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A Homozygous Missense Variant in *PPP1R1B/DARPP-32* is Associated with Generalized Dystonia

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Conflict of Interest

The authors declare no conflict of interest.

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Abstract

Background

Dystonia is a heterogeneous group of hyperkinetic disorders characterized by sustained or intermittent muscle contractions causing abnormal movements and/or postures. Although more than 200 causal genes are known, many cases of primary dystonia still remain without a clear genetic cause.

Objectives

To identify the causal gene in a consanguineous family with three siblings affected by a complex persistent generalized dystonia.

Methods

We performed exome sequencing in the parents and two affected siblings and characterized the expression of the identified gene by immunohistochemistry in human and zebrafish brains.

Results

We identified a novel missense variant (c.142G>A (NM_032192); p.Glu48Lys) in the protein phosphatase 1 regulatory inhibitor subunit 1B gene (*PPP1R1B*) that was homozygous in all three siblings and heterozygous in the parents. This gene is also known as Dopamine and cAMP-Regulated Neuronal Phosphoprotein 32 (*DARPP-32*) and has been involved in the pathophysiology of abnormal movements. The uncovered variant is absent in public databases and modifies the conserved glutamate 48 localized close to the serine 45 phosphorylation site. The *PPP1R1B* protein was shown to be expressed in cells and regions involved in movement control including projection neurons of the caudate-putamen, substantia nigra neuropil and cerebellar Purkinje cells. The latter cells were also confirmed to be positive for *PPP1R1B* expression in the zebrafish brain.

Conclusions

This is the first report associating a *PPP1R1B* variant with generalized dystonia in man. It might be relevant to include the sequencing of this new gene in the diagnosis of patients with otherwise unexplained movement disorders.

Introduction

Dystonia is a clinically and genetically highly heterogeneous hyperkinetic movement disorders characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive, movements, postures, or both [1-3]. Dystonia can manifest from infancy (from birth to 2 years) to late adulthood (> 40 years) and is classified according to the body distribution (focal, segmental, multifocal, generalized *i.e.* the trunk and at least two other sites are involved) and temporal pattern (static or progressive, and persistent, paroxysmal, diurnal and/or action-specific). Moreover, dystonia may be isolated (no other feature), combined (another movement disorders) or complex (other manifestation including non-neurological) [4].

Dystonia may be acquired or inherited in an autosomal recessive (AR), autosomal dominant (AD) or X-linked fashion [2, 5]. More than 200 genes have been reported to date as responsible, when mutated, for dystonia as part of their phenotypic spectrum (<http://www.omim.org>). Given the genetic and clinical heterogeneity many cases of primary dystonia, even if familial, remain without a clear genetic cause [6].

Herein, we describe a Pakistani kindred exhibiting AR generalized dystonia associated with a novel homozygous missense variant in the protein phosphatase 1 regulatory inhibitor subunit 1B gene (*PPP1R1B*) also known as Dopamine and cAMP-Regulated Neuronal Phosphoprotein 32 (*DARPP-32*).

Patients and Methods

Recruitment of Participants

A four-generation consanguineous family presenting AR generalized dystonia from the Khyber Pakhtunkhwa (KPK) province of Pakistan was recruited (Figure 1A). Written informed consent was obtained from all analysed subjects. All affected members were examined by a local neurologist, geneticist and medical doctor.

DNA Extraction and Quantification

Genomic DNA of the patients (IV:3, IV:5, IV:8), normal siblings (IV:1, IV:2, IV:4, IV:6, IV:7, IV:9) and parents (III:1, III:2) were extracted from whole blood using the QIAamp® kit and following manufacturer's instructions (Qiagen, Hilden, Germany). The extracted DNA was quantified using a NanoDrop™ 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Exome Sequencing and Data Analysis

Exome sequencing libraries were prepared with the Twist Human Core Exome Kit (Twist Bioscience, San Francisco, USA) following the manufacturer's recommendations. Paired-end (2 × 75 bp) sequencing was performed on a NextSeq500 sequencer (Illumina, San Diego, CA, USA). Sequence data were aligned to the human assembly hg19 reference genome using Isaac Genome Alignment Software 2.1.0 (Illumina, San Diego, CA, USA). On average 78X depth of coverage was achieved and 96.7% of the target region was covered by at least 20 reads. We focused only on protein-altering variants with minor allele frequencies (MAF) below 0.01 in the Exome Variant Server (EVS, <https://evs.gs.washington.edu/EVS/>), Genome Aggregation Database (gnomAD, <https://gnomad.broadinstitute.org/>) and an in-house database of ~1000 exomes. To identify potential causal variants, we further filtered the variants based on a recessive mode of inheritance.

