequence alignment to compare PPP2R5E with other PPP2R5 proteins. The percentage of identity between PPP2R5E and the other PPP2R5 proteins was calculated using the Uniprot sequence alignment tool (https://www.uniprot.org/) (accessed on 25 August 2024). The plot depicting the percentage of identity was generated with R Studio version 3.4.3., adopting ggplot2 and pheatmap packages. The expression patterns of *PPP2R5E* gene were analysed by The Human Protein Atlas (https://www.proteinatlas.org, accessed on 8 February 2024).

### 2.3. Biochemical assays

The identified *de novo PPP2R5E* variant (p.E191K) was generated by PCR-based site-directed mutagenesis using a eukaryotic B56ε pEGFP-C1 expression plasmid as template (**Lenaerts et al., 2021**) and two complementary primers harboring the mutation (for. 5'-GGAGCTATTTGACAGCAAAGACCCTCGGGAACGGG-3' and rev. 5'-CCCGTTCCCGAGGGTCTTTGCTGTCAAATAGCTCC-3'). Following transfection of pEGPF-C1 (negative control), B56ε wildtype (WT) pEGFP-C1 (positive control) and B56ε E191K pEGFP-C1 in HEK239T cells, binding of endogenous PP2A A and C subunits was assessed in GFP trapping experiments, as previously described for *PPP2R5D* variants (**Oyama et al., 2023**).

## 3. Results

### 3.1. Clinical report

The subject is a male child born to healthy, non-consanguineous parents, with four healthy siblings. He was delivered at 38 weeks' gestation via normal delivery following a pregnancy marked by threatened miscarriage in the third trimester. At birth, his weight was 3250 g, and there were no indications of perinatal distress. He commenced walking at the age of one but experienced daytime and nighttime enuresis until the age of four. At 12 years old, he received a diagnosis of a motor coordination disorder and mixed learning disabilities. Array Comparative Genome Hybridization (aCGH) performed at age of 12 revealed no genomic imbalances. The subject displayed an elongated face with a high forehead, and normal head circumference (50th percentile). Brain Magnetic Resonance Imaging (MRI) showed no signs of parenchymal distress or malformations, although a slight asymmetry in the lateral ventricles was noted due to a minor increase in the right ventricular volume. The head circumference was within the normal range, at the 50th percentile both at birth and currently. At the age of 14, he was admitted to the hospital due to intestinal obstruction, which was found to be associated with dolichosigmoid. The individual experienced constipation issues both before and after the hospital admission. At 15 years old, both Holter ECG and echocardiography yielded normal results. Prior to hospitalization, he showed difficulties in reading, writing, and arithmetic, as well as social challenges, fine motor skill issues, sleep disturbances, and

increased fatigue. Upon examination during his hospital stay at 16 years old, he displayed an asthenic physique with sunken shoulders and drooping arms, abdominal muscle hypotonia, right-convex dorsal spine scoliosis, dorsal hyperkyphosis, and ventral hyperlordosis. Electroneurography (ENG) showed normal findings while the Electromyography (EMG) examination revealed, at rest, clear denervation activity in the right biceps brachii (BB) and vastus lateralis (VL) muscles and membrane instability in all remaining muscles examined. During voluntary contraction, the electromyographic pattern showed Motor Unit Potentials (MUPs) of low amplitude and polyphasic morphology mixed with MUPs of slightly increased amplitude and duration, as an expression of a reorganization of the Motor Unit. Automated interference pattern analysis (IPA, "Willison analysis turns/amplitude) and Quantitative assessment of the motor unit potential (QMUP) were performed on the BB and VL muscles which confirmed the significant prevalence of low amplitude MUPs and an increase in polyphasic potentials (Turns > 30%). Neurography excluded involvement of the peripheral nerve trunks, strengthening the hypothesis of a primary muscular pathology (**Tab. 1**).

**Table 1**. Variation of the electromyography (EMG) parameters among the different nerves examined.

| Nerves Examined | SNAP | | | MAP | | |
|---|---|---|---|---|---|---|
| | CV (m $s^{-1}$) | AMP (mV) | LAT (ms) | CV (m $s^{-1}$) | AMP (mV) | LAT (ms) |
| MEDIAN R (elbow-wrist) | 68 | 118 | 2.2 | 62 | 8 | 2.5 |
| EPSN R (knee-ankle) | - | - | - | 57 | 11 | 2.3 |
| SURAL R (calf-ext. mall.) | 64 | 43 | - | - | - | - |

MEDIAN R: right median nerve; EPSN: external popliteal sciatic nerve; SURAL R: right sural nerve; SNAP: sensory action potential; MAP: motor action potential; CV: conduction velocity; AMP: amplitude; LAT: latency.

