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ORIGINAL INVESTIGATION

De novo missense variants in *PPP1CB* are associated with intellectual disability and congenital heart disease

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Abstract Intellectual disabilities are genetically heterogeneous and can be associated with congenital anomalies. Using whole-exome sequencing (WES), we identified five different de novo missense variants in the protein phosphatase-1 catalytic subunit beta (*PPP1CB*) gene in eight unrelated individuals who share an overlapping phenotype of dysmorphic features, macrocephaly, developmental delay or intellectual disability (ID), congenital heart disease, short stature, and skeletal and connective tissue abnormalities. Protein phosphatase-1 (PP1) is a serine/threonine-specific protein phosphatase involved in the dephosphorylation of a variety of proteins. The *PPP1CB* gene encodes a PP1 subunit that regulates the level of protein phosphorylation. All five altered amino acids we observed are highly conserved among the PP1 subunit family, and all are predicted to disrupt PP1 subunit binding and impair dephosphorylation. Our data suggest that our heterozygous de novo *PPP1CB* pathogenic variants are associated with syndromic intellectual disability.

Introduction

Developmental delay and intellectual disability are observed in ~1–3 % of the population, and congenital heart defects are present in ~1 % of all live births (Zablotsky et al. 2015; van der Linde et al. 2011). De novo predicted pathogenic sequence variants are more frequently identified in individuals with congenital heart disease who also have neurodevelopmental disorders and/or other congenital anomalies compared to individuals with isolated congenital heart disease (Homsy et al. 2015). The underlying etiology for most individuals with neurodevelopmental disorders

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and/or congenital heart disease is unclear due to the heterogeneous causes of both conditions (Homsy et al. 2015; Vissers et al. 2016). Whole-exome sequencing (WES) has proven to be a successful strategy to identify disorders due to de novo pathogenic variants in a significant portion of individuals with disorders that affect reproductive fitness (Shang et al. 2016).

Reversible protein serine/threonine phosphorylation, catalyzed by kinases and phosphatases, is a common mechanism for the regulation of many cellular functions. De novo heterozygous pathogenic variants in protein phosphatase-2 regulatory subunit B delta (*PPP2R5D*) were recently identified using WES in 7 out of 2790 (0.25 %) patients with neurodevelopmental disorders and additional features of dysmorphic facial features, developmental delay, autistic features, macrocephaly, hypotonia, and seizures (Shang et al. 2016). This finding highlights the importance of protein phosphatases in the brain development. There are seven major serine/threonine-specific protein phosphatases, including PPP1-7, in eukaryotic cells. Protein phosphatase 1 is the major serine/threonine phosphatase that regulates diverse cellular processes, such as cell-cycle progression, protein synthesis, muscle contraction, carbohydrate metabolism, transcription, and neuronal signaling (Aggen et al. 2000). Protein phosphatase 1 regulates neurotransmitters and synaptic activity and is regulated by multiple factors (Bausen et al. 2010; Kanematsu et al. 2006; Pribiag and Stellwagen 2013). Protein phosphatase 1 catalytic subunit (PP1C) mediates dephosphorylation of dishevelled (Dvl) protein, a key component in Wnt signaling, via its C-terminal domain. PP1C cooperates with homeodomain-interacting protein kinase 2 (Hipk2) to stabilize Dvl and form Dvl-Hipk2-PP1c complex to sustain Wnt signaling. In the absence of PP1c catalytic activity, Dvl proteins are hyperphosphorylated and consequently degraded by the proteasome (Shimizu et al. 2014). Protein phosphatase type 1 beta regulates and forms a complex with the tumor suppressor genes Merlin and Moesin (Yang et al. 2012). Protein phosphatase-1 catalytic subunit beta (PPP1CB) interacts with the myosin-binding subunit, which directs the phosphatase activity of the enzyme to specifically dephosphorylate the myosin, troponin complex, and myosin light chain in smooth and skeletal muscles (Pereira et al. 2011). A de novo heterozygous frameshift pathogenic variant c.909dupA, p.Tyr304Ilefs*19 in protein phosphatase 1 catalytic subunit beta (*PPP1CB*), was identified by Hamdan et al. in 1 out of 41 subjects with intellectual disability, developmental delay, postnatal growth deficiency, and some minor dysmorphic features (Hamdan et al. 2014). Another study reported two de novo missense pathogenic variants in *PPP1CB* in four patients; three of these individuals had a recurrent *PPP1CB* c.146G>C, p.Pro49Arg variant, while the fourth had a c.166G>C, p.Ala56Pro variant (Gripp et al. 2016). These four patients were initially considered to have a recognizable phenotype closely resembling Noonan syndrome with loose anagen hair (Gripp et al. 2016). In this study, we present eight additional unrelated individuals with de novo variants in *PPP1CB*, four of them with the recurrent p.Pro49Arg variant, with common features of dysmorphic features, macrocephaly, neurodevelopmental delay, congenital heart disease, short stature, and skeletal and connective tissue abnormalities. We present data to further show that de novo variants in *PPP1CB*, a catalytic subunit of PP1, cause a distinct disorder related to intellectual disability.