***In silico* Prediction Analysis**

Different *in silico* tools including PolyPhen-2 [7], Sorting Intolerant from Tolerant (SIFT) [8], Protein Variation Effect Analyzer (PROVEAN) [9], Mutation Taster [10], Varsome [11], Mutation assessor [12] and Combined Annotation Dependent Depletion (CADD) [13] were used for functional effect prediction of the identified variants. The degree of amino acid conservation between species was assessed through protein multiple sequence alignment using Clustal Omega (<http://www.clustal.org/omega/>), PhyloP [14] and GERP++ [15]. The mutant protein stability was predicted with the online tools MUpro [16] and I-Mutant v2.0

(<http://folding.biofold.org/i-mutant/i-mutant2.0>). Finally, for the interpretation of variants, the American College of Medical Genetics and Genomics (ACMG) 2015 guidelines were used [17].

Sanger Sequencing

The following primers were used to PCR amplify the genomic region carrying the variant: forward, 5'-AGTGGTGTACCTTCAGAAGCTGGATACA-3'; reverse, 5'-GGGAAGAGTGGTGTCAATTCCGGT-3'. The amplification was carried out in 25 µl solution containing 1.5 mM MgCl₂, 0.2 mM dNTP, 1 mM of each primer, 10 ng of genomic DNA, and 1 U of Platinum® Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA). After confirming the purity of the amplicon by agarose gel electrophoresis, the PCR product was purified using the ExoStar Kit (GE Healthcare Life Sciences, Marlborough, MA, USA). The PCR product was sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA) and the ABI PRISM 3730xl Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Finally, the Sanger sequencing results were examined with the Chromas Lite software (Technelysium Pty Ltd, Brisbane, Australia).

Immunohistological stainings of human brain tissue

Immunohistological analysis was performed on normal postmortem human brain samples provided by the Pathology Department of Strasbourg University Hospital. The study has been approved by the local ethics committee and complied with the declaration of Helsinki. Tissue samples were fixed in 4% buffered saline formalin for 4 weeks. The expression of PPP1R1B protein was analyzed on 4 µm paraffin section by immunohistochemistry with a PPP1R1B monoclonal antibody (sc-271111; Santa Cruz Biotechnology Inc., Dallas, TX, USA). The multiplex analysis was performed by an automated chromogenic multiplexed immunohistochemistry assay on the Discovery Ultra automated immunostainer (Ventana Medical Systems, Tucson, USA) with 2 antibodies targeting GFAP (clone 6F-2) and

neurofilament (clone 2F11). All samples were analyzed by 2 neuropathologists and archived by a slide scanner (Ventana DP 200, Tucson, USA).

Preparation of adult zebrafish brains, immunohistochemistry and imaging

Six month old *TgBAC(GFAP:GFP)* [18] adult zebrafish brain were dissected and sectioned with a vibratome (VT1000S, Leica, Wetzlar, Germany) as described in Schmidt et al (2014)[19]. For immunohistochemistry, primary antibodies included: chicken anti-GFP (1:1000, Aves labs, Davis, CA, USA), rabbit anti-HuC/D (1:500, Abcam, Cambridge, UK) and PPP1R1B monoclonal antibody (1:200; sc-271111; Santa Cruz Biotechnology Inc., Dallas, TX, USA). Secondary antibodies were conjugated with Alexa fluor dyes and include anti-chicken Alexa 488, anti-mouse Alexa 546 and anti-rabbit Alexa 633 (1:1000, Invitrogen, Waltham, MA, USA). Immunohistochemically stained brain slices were mounted using Aqua-Poly/Mount (Polysciences Inc, Warrington, PA, USA) with coverslips (0.17mm thickness) and imaged with a laser scanning confocal microscope (Leica TCS SP5, Leica, Wetzlar, Germany). To obtain single-cell resolution images, an HCX PL APO CS x63/1.2NA objective was used with the pinhole size set to 1-airy unit. Fluorescent images for green (GFP), red (DARPP-32/PPP1R1B), and infrared channel (HuC/D) were acquired sequentially in 16-bit color depth with excitation/emission wavelength combinations of 488 nm/492-550 nm, 561 nm/565-605 nm, and 633 nm/650-740 nm, respectively. Pixel resolution for XY and Z planes are 0.24 and 0.50 μm , respectively.