The electrophysiological findings (EMG, ENG) are indicative of primary muscular distress. Psychological evaluation unveiled SLDs, motor coordination disorders (**Henderson et al., 2007**), and emotional dysregulation primarily manifested through mood swings and anxiety. SLDs were identified through MT tests, commonly utilized in Italy to evaluate reading, text comprehension, and mathematical skills in individuals aged 6 to 16 (**Cornoldi and Colpo, 2012**).

### 3.2. Next generation sequencing

WES analysis unveiled a novel heterozygous *de novo* variant, specifically c.571G>A, within the *PPP2R5E* gene (NM_006246). The observed amino acid variation in the PPP2R5E protein was located in an extra domain region at position 191 (p.Glu191Lys) of the amino acid chain. In correspondence of the variant position, the total reads count ascertained for the individual was 241, showing ~54% of Wild Type allele (G) and ~45% of the *de novo* allele (A). It was not detected as parental somatic mosaicism; in fact, only wild type allele (G) accounted for a total of 298 and 256 number of reads for the father and mother respectively. Sanger sequencing conducted on both the subject and the parents confirmed the presence of

the identified *de novo* nucleotide variation (**Fig. 1**). Remarkably, the novel variant, as expected, was not observed in the four healthy brothers.

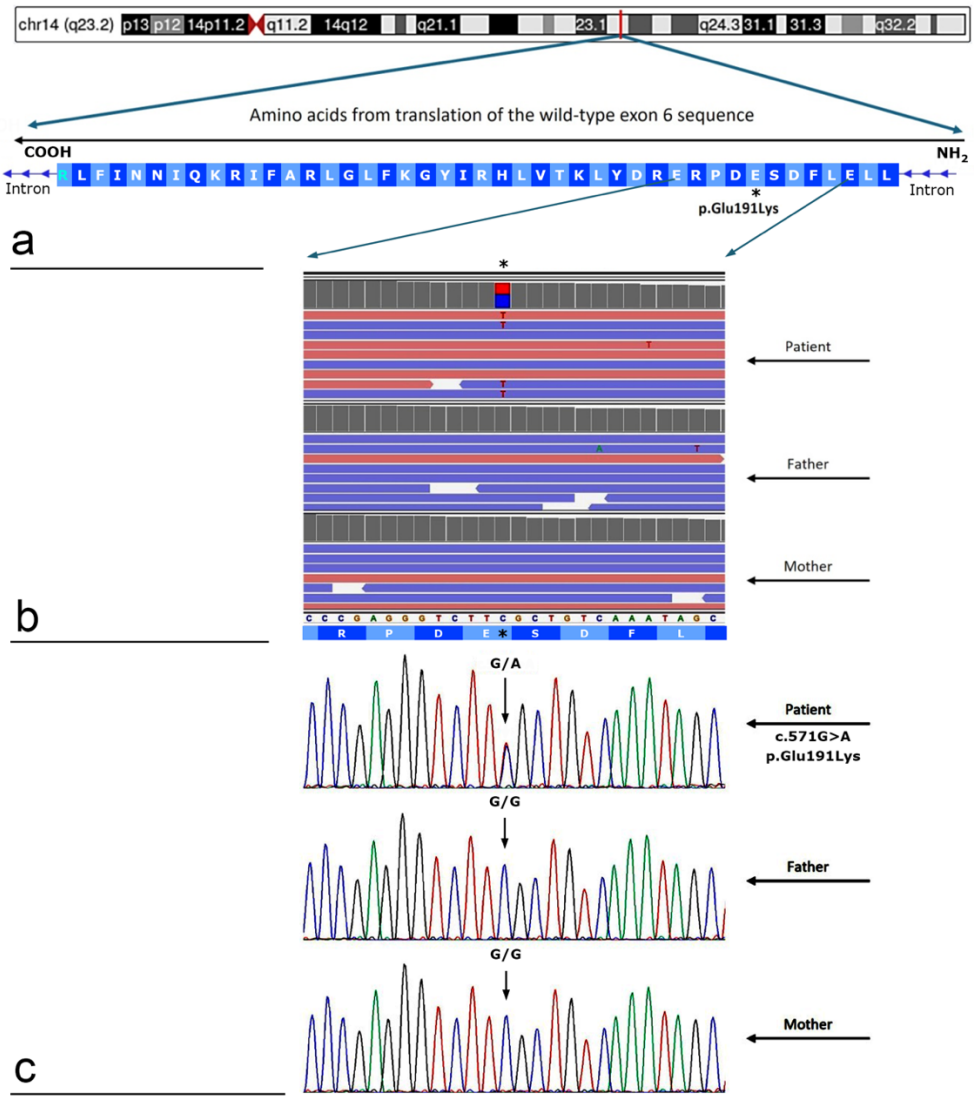


**Figure 1. Description of the observed variant within the *PPP2R5E* gene**. (a) Graphical representation of the amino acid sequence related to the translation of the DNA sequence from the exon 6. Horizontal arrow above the amino acidic sequence indicates NH2 → COOH direction. The representation was retrieved and modified from UCSC Genome Browser (http://genome.ucsc.edu, accessed on 18/03/2024). (b) Variant c.571G>A detected through WES in the subject, and not in the father, and the mother. Images were obtained by the Integrative Genome Viewer (IGV). Asterisks indicate the amino acid variation (p.Glu191Lys) within PPP2R5E. (c) Variant confirmation through Sanger sequencing for the variant c.571G>A. Figure 1c displays a portion of the amplicon generated by the reverse primer. The genotype of subject, father, and mother are indicated in the electropherogram.

### 3.3. In silico variant prediction

Several algorithms employed for *in-silico* prediction analysis hinted to the pathogenic significance of the identified variant (**Tab. S2 and Tab. S3**). Specifically, 6 tools (BayesDeladdAF, EIGEN, EIGEN PC, LIST-S2, Mutation assessor and PrimateAI) classified it as 'pathogenic moderate'; 9 tools (BayesDelnoAF, MetaRNN, CADD, DANN, DEOGEN2, FATHMM-MKL, LRT, MutPred and SIFT) as

'pathogenic supporting'. In contrast, 7 tools classified the mutation as 'of uncertain significance' (MetaLR, MetaSVM, BLOSUM, FATHMM-XF, MutationTaster, PROVEAN and SIFT4G), while 3 tools described it as 'benign supporting' (REVEL, M-CAP and MVP). Additionally, the mutated residue (Glu) introduces a negative charge, whereas the wild-type amino acid (Lys) carries a positive charge. This mutation occurs within a conserved LFDSEDPRER motif present in all PPP2R5 subunits (**Fig. S1**). As clearly depicted in **Figure S2**, the similarity between PPP2R5E and the five PPP2R5 proteins ranges from 63.09% with PPP2R5A to 77.29% with PPP2R5D.

The *in-silico* predictive analysis conducted by DOMINO identified an autosomal dominant (AD) inheritance pattern, assigning a probability score of 0.8602 for autosomal dominance. This score suggests a highly probable dominant inheritance model.

### 3.4. Biochemical analysis showed decreased PP2A trimeric complex formation for the variant

To test whether PP2A-B56ε holoenzyme formation might be affected, wildtype (WT) *PPP2R5E* and the p.E191K *PPP2R5E* variant were expressed as GFP-tagged proteins in HEK293T cells and assessed for their ability to interact with endogenous PP2A A and C subunits (**Fig. 2**).