Materials and methods

Consent

Informed consent was obtained from all parents of participants included in this study. This study was approved by the Institutional Review Boards of Columbia University and Baylor College of Medicine.

Whole-exome sequencing

Patients 1, 2, and 5 through 8 had whole-exome sequencing (WES) at GeneDx along with their parents. The testing was performed and data were analyzed on the HiSeq 2000, 2500, or 4000 sequencing system, as described previously (Shang et al. 2016). Identified de novo *PPP1CB* variants were confirmed in all affected probands, and their absence confirmed in their parents with a second independent DNA preparation by dideoxy Sanger sequencing using an ABI3730 DNA Sequencer (Life Technologies, Carlsbad, CA, USA).

Patient 3 had WES as a singleton at the Baylor College of Medicine Human Genome Sequencing Center (BCM-HGSC). Briefly, DNA sample was prepared into Illumina paired-end libraries. Capture was performed using BCM-HGSC core design (52 Mb, Roche NimbleGen, Inc) and sequenced on the Illumina HiSeq 2000 platform (Illumina, Inc) with an ~150× depth of coverage. Data produced were aligned and mapped to the human genome reference sequence (hg19) using the Mercury pipeline. Variants were called using the ATLAS variant calling method and SAMtools (The Sequence Alignment/Map) and annotated using the in-house-developed "Cassandra" annotation pipeline that uses ANNOVAR (Li et al. 2009; Wang et al. 2010; Bainbridge et al. 2011; Lupski et al. 2013). Potentially pathogenic variants were confirmed in the proband and evaluated in the parents by Sanger sequencing.

Patient 4 underwent WES at Ambry Genetics along with both parents. Exome library preparation, sequencing,

bioinformatics, and data analysis were performed as previously described (Farwell et al. 2015). Briefly, samples were prepared using the IDT xGen Exome Research Panel V1.0 and sequenced using paired-end, 150-cycle chemistry on the Illumina HiSeq 2500 (Illumina, San Diego, CA, USA). Identified candidate alterations were confirmed using automated fluorescence dideoxy sequencing. Co-segregation analysis was performed using each available family member.

Results

WES was performed in 5964 probands at GeneDx with developmental delay and/or ID, with or without additional clinical features. 4624 cases were submitted as complete trios with both parents. Ten individuals (0.17 %) were identified to have de novo predicted pathogenic variants in *PPP1CB* that were all confirmed with Sanger sequencing. The probability of identifying 10 individuals with de novo variants in *PPP1CB* among 4624 trios is 3.2×10^{-14} by a Poisson test and 1.7×10^{-9} by TADA (He et al. 2013). Given the mutation rate for *PPP1CB,* the probability of identifying 10 de novo variants is 8.6×10^{-6}. Four of those ten individuals declined to be included in this paper, since they were already involved in another publication. Of the remaining six samples tested at GeneDx, WES produced an average of ~10 GB of sequence per sample. Mean coverage of captured regions was ~110X per sample, with >95 % covered with at least 10x coverage, an average of 91 % of base call quality of Q30 or greater, and an overall average mean quality score of >Q36.