Results

Clinical description

We report on three patients (2 males IV:3 and IV:5, 1 female IV:8) with generalized dystonia in a single generation of a consanguineous family from the Khyber Pakhtunkhwa province of Pakistan (Figure 1A). Their parents (III:1 and III:2) and six siblings (IV:1, IV:2, IV:4, IV:6, IV:7, IV:9) did not show any abnormal features. The patients IV:3, IV:5 and IV:8 were examined at ages of 38, 34 and 28 years, respectively. They were referred to the department of

neurology, District Head Quarter Hospital Bannu and Khyber Teaching Hospital Peshawar, Khyber Pakhtunkhwa Province, Pakistan. All three patients shared typical phenotypes of generalized dystonia with a likely AR mode of inheritance. Detailed clinical features are presented in Table 1. The family's medical background was unremarkable with no history of dystonic disease.

Patient 1 (IV:3)

During childhood, the proband, patient 1 (IV:3), presented speech delay and learning disability followed, at about 5-6 years of age, by involuntary movements mimicking tremor of upper limbs gradually spreading to head and trunk then associated with blepharospasm. He showed more clearly dystonia when he was 6-7 years old in primary school. At 8 years of age, epileptic seizures appeared which were later treated with valproic acid (250 mg twice a day). Dysphonia, dysarthria, dysphagia and oromandibular dystonia were noted as well as impaired gait due to limbs and trunk dystonia. At the age of 9-10 years, he was able to eat, drink, play and write homework by himself, but later, at the age of 15 years, he lost all these abilities due to severe phasic movements and tremor of the hand and whole body. His sleep was normal but dystonic tremor affecting the trunk and upper limbs appeared 20-25 min after wake-up. On his last physical examination at 38 years of age, he had a weight of 79 kg, a height of 178 cm, and a head circumference of 61 cm. He had normal eyesight, thick eyebrows, and normal teeth. There were no ataxia and hypotonia present (Video segments 1 and 3). No social or behavioural abnormalities were observed and there was no cognitive decline, however, depression and anxiety were noted. His hearing, cardiac, respiratory, renal and limb functions were normal. MRI scan was not feasible due to the abnormal movements of the head not attenuated enough by pharmacological sedation.

Patient 2 (IV:5)

Patient 2 (IV:5), the brother of the proband, had prominent generalized dystonia that started at 5-7 years of age. He lost his teeth after severe epileptic attacks that started at 5-9 years of

age; he was treated with valproic acid (250mg per day). He left school at the age of 12 due to severe head and hand tremor which hampered his eating, drinking and writing. At the last examination (34 years), his eyesight was 6/6, weight 71 kg, height 175 cm, and head circumference 58 cm. He showed thick eyebrows, blepharospasm, nystagmus, dysphonia, dysarthria, dysphagia, impaired gait and oromandibular dystonia (Video segments 2 and 3). Detailed clinical assessment revealed normal cognition, hearing, respiratory, kidney and cardiac functions. No facial dysmorphism was noted and social and behavioural activities were normal. However, depression and anxiety were noted. Alike the proband, the patient did not respond to pharmacological sedation making the MRI not feasible.

Patient 3 (IV:8)

The Patient 3 (IV:8) was diagnosed with febrile seizures at 6 years of age. At age of 7-8, her symptoms were minor; she was able to write, eat and drink by herself. The symptoms however worsened including mainly generalized dystonia and she left school at the age of 10 due to the disability related to dystonia. Her sleep was normal but dystonic tremor affecting the trunk and upper limbs appeared 20-25 min after wake-up. At last examination (28 years), she presented with blepharospasm, nystagmus, dysphonia, dysarthria, dysphagia, impaired gait, oromandibular dystonia and severe generalized dystonia. Clinical examination revealed normal eyesight (6/6), a height of 168.5 cm, a weight of 60 kg and a head circumference of 56 cm. Depression and anxiety were also noted. Hearing, respiratory, kidney and heart functions were normal.

Molecular analysis

The two affected siblings (IV:3 and IV:5) and their unaffected parents (III:1 and III:2) were directly subjected to exome sequencing for molecular diagnosis. The variants were filtered according to an AR mode of inheritance. Only one homozygous coding variant in the protein phosphatase 1 regulatory inhibitor subunit 1B gene (*PPP1R1B*; MIM: 604399, also known as *DARPP-32*) was common amongst the three affected individuals: c.142G>A (NM_032192);

p.Glu48Lys. This variant was validated by bi-directional Sanger sequencing and confirmed to be homozygous in patient 3 (IV:8) and heterozygous or absent in all other family members (Figure 1B). It was also predicted as damaging or pathogenic by most of the tested bioinformatics tools (Table 2). Furthermore, the variant is not reported Exome Variant Server (EVS, <https://evs.gs.washington.edu/EVS/>), Genome Aggregation Database (gnomAD, <https://gnomad.broadinstitute.org/>), 1000 Genomes project (<http://www.1000genomes.org/>), and our in-house database of ~1,000 exomes (Table 1). The variant Glutamic acid 48 (Glu48) is conserved across various vertebrate species and is located close to the serine 45 (Ser45) phosphorylation site of the protein (Figure 1C and D).

Immunohistological analysis in human brain

Immunohistological stainings revealed only a few cells and processes expressing PPP1R1B in the neocortex (Figure 2A). In the putamen and caudate nucleus, bundles of fibers of passage expressed strongly PPP1R1B as well as some fibers in the internal capsule, probably the caudo-pallidal fibers (Figure 2B). Within the caudate nucleus, neuronal cell bodies and processes expressed PPP1R1B with an even stronger expression in striosomes as compared with matrix (Figure 2C). Substantia nigra had a strong PPP1R1B expression contrasting with the adjacent thalamus showing no labelling (Figure 2D). This labeling was only observed in the neuropil and not in pigmented neuron cell bodies which remained negative (Figure 2E). In the cerebellar cortex, PPP1R1B expression was mainly localized in cell bodies, dendritic tree and Purkinje cells axons (Figure 2F-G). Finally, in the dentate nucleus of the cerebellum, neuronal cell bodies did not express PPP1R1B, whereas the neuropil including axonal terminals from Purkinje cells was strongly positive (Figure 2H).

Immunohistological analysis in zebrafish brain

No PPP1R1B protein staining was detected in the zebrafish telencephalon, but very strong and specific labelling was observed in the optic tectum and in the cerebellum of the zebrafish adult brain (Figure 3). In the cerebellum, the expression of PPP1R1B was associated with

HuC/D expressing Purkinje cells and their dendritic tree and axons (Figure 3). No or only very weak expression of PPP1R1B was detected in the granular cell layer (GCL). We also did not observe any cells co-expressing GFAP and PPP1R1B in the cerebellum.

Discussion

Here we present a large consanguineous Pakistani family affected by generalized dystonia associated with generalized epilepsy and mild intellectual deficiency in three out of nine siblings. By exome sequencing, we identified a novel missense variant (c.142G>A; p.Glu48Lys) in the *PPP1R1B* gene, also known as *DARPP-32*, that segregated with the disease and showed a recessive inheritance pattern.

PPP1R1B has been implicated in a number of psychiatric and neurological disorders such as Parkinson's and Huntington's diseases as well as schizophrenia [20, 21]. *PPP1R1B* knock-out mice show impaired behavioural and molecular responses to dopamine, psychotropic substances, and antipsychotic medication, suggesting that *PPP1R1B* plays a central role in regulating the efficacy of dopamine neurotransmission [22]. Brain dopamine is indeed known to be involved in multiple functions, including movement, rewards, motivation and stress [23]. Interestingly, L-DOPA-induced dyskinesia were shown to be attenuated in *PPP1R1B* knock-out mice, indicating that the protein may be involved in the emergence of these abnormal movements [24]. Finally, mutant mice lacking CK2 in Dopamine receptor1-expressing spiny neurons, a kinase that is known to phosphorylate Ser45 in PPP1R1B, have defective novelty-induced hyperlocomotion and exploratory behavior, defective motor control, and motor learning [25].

PPP1R1B is an intrinsically unstructured protein that is activated by several neurotransmitters including dopamine or glutamate, but also by multiple psychoactive substances such as cocaine or nicotine [26]. The protein regulates the activity of protein phosphatase 1 (PP1, also known as PPP1), a phosphatase that removes phosphoryl groups from serine and threonine of hundreds of protein substrates [27, 28]. PPP1R1B binds and inhibits PP1 when phosphorylated at threonine 34 (Thr34) by cAMP-dependent protein

kinase (PKA) or activates PP1 when phosphorylated at threonine 75 by cyclin dependent kinase 1/5 (CDK1/5). In other words, PPP1R1B acts as a modulator of PP1 activity in a phosphorylation-dependent manner in response to extracellular small molecules.

PPP1R1B is also a substrate for casein kinase II (CK2), which is broadly expressed and highly active in the brain [29]. CK2 phosphorylates Ser45 in PPP1R1B which is close to the Glu48 that is mutated in the homozygous state in our three patients [30]. Glu48 is conserved across the paralogues *PPP1R1A*, *PPP1R1B* and *PPP1R1C*, and the replacement of a glutamic acid by a lysine is predicted to have damaging impact on protein function through different bioinformatics tools, supporting the potential pathological role of this specific amino acid substitution (Table 2). The change of Glu48 into the oppositely charged lysine may in particular affect the efficiency of the phosphorylation of Ser45. As the phosphorylation of Ser45 has been linked to increased phosphorylation by PKA on Thr34 [30], the p.Glu48Lys variant might affect more generally the regulatory function of *PPP1R1B* in the brain.

The neurotransmitter dopamine has been confirmed by various biological techniques to be unevenly distributed in the mammalian central nervous system [31, 32]. Interestingly, PPP1R1B displays a remarkable regional distribution in brain, roughly similar to that of dopamine innervation [31-35]. PPP1R1B has two isoforms: the full-length protein of 204 amino acid (transcript NM_032192) and a truncated form called t-DARPP lacking the first 36 amino acids (transcript NM_001242464) which include the PP1 binding site that is involved in the modulation of dopaminergic neurotransmission [36]. The majority of PPP1R1B expression data reported in the literature has been generated in animals or brain lysates or have used antibodies that cannot distinguish between the two PPP1R1B isoforms [34, 37, 38]. Here we characterized the expression of the full length PPP1R1B in human brain using an antibody targeting the N terminal domain of the protein (amino acids 2-34). PPP1R1B was mainly expressed in striatal projection neurons (SPN) that are localized in the substantia nigra neuropil, the caudate-putamen (CP) and striosomes. The CP is the principal structure of the basal ganglia, which has multiple functions including motor control, motor learning, executive functions and motivation. The CP is mainly composed of two classes of medium

spiny neurons (MSN), which participate in the regulation of movement through dopamine receptor 1 (D1), or dopamine receptor 2 (D2) [21]. Interestingly, PPP1R1B is also known to be enriched in the striosome compartment in mice [39]. The striosome is thought to contain the only striatal neurons that have direct projections to the pars compacta substantia nigra containing the dark-pigmented dopaminergic neurons [40]. Interestingly, PPP1R1B protein expression appears to be associated with the neuropil rather than the cell bodies of the pars compacta. Our data also show a strong PPP1R1B expression in Purkinje cells including its dendritic tree and projecting efferent outputs to dentate nucleus which is the main cerebellar nucleus participating in the control of voluntary movements. The confirmation of PPP1R1B expression in Purkinje cells of zebrafish brain argues for a functional conservation of the role of this gene family in vertebrates.

A limitation of our study is that although the variant is present at the homozygous state in three affected siblings and the segregation in this large pedigree is a strong evidence for the involvement of *PPP1R1B* in the disease, we were only able to find a single family with variants in this gene. The absence of response to ketamine, which incidentally might be linked to the *PPP1R1B* variant [41], did not allow us to collect and report MRI data. Finally, expression data of the mutant protein in patient samples is missing. Further studies are needed to uncover the detailed functional mechanism behind *PPP1R1B* associated dystonia.

Conclusions

To our knowledge, this is the first report associating a *PPP1R1B* variant with generalized dystonia in man. Based on these findings, it might be relevant to include the sequencing of this new gene in the diagnostic workup of patients with otherwise unexplained movement disorders.

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Author Roles

(1) Research Project: A. Conception, B. Organization, C. Execution; (2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique; (3) Manuscript: A. Writing of the First Draft, B. Review and Critique. AK: 1B, 1C, 3A; AM: 1B, 1C; SM: 1C, 3B; GZ: 1C; BR: 1C; BL: 3B; BLH: 3B; MU: 1B; SA: 1C, 2B; SF: 1B, 3B; SR: 1A, 3B; MA: 1A, 1B, 3B; SB: 1A, 1B, 3B; RC: 1A, 1B, 3A.

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Tables

Table 1. Clinical features of affected subjects (IV:3, IV:5 and IV:8).

General Features			
Subject	IV:3	IV:5	IV:8
Gender	Male	Male	Female
Origin	Pakistan	Pakistan	Pakistan
Dystonia onset (years)	5-6	5-7	7-8
Age at the last examination (years)	38	34	28
Consanguinity	+	+	+
Pregnancy	Normal	Normal	Normal
Developmental Features			
Developmental delay	+	+	+
Speech delay/impairment	+	+	+
Learning disability	+	+	+
Dysmorphia /skeletal deformities	-	-	-
Head circumference	61 cm	58 cm	56 cm
Height	178 cm	175 cm	168.5 cm
Weight at the last examination	79 kg	71 kg	60 kg
Eyesight	Normal	Normal	Normal
Hearing	Normal	Normal	Normal
Respiration	Normal	Normal	Normal
Behavioral Features			
Impairment of social interaction	Mild	Mild	Mild
Amnesia	+	+	+
Neurological Features			
Intellectual disability	+	+	+
Anxiety/psychiatric	++	++	++
Feeding Difficulty	+	+	+
Epilepsy onset	8 years	5-9 years	6 years
Epilepsy medication	valproic acid	valproic acid	valproic acid
Dystonia	Generalized	Generalized	Generalized

+, present; ++, severe phenotype; -, absent

Table 2. *In silico* analysis of the homozygous *PPP1R1B* variant

Gene Name	<i>PPP1R1B</i>
Alternative names	<i>DARPP32; DARPP-32</i>
Chromosome location	17q12
MIM Number	604399
GenBank accession	NM_032192.4
Ensembl ID	ENST00000254079
Total number of exons	9
DNA Change (NM_032192)	c.142G>A
Protein Change	p.Glu48Lys
Variant Exonic Location	Exon 2
Variant Chromosome Location (hg19)	Chr17:37785483
Variant Type	Missense
SIFT	0.019 (damaging)
Polyphen-2_HDIV	0.997 (probably damaging)
Polyphen-2_HVAR	0.989 (probably damaging)
LRT	0 (deleterious)
MutationTaster	1 (disease causing)
PROVEAN	-2.98 (damaging)
CADD	31 (damaging)
MUpro ($\Delta \Delta G$)	-0.77046956 (decreased stability)
I-Mutant	-0.68 (decreased stability)
GERP++	5.41 (conserved)
phyloP	4.378 (conserved)
ACMG Classification	PM2 and PM3 (Pathogenic Moderate and Supporting)

Figures

Figure 1.

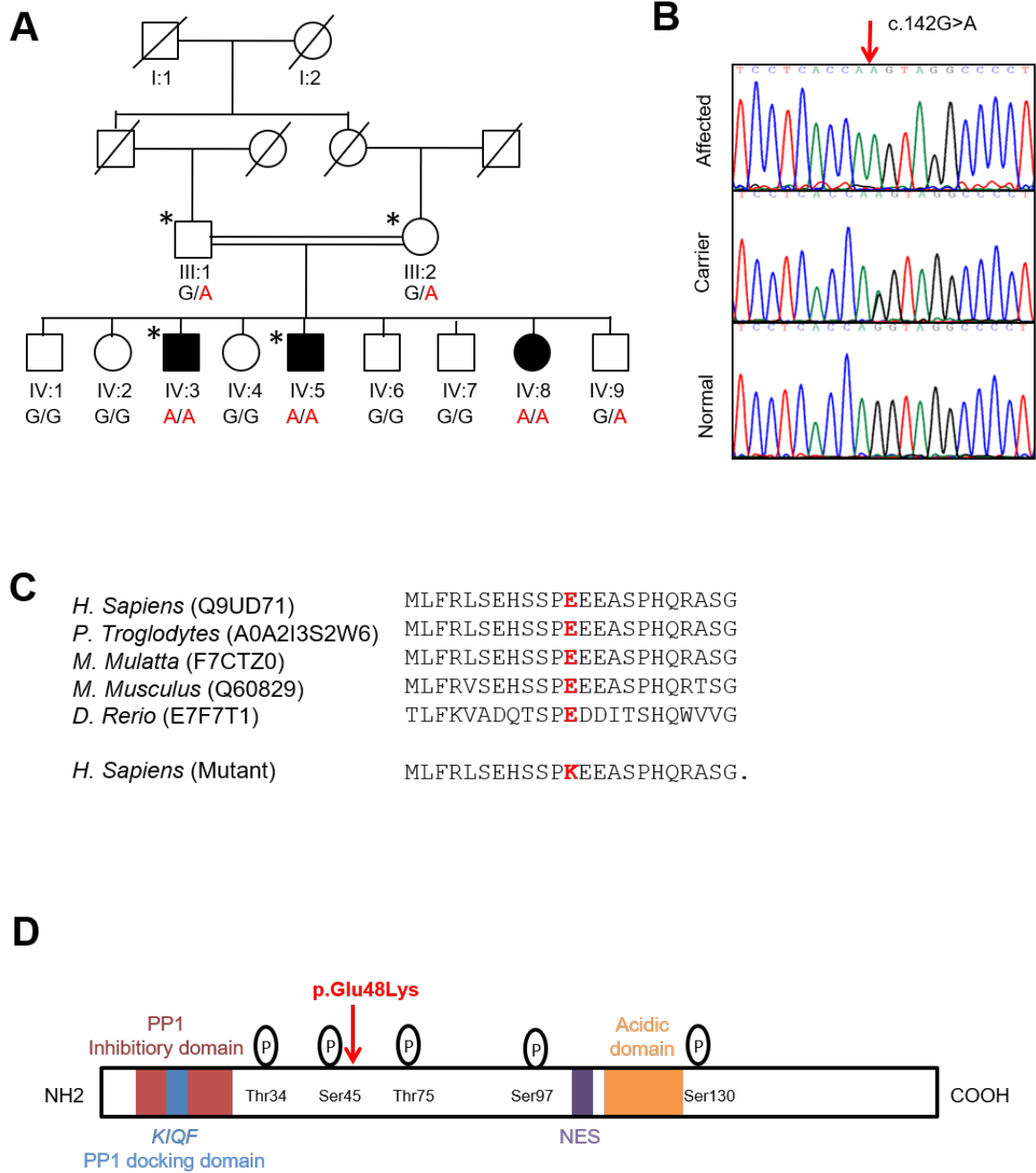


Figure 2.

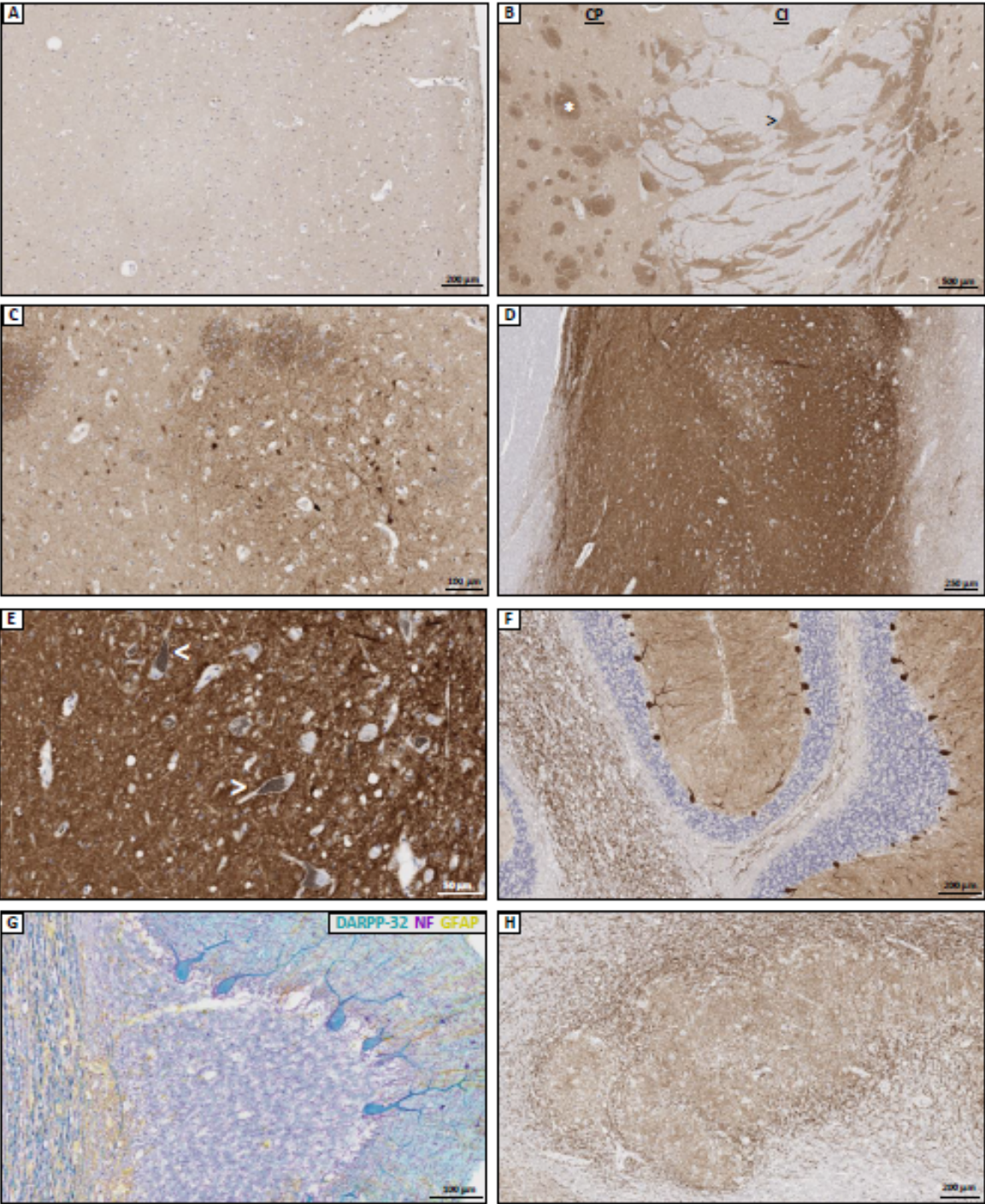


Figure 3.

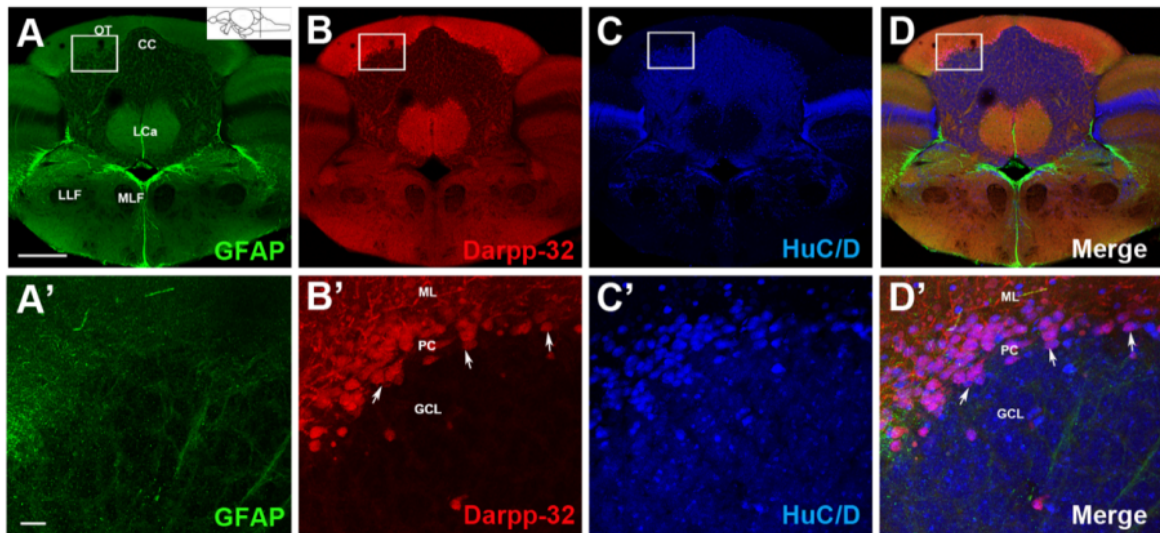


Figure Legends

Figure 1. Pedigree and *PPP1R1B* variant.

(A) Pedigree of the family showing an autosomal recessive segregation of the disease. Double lines indicate a consanguineous union. White circles and squares represent healthy females and males, respectively. Black symbols represent affected individuals. The stars indicate the members of the family subjected to exome sequencing. (B) Representative Sanger sequence electropherogram of one affected individual (upper panel), one healthy heterozygous carrier (middle panel) and one healthy non-carrier individual (lower panel). The red arrow indicates the position of the DNA variant (c.142G>A). (C) *PPP1R1B* amino acid alignment and conservation analysis across different species. Protein accession numbers for each species are indicated. (D) Secondary structure of *PPP1R1B* protein. The red arrow indicates the protein variant position (p.Glu48Lys). The main phosphorylated amino acid residues are indicated by the circled letter P. Other abbreviations: KIQF, consensus motif (Lys-Ile-Gln-Phe) that binds PP1 and contributes to its inhibition; NES, nuclear export sequence; PP1, protein phosphatase 1.

Figure 2. Immunohistology of PPP1R1B in human brain

The figure shows representative examples of PPP1R1B protein expression in human neocortex (A), basal ganglia (B-E), and cerebellum (F-H). Standard immunohistochemical PPP1R1B detection by DAB staining (brown) (A-F and H) and multiplex chromogenic immunohistochemistry of neurofilament (NF) by purple staining, teal-blue staining of PPP1R1B and yellow staining of GFAP (G) are shown. (A) neocortical grey matter. (B) caudate-putamen (CP, left) and internal capsule (CI, right). The star shows fiber of passage and the black arrow indicates caudo-pallidal fibers. (C) Striosome compartment. (D) substantia nigra. (E) dark-pigmented neurons of substantia nigra (white arrow). (F-G) cerebellarcortex. (H) dentate nucleus.

Figure 3. Immunohistology of PPP1R1B in zebrafish brain

(A-D') Immunostaining with PPP1R1B/DARPP-32 (red), HuC/D (blue) and GFP (green) antibodies on transverse cerebellum sections of a GFAP::GFP transgenic line. Note the strong expression of DARPP-32 in the Purkinje cells and their dendrites in the molecular layer (B and B'). The expression of DARPP-32 in the Purkinje cells overlaps with the post mitotic neural marker HuC/D (C and C'). White rectangles (A-D) represent the region magnified in (A'-D'). OT, optic tectum; LCa, caudal lobe of cerebellum; CC, corpus cerebelli; MLF, medial longitudinal fascicle; LLF, lateral longitudinal fascicle; GCL, granular cell layer. Scale bar =100µm (A-D) and 20µm (A'-D').

Video legends

Video segments 1 and 2 show dystonic movements in patient 1 (IV:3) and patient 2 (IV:5), respectively. Video segment 3 shows patients 1 and 2 walking side by side.