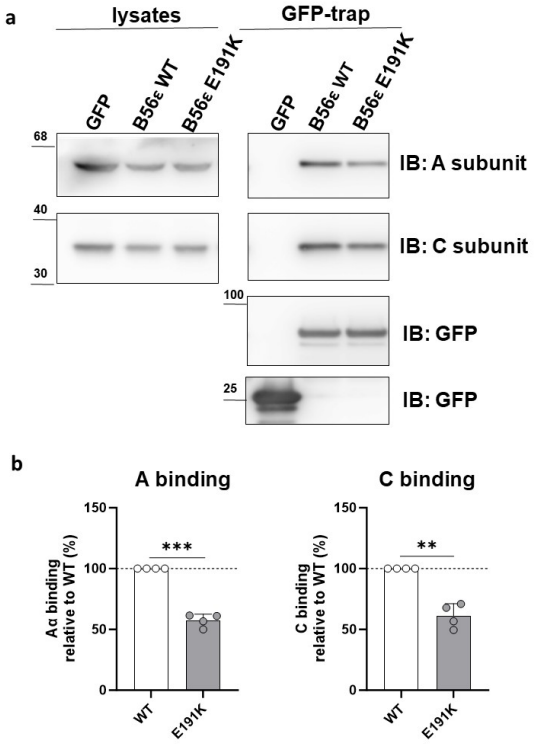


**Figure 2. Functional characterization of the *PPP2R5E* p.E191K variant.** (a) GFP, GFP-tagged wildtype (WT) B56ε and GFP-tagged mutant B56ε were purified from transfected HEK293T cells by GFP-trapping. Interaction of co-precipitating endogenous PP2A A and C subunits was determined by immunoblotting (IB) with antibodies against GFP, PP2A A and PP2A C. A representative assay of four independent experiments is shown. (b) Results were quantified and depicted as the average ± SD of the ratios of the quantified endogenous protein signal to the quantified GFP signal in ratio to the WT B56ε interaction (set to 100% in each experiment), for n=4. A one-sample t-test (compare to 100%) was used to determine statistical significance (**: p≤0.01; ***: p≤0.001).

Upon GFP-trapping, a statistically significant binding defect of up to 50% was found for both the A and the C subunit, not only suggesting impaired PP2A holoenzyme formation, but also underscoring a dominant-negative effect of the variant, as demonstrated before for the PPP2R5D-related disorder (**Houge et al., 2015**).

## 4. Discussion

In this study, we report the clinical profile of a 20-year-old male individual presenting a broad spectrum of symptoms, including SLDs, mood and emotional dysregulation, hypotonia, and motor coordination disorders. Moreover, EMG revealed the presence of myopathy. WES did not uncover mutations in genes previously associated with the subject's symptoms. However, the identified *de novo* missense variation in *PPP2R5E* resulted in a charge-reversing alteration of the PP2A B56ε protein at position Glu191 (Glu was changed into a Lysine, p.E191K). The charge alteration occurred within the highly conserved LFDSEDPRER motif, which is present in all PPP2R5 proteins (**Fig. S1**). Currently, the *PPP2R5E* gene does not have an MIM phenotype entry code, which associates the gene with a specific phenotype; in fact, it was rarely reported in the HGMD database. The only three variants associated to ID, ASD and DD were classified as "DM?" indicating some degree of doubt. Furthermore, DOMINO analysis indicated a high probability rate (0.8602) for autosomal dominant inheritance of the observed variant. Parental somatic mosaicism was not observed in our case, and the variant was not detected in the four healthy brothers. The *in-silico* predictions reported in **Table S2,** described the variant as likely pathogenic, and this was experimentally confirmed in our A/C binding assays, demonstrating significantly decreased PP2A holoenzyme formation, and thus, a likely dominant-negative disease mechanism (**Fig. 2**).

To provide a structural explanation for the observed binding defects, we generated a graphical prediction through analysis of macromolecular structures and sequences related to the PP2A complex (PPP2R5E, PPP2R1B and PPP2CA) by UCSF ChimeraX (**Fig. 3**).

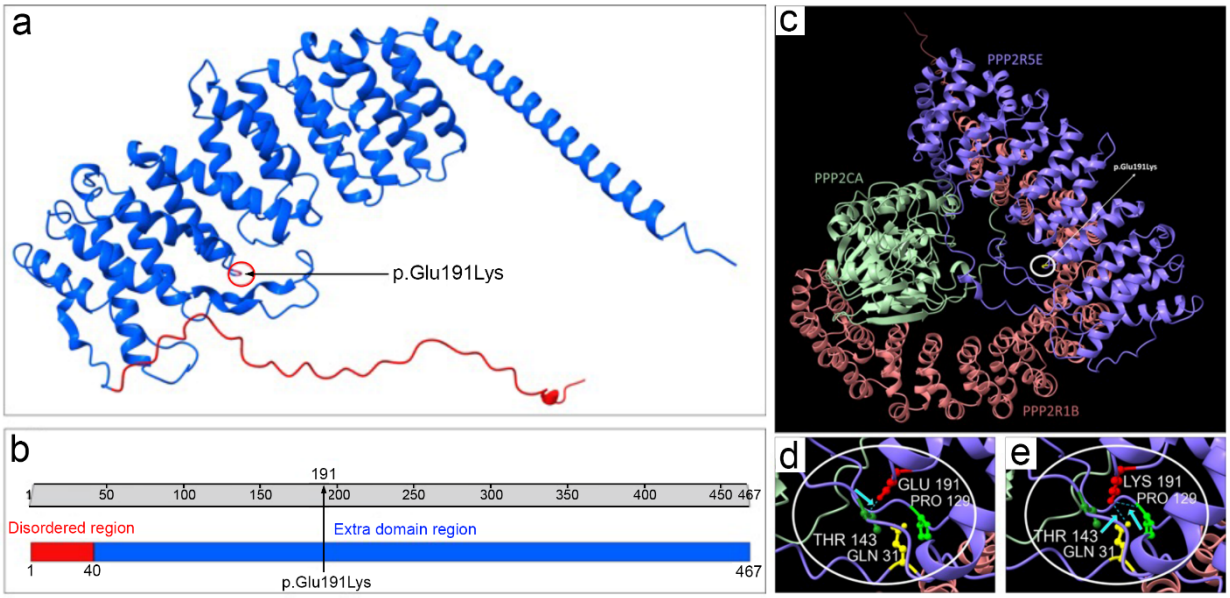


**Fig. 3. PPP2R5E protein organization and domains.** (a) Graphical representation of the entire PPP2R5E protein with different colours for each domain. (b) Domain organization underscoring with a specific label the mutation site, located in the extra domain region. The protein prediction model was developed using UCSF ChimeraX while Figure 3b was retrieved and modified from Uniprot database (https://www.uniprot.org/ accessed on 18/03/2024). (c) PP2A complex: PPP2R5E (purple chain), PPP2R1B (pink chain) and PPP2CA (green chain). (d) Model of the wild-type amino acid (Glu) at position 191 of the amino acid chain, displaying only one hydrogen bond with the amino acid at position 143 (Thr). (e) Model of the mutated amino acid (Lys) at position 191, exhibiting two interprotein hydrogen bonds (one less more the wild-type protein). Precisely, the hydrogen bonds were established with the aa 31 (Gln) and the aa 129 (Pro) of the same polypeptide chain (Purple).

First, this model showed that the amino acid variant p.Glu191Lys is located in one of the intrahelical loops of the PPP2R5E protein in a structural position relevant for the interaction with other subunits of the PP2A complex (**Fig. 3a-3c**). Second, the sequence-structure analysis revealed 1345 and 1330 hydrogen bonds within the PP2A complex for the PPP2R5E-Glu191 (WT) and PPP2R5E-Lys191 (mutated), respectively. Specifically, the substitution of glutamic acid (Glu) with lysine (Lys) led to an increase of intra-protein hydrogen bonds, from one to two at this position (**Fig. 3d and 3e**). In particular, the wild type PPP2R5E exhibited one intra-protein hydrogen bond between Glu191 and a threonine (Thr) at position 143 (**Fig. 3d**), while the mutated PPP2R5E exhibited two intra-protein hydrogen bonds involving Lys191: one with a glutamine (Gln) at position 31 and one with a proline (Pro) at position 129 (**Fig. 3e**). Furthermore, the reduction of 15 hydrogen bonds in the variant protein may induce a substantial alteration in the complex's three-dimensional structure, potentially affecting its normal activity (**Morozov et al., 2005; Hubbard et al., 2010; Pace et al., 2014; Kemp et al., 2021**).

To date, *PPP2R5E* has not been convincingly associated with neurological conditions or skeletal muscle diseases. Nevertheless, mutations in the *PPP2CA*, *PPP2R1A* and *PPP2R5D* genes (all encoding subunits of the multifunctional enzymatic PP2A complexes) have been causally linked to neurological impairments (**Verbinnen et al., 2021**). The potential causal link between *PPP2R5E* and the subject's symptoms is

supported by our functional assays of the *de novo* p.E191K variant, and by the high percentage of similarity with *PPP2R5D* (**Fig. S2**), which encodes the related B56δ subunit. Defects in this subunit have been associated with neurological and muscular disorders, including hypotonia (**Houge et al., 2015**; **Shang et al., 2016; Oyama et al., 2023**). Notably, a pathogenic *de novo* p.E250K *PPP2R5D* variant, affected at the orthologous position of *PPP2R5E* p.E191 by an identical amino acid substitution (E to K), was identified in an individual with delayed early developmental milestones, moderate ID, macrocephaly, ADHD and juvenile-onset parkinsonism (**Walker et al., 2021**), further underscoring the pathogenicity of the identified *PPP2R5E* variant in the examined subject. Indeed, the latter experienced both motor coordination problems and hypotonia, which are features that can contribute to juvenile parkinsonism (**Leuzzi et al., 2021**).

Due to high expression of *PPP2R5E* in both skeletal muscle and brain (**McCright et al., 1996; Theendakara et al., 2017**), we hypothesize a potential association between the observed variant in both learning disorders and myopathy through the potential regulation of microtubule dynamics and microtubule-guided (axonal) transport by PPP2R5E (**Hyodo et al., 2016; Nunes Bastos et al., 2016; Rudrabhatla, 2014; Cheng et al., 2015; Hoffman et al., 2017**; **Georgitsi et al., 2021**). Alterations in microtubule activity have also been linked to impairments in memory and learning, as well as anxiety (**Soetanto et al., 2010; Marchisella et al., 2016; Koenning et al., 2021; Treccarichi et al. 2024**). Different PP2A complexes are intricately involved in diverse signaling cascades engaging muscular processes, including those related to muscle development, contraction and metabolism (**Veloso et al., 2019; Wang et al., 2019**) and influencing processes such as myogenesis, muscle cell differentiation and the regulation of proteins involved in muscle function, such as the assembly and dephosphorylation of myosin (**Terry et al., 2006; Reynhout and Janssens, 2019**). Moreover, microtubules in mature myofibers play a crucial role in muscle biology, contributing to myoblast fusion, nuclear localization, sarcomere assembly, and transport/signaling (**Becker et al., 2020; Denes et al., 2021; Lucas et al., 2023**). Alterations in microtubule dynamics can disrupt the cytoskeletal organization, potentially resulting in myopathy (**Ross et al., 2019; Gómez-Oca et al., 2021**). In this respect, a study in *C. elegans* nicely demonstrated the presence of nearly all PP2A subunits, including PPTR-1 (orthologue of B56α/β/ε) and PPTR-2 (orthologue of B56γ/δ) in sarcomeres of striated muscle, with animals with decreased *pptr1* or *pptr2* expression exhibiting structural sarcomere defects. Moreover, the additive phenotype of the double *pptr* mutant could indicate partially redundant functions of PPTR-1 and PPTR-2 in muscle, or more likely, that the two proteins disrupt separate processes in muscle, supported by their distinctly different localizations within the sarcomere (**Qadota et al., 2018**).

The examined individual will be further monitored over time to assess the severity of his learning and motor conditions, to determine whether the symptoms could be classified as parkinsonism. Additional functional studies are essential for validating *PPP2R5E* involvement in the learning difficulties, motor coordination problems and hypotonia, and identifying the relevant physiologic PP2A-B56ε substrate(s)

affected. Within this framework, the utilization of *in vitro* or *in vivo* studies, and a larger cohort of individuals would be imperative to gain a more comprehensive understanding of this new PP2A-related disorder. Additionally, a muscular biopsy may be advisable to validate the potential association between the observed variant and myopathy.

In conclusion, our work identified *PPP2R5E* as a novel gene causally related to specific learning problems, mood and emotional problems, hypotonia and myopathy in the examined subject, thereby enlarging the spectrum of PP2A-related disease genes.

**Author contribution**: Conceptualization: A.M., M.V, F.C.; methodology: A.M, S.T., D.G., A.G.V; investigation: A.M., M.V., S.T., V.C., D.G., A.G.V., C.F., S.S., F.C., I.V., E.N.; data curation: A.M., M.V, S.S., F.C.; original draft preparation: A.M., M.V, V.C., S.S., F.C.; manuscript review and editing: A.M., M.V., A.G.V., C.F., S.S., F.C., V.J.; supervision: A.M., M.V., S.S., F.C., V.J. All authors have read and agreed the final version of the manuscript.

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**Institutional Review Board Statement**: All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was conducted in accordance with the Declaration of Helsinki and approved by the local Ethics Committee "Comitato Etico IRCCS Sicilia - Oasi Maria SS". Prot. CE/193, as of 5th April, 2022, approval code: 2022/04/05/CE-IRCCS-OASI/52.

**Informed Consent Statement**: Written informed consent has been obtained from the subjects to publish this article.

**Data Availability Statement:** The data presented in this study, not concerning personal data of the participating subjects, are available on request from the corresponding author. Figure 3b data are from Uniprot protein database. Accession: https://www.uniprot.org/uniprotkb/Q16537/entry, family & domains section. Accessed on 18/03/2024.

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**Competing Interest**: The authors declare no competing interest.

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