At Ambry, WES was performed in 1798 probands with developmental delay and/or ID, with or without additional clinical features. 1358 cases were submitted as complete trios with both parents. Three individuals (0.22 %) were identified to have de novo predicted pathogenic variants in *PPP1CB* that were all confirmed with Sanger sequencing. Two of those three individuals declined to be included in this paper. Of the remaining sample tested at Ambry, WES produced an average of ~11 GB of sequence per sample. Mean coverage of captured regions was ~133× per sample, with >95 % covered with at least 10x coverage, an average of 91 % of base call quality of Q30 or greater, and an overall average mean quality score of >Q36.

In combining cases from both GeneDx and Ambry, the probability of identifying 13 individuals with de novo variants in *PPP1CB* among 5982 trios is 2.49×10^{-13} by a Poisson test and 2.22×10^{-16} by TADA (He et al. 2013).

In addition to the six patients analyzed at GeneDx, one patient underwent WES at BCM-HGSC and Sanger sequencing confirmed the presence of the *PPP1CB* variant in the proband and absence in the unaffected parents

Table 1 Novel variants in PPP1CB and pathogenicity predictions

Chr	Position	Ref	Alt	Amino-acid change	AA Change.ref gene	SIFT_pred	Polyphen2_pred	Mutation Taster_pred	MetaSVM_pred	CADD_phred	GERP++_RS
2	28999810	C	G	p.Pro49Arg	PPP1CB:NM_002709:exon2:c.C146G:p.P49R,PPP1CB:NM_206876:exon3:c.C146G:p.P49R	Damaging	Damaging	Damaging	Damaging	24.7	4.49
2	29006800	A	C	p.Glu183Ala	PPP1CB:NM_002709:exon5:c.A548C:p.E183A,PPP1CB:NM_206876:exon6:c.A548C:p.E183A	Damaging	Benign	Damaging	Tolerated	24.5	5.87
2	29006800	A	T	p.Glu183 Val	PPP1CB:NM_002709:exon5:c.A548T:p.E183 V,PPP1CB:NM_206876:exon6:c.A548T:p.E183V	Damaging	Benign	Damaging	Tolerated	29.8	5.87
2	29016738	G	T	p.Asp252Tyr	PPP1CB:NM_002709:exon7:c.G754T:p.D252Y,PPP1CB:NM_206876:exon8:c.G754T:p.D252Y	Damaging	Damaging	Damaging	Damaging	25.8	5.13
2	29016804	G	A	p.Glu274Lys	PPP1CB:NM_002709:exon7:c.G820A:p.E274K,PPP1CB:NM_206876:exon8:c.G820A:p.E274K	Damaging	Possibly damaging	Damaging	Tolerated	26.1	5.31

```
PPP1CB      ------------------------------------------------------------
PPP2CA      ------------------------------------------------------------
PPP2CB      ------------------------------------------------------------
PPP4C       ------------------------------------------------------------
PPP5C       MAMAEGERTECAEPPRDEPPADGALKRAEELKTQANDYFKAKDYENAIKFYSQAIELNPS
PPP6C       ------------------------------------------------------------
PPP7C       --------MGCSSSSTKTRRSDT-------------------------------------


PPP1CB      ------------------------------------------------------------
PPP2CA      ------------------------------------------------------------
PPP2CB      ------------------------------------------------------------
PPP4C       ------------------------------------------------------------
PPP5C       NAIYYGNRSLAYLRTECYGYALGDATRAIELDKKYIKGYYRRAA----------------
PPP6C       ------------------------------------------------------------
PPP7C       ------------------------SLRAALIIQNWYRGYKARLKARQHYALTIFQSIEYA


PPP1CB      ----------------------------------------------------MADGELNVD
PPP2CA      ----------------------------MDEKVFTKELDQWIEQLNECKQLSESQ----
PPP2CB      ----------------------------MDDKAFTKELDQWVEQLNECKQLNENQ----
PPP4C       --------------------------------MAEISDLDRQIEQLRRCELIKESE----
PPP5C       ---SNMALGKFR-AALRDYETVVKVKPHDKDAKMKYQECNKIVKQKAFERAIAGDEHKRS
PPP6C       --------------------------------MAPLDLDKYVEIARLCKYLPENDLKVS
PPP7C       DEQGQMQLSTFFSFMLENYTHIHKEE-----LELRNQSLE-------SEQDMRDR---WD
                                                                    :

PPP1CB      SLITRLLEVRGC-----------RPGKIVQMTE--------AEVRGLCI-----KSREIF
PPP2CA      ------------------------------------------VKSLCE-----KAKEIL
PPP2CB      ------------------------------------------VRTLCE-----KAKEIL
PPP4C       ------------------------------------------VKALCA-----KAREIL
PPP5C       VVDSLDIESMTIEDEYSGPKLEDGKVTISFMKELMQWYKDQKKLHRKCAYQILVQVKEVL
PPP6C       PICG--LAPSGCGAPAGRPFLSPGPPPVFHFLR--------FLKERLCD-----YVCDLL
PPP7C       -----YVDSIDVPDSYNGPRLQF-PLTCTDIDLLLEAFKEQQILHAHYVLEVLFETKKVL
                                                      .           .::

PPP1CB      LSQPI----LLELEAPLKICGDIHGQYTDLLRLFEYGGFPPEAN-YLFLGDYVDRGKQSL
PPP2CA      TKESN----VQEVRCPVTVCGDVHGQFHDLMELFRIGGKSPDTN-YLFMGDYVDRGYYSV
PPP2CB      TKESN----VQEVRCPVTVCGDVHGQFHDLMELFRIGGKSPDTN-YLFMGDYVDRGYYSV
PPP4C       VEESN----VQRVDSPVTVCGDIHGQFYDLKELFRVGGDVPETN-YLFMGDFVDRGFYSV
PPP5C       SKLSTLVETTLKETEKITVCGDTHGQFYDLLNIFELNGLPSETNPYIFNGDFVDRGSFSV
PPP6C       LEESN----VQPVSTPVTVCGDIHGQFYDLCELFRTGGQVPDTN-YIFMGDFVDRGYYSL
PPP7C       KQMPNFTHIQTSPSKEVTICGDLHGKLDDLFLIFYKNGLPSERNPYVFNGDFVDRGKNSI
             .             :.:*** **:  **  :*   *   : * *:* **:****  *:

PPP1CB      ETICLLLAYKIKYPENFFLLRGNHECASINRIYGFYDECKRRF-N--IKLWKTFTDCFNC
PPP2CA      ETVTLLVALKVRYRERITILRGNHESRQITQVYGFYDECLRKYGN--ANVWKYFTDLFDY
PPP2CB      ETVTLLVALKVRYPERITILRGNHESRQITQVYGFYDECLRKYGN--ANVWKYFTDLFDY
PPP4C       ETFLLLLALKVRYPDRITLIRGNHESRQITQVYGFYDECLRKYGS--VTVWRYCTEIFDY
PPP5C       EVILTLFGFKLLYPDHFHLLRGNHETDNMNQIYGFEGEVKAKY-T--AQMYELFSEVFEW
PPP6C       ETFTYLLALKAKWPDRITLLRGNHESRQITQVYGFYDECQTKYGN--ANAWRYCTKVFDM
PPP7C       EILMILCVSFLVYPNDLHLNRGNHEDFMMNLRYGFTKEILHKYKLHGKRILQILEEFYAW
            * .  *      : : : : *****   :.  ***  *   ::        .   . :
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Fig. 1 De novo variants identified in patients and protein alignment of PPP1CB with all other members of protein phosphatase family: PPP2AC, PPP2BC, PPP4C, PPP5C, PPP6, and PPP7C (all human). Variants identified in our patients are *highlighted* at amino-acid residues 49, 183, 252, and 274. Identical (*), conservative (:), and similar (.) residues are indicated. Amino acids Glu183 and Asp252 are conservative; Glu274 is similar, while Pro49 is not conserved among proteins in PPP family

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PPP1CB      LPIAAIVDEKIFCCHGGLS-PDLQSMEQIRRIMRPTD----------------VPDTGLL
PPP2CA      LPLTALVDGQIFCLHGGLS-PSIDTLDHIRALDRLQE----------------VPHEGPM
PPP2CB      LPLTALVDGQIFCLHGGLS-PSIDTLDHIRALDRLQE----------------VPHEGPM
PPP4C       LSLSAIIDGKIFCVHGGLS-PSIQTLDQIRTIDRKQE----------------VPHDGPM
PPP5C       LPLAQCINGKVLIMHGGLFSEDGVTLDDIRKIERNRQ----------------PPDSGPM
PPP6C       LTVAALIDEQILCVHGGLS-PDIKTLDQIRTIERNQE----------------IPHKGAF
PPP7C       LPIGTIVDNEILVIHGGISETTD--LNLLHRVERNKMKSVLIPPTETNRDHDTDSKHNKI
            * :   :: :::  ***:       :: :: : *                    .   :

PPP1CB      CDLLWSDPDKDVQGWGENDRGVSFTFGADVVSKFLNRHDLDLICRAHQVVEDGYEFFAKR
PPP2CA      CDLLWSDPD-DRGGWGISPRGAGYTFGQDISETFNHANGLTLVSRAHQLVMEGYNWCHDR
PPP2CB      CDLLWSDPD-DRGGWGISPRGAGYTFGQDISETFNHANGLTLVSRAHQLVMEGYNWCHDR
PPP4C       CDLLWSDPE-DTTGWGVSPRGAGYLFGSDVVAQFNAANDIDMICRAHQLVMEGYKWHFNE
PPP5C       CDLLWSDPQ-PQNGRSISKRGVSCQFGPDVTKAFLEENNLDYIIRSHEVKAEGYEVAHGG
PPP6C       CDLVWSDPE-DVDTWAISPRGAGWLFGAKVTNEFVHINNLKLICRAHQLVHEGYKFMFDE
PPP7C       IDILWSDPRGKNGCFPNTCRGGGCYFGPDVTSKILNKYQLKMLIRSHECKPEGYEICHDG
             *::****         . ** .  ** .:   :     :  : *:*:   :**:

PPP1CB      QLVTLFSAPNYCGEFDNAGGMMSVDET-LMCSFQILKPSEKKAKYQYGGL----------
PPP2CA      NVVTIFSAPNYCYRCGNQAAIMELDDT-LKYSFLQFDPAPRRG----------------
PPP2CB      NVVTIFSAPNYCYRCGNQAAIMELDDT-LKYSFLQFDPAPRRG----------------
PPP4C       TVLTVWSAPNYCYRCGNVAAILELDEH-LQKDFIIFEAAPQET----------------
PPP5C       RCVTVFSAPNYCDQMGNKASYIHLQGSDLRPQFHQFTAVPHPNVKP--------------
PPP6C       KLVTVWSAPNYCYRCGNIASIMVFKDV-NTREPKLFRAVPDSE----------------
PPP7C       KVVTIFSASNYYEEGSNRGAYIKLCSG-TTPRFFQYQVTKATCFQPLRQRVDTMENSAIK
              :*::** **  .  * .. : .

PPP1CB      -NSGRPVTPPRTANPPKKR-------------------------------------------
PPP2CA      ----EPHV-TRRT-PDYFL-------------------------------------------
PPP2CB      ----EPHV-TRRT-PDYFL-------------------------------------------
PPP4C       ----RGIPSKKPV-ADYFL-------------------------------------------
PPP5C       ---------MAYANTLLQLGM------M---------------------------------
PPP6C       ----RVIPPR-TT-TPYFL-------------------------------------------
PPP7C       ILRERVISRKSDLTRAFQLQDHRKSGKLSVSQWAFCMENILGLNLPWRSLSSNLVNIDQN

PPP1CB      ------------------------------------------------------------
PPP2CA      ------------------------------------------------------------
PPP2CB      ------------------------------------------------------------
PPP4C       ------------------------------------------------------------
PPP5C       ------------------------------------------------------------
PPP6C       ------------------------------------------------------------
PPP7C       GNVEYMSSFQNIRIEKPVQEAHSTLVETLYRYRSDLEIIFNAIDTDHSGLISVEEFRAMW

PPP1CB      --------------------------------------------------------
PPP2CA      --------------------------------------------------------
PPP2CB      --------------------------------------------------------
PPP4C       --------------------------------------------------------
PPP5C       --------------------------------------------------------
PPP6C       --------------------------------------------------------
PPP7C       KLFSSHYNVHIDDSQVNKLANIMDLNKDGSIDFNEFLKAFYVVHRYEDLMKPDVTNLG
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Fig. 1 continued

and siblings, thus confirming it was de novo. One additional unrelated patient was evaluated by WES at Ambry and found to have a de novo predicted pathogenic variant in *PPP1CB* that was confirmed with Sanger sequencing. Five novel de novo heterozygous missense variants in *PPP1CB* were identified in a total of eight unrelated individuals. One variant was previously reported (Gripp et al. 2016), four variants are novel and have not been reported in the Database of Single Nucleotide Polymorphisms (dbSNP, http://www.ncbi.nlm.nih.gov/SNP/), 1000 Genomes (http://www.1000genomes.org/), Exome Variant Server (ESP), or in GeneDx's internal database of over 15,000 control exomes. Although 17 loss-of-function variants were expected in *PPP1CB*, no loss-of-function variants were observed in exome sequencing data from control subjects catalogued in the ExAC Browser (http://exac.broadinstitute.org/). Hence, *PPP1CB*'s probability of loss-of-function intolerance (pLI) is 1.0. All variants are predicted to be deleterious by SIFT (http://sift.jcvi.org/), Mutation Taster (http://www.mutationtaster.org/), and CADD (http://cadd.gs.washington.edu/). PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) and MetaSVM (https://sites.google.com/site/jpopgen/dbNSFP) predict two of the variants (Pro49Arg and Asp252Tyr) to be deleterious (Table 1). The five variants occur in amino acids that are highly conserved across species (Fig. 1). Four of the eight patients have the same recurrent de novo variant, Pro49Arg, and two variants alter amino acid Glu183 to either Valine to Alanine. The catalytic subunit of PP1 is composed of 9 alpha helices and 14 beta strands comprising 3 beta sheets (Maynes et al. 2001; Peti et al. 2013). According to the secondary structure of the protein, Pro49 is located between alpha helices A and beta loop 1. Glu183 is located in an alpha helix, while Asp252 is in the beta 10–11 loop (Fig. 2). All three variants are located in catalytic core (amino acids 41–269) of PP1 (Ansai et al. 1996) which possesses phosphatase activity. Deletion of ten amino-acid residues from either the N or the C termini of the PP1 catalytic core, in which Pro49 and Asp252 are located, destroyed the phosphatase activity, indicating the critical role of these amino acids to the catalytic function (Ansai et al. 1996). Glu274 is in the active site region in beta 12–13 loop, and changing Glu274 to Lys may affect the intramolecular hydrogen bond with toxin Okadaic acid (Maynes et al. 2001).

Clinically, the eight individuals with de novo *PPP1CB* variants range in age from 2 to 20 years, although only four are older than age five. Three are females and five are males. All individuals were either developmentally delayed or intellectually disabled. All individuals have dysmorphic features (Table 2). Four individuals have hypertelorism and two have webbed necks. Other variable dysmorphic features include arched eyebrows, broad nose, long philtrum, low-set ears, preauricular pit, triangular mouth with thin upper lip, ankyloglossia, and bifid uvula. Representative facial characteristics are shown in Fig. 3. Six individuals had congenital heart disease, including atrial septal defect, mitral and tricuspid valve insufficiency, hypoplastic left-sided aortic arch, coarctation of aorta, dilated aortic root, and peripheral pulmonic stenosis. One individual had macrocephaly at birth and most had relative or absolute macrocephaly at the time of evaluation. Three had abnormalities of the brain on MRI, including septo-optic dysplasia, increased white matter signal intensity in the right frontal region and prominent subarachnoid spaces. Six individuals had failure to thrive and/or short stature. Three of the individuals had hypotonia, three had pectus excavatum,

Fig. 2 PPP1CB sequence and structure. Secondary structural elements of PPP1CB protein are indicated above the sequence with *cylinders* represent alpha helices and *arrows* indicate beta-strands. Variants identified in this study are highlighted in *red*

Table 2 Phenotypic features of individuals with de novo missense variants in *PPP1CB*

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
PPP1CB variant	c.146C>G; p.Pro49Arg	c.146C>G; p.Pro49Arg	c.146C>G; p.Pro49Arg	c.146C>G; p.Pro49Arg	c.548A>C; p.Glu183Ala	c.548A>T; p.Glu183Val	c.754G>T; p.Asp252Tyr	c.820G>A; p.Glu274Lys
Sex	M	F	M	M	F	M	F	M
Current age	2	6	8	2	4	20	2	6
Growth parameters	At 2 years: HT 88.5 cm (50–75 ‰) WT 13.3 kg (50–75 ‰) OFC 55.5 cm (>95 ‰)	At 6 years: HT 105 cm (2 ‰) WT 18.9 kg (28 ‰) OFC 54 cm (99 ‰)	At 8 years: HT 112 cm (<1 ‰) WT 18 kg (<1 ‰) OFC 55 cm (98 ‰)	At birth: HT 48.3 cm (30 ‰) WT 32 kg (50 %‰) OFC 36 cm (90 %ille)	At 3 years: HT 95.8 cm (53 ‰) WT 14.8 kg (67 ‰) OFC 52.0 cm (99 ‰)	At 17 years: HT 163.8 cm (9 ‰) WT 54.98 kg (17 ‰) OFC 57 cm (91 ‰)	Shortening of the long bones on prenatal ultrasound. At 2 years: HT 76.7 cm (<1 ‰) WT 8.2 kg (<1 ‰) OFC 46.9 cm (34 ‰)	Absent growth during the last 2 weeks of gestation and was born prematurely at 35 weeks. At 4 years: HT 87.6 cm (<1 ‰) WT 11.5 kg (<1 ‰) OFC 48.5 cm (10 ‰)
Dysmorphism	Hypertelorism, bifid uvula	Arched eyebrows, downslanting palpebral fissures, hypertelorism, low set and posteriorly rotated ears, thickened helices, anterior ear pit, broad, pear-shaped nose, bulbous nasal tip, hypoplastic alae nasi, thin upper lip, small, widely spaced teeth, prominent chin, broad neck, high anterior hairline, widely spaced nipples	Frontal bossing, hypertelorism, prominent, low-set ears, triangular mouth, webbed neck, low posterior hairline	Prominent forehead, large anterior fontanelle, low nasal bridge, small nose with upturned tip, smooth philtrum, microstomia, narrow chin, overfolded helices, bilateral anterior ear lobe dimples	Prominent forehead, broad eyebrows, broad nose, thin upper lip	Broad nasal tip	Hypertelorism, upslanted palpebral fissures, depressed nasal bridge, long philtrum, small posteriorly rotated ears, preauricular pit, ptosis, triangular mouth, thin upper lip	Prominent forehead, short palpebral fissures, hypertelorism, broad nasal root
Hypotonia	Y	Y	N	Y	Y	N	Y	N
Development	DD, SD	DD, SD	DD, SD	DD, ID	DD, SD	DD, SD	DD, SD	DD, SD
Cardiovascular	Dilated aortic root (Z-score +2.2)	N	Mild mitral and tricuspid valve insufficiency	Patent foramen ovale	Atrial septal defect	Aortic coarctation	Peripheral pulmonary stenosis, aortic coarctation, dilation, and tortuosity Patent foramen ovale	Hypoplastic left aortic arch

Table 2 continued

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Skeletal	N	Cervical fusion, pectus excavatum, clinodactyly, delayed bone age	Pectus excavatum, delayed bone age	N	N	Delayed bone age	Short limbs, brachydactyly, clinodactyly, pectus excavatum, spinal stenosis	2,3 toe syndactyly
Connective tissue	N	High-arched palate, joint hypermobility	Joint hypermobility, thin and loose skin	High-arched palate	Joint hypermobility, doughy skin	N	Joint hypermobility, translucent skin	High-arched palate
Other	N	Bilateral hydronephrosis, slow growing hair, prominent subarachnoid spaces, gastrointestinal reflux, constipation, reduced sweating	Brittle nails, bilateral inguinal hernia, cryptorchidism	N	Constipation, dysphagia	Abnormal sleep patterns, dental crowding, gliosis-versus migrational abnormality, kidney cysts, epistaxis, heat intolerance, slow growing hair and nails	Feeding difficulties, iris coloboma, vesicoureteral reflux, thin, slow growing, bright orange hair, neonatal teeth	Septo-optic dysplasia, feeding difficulties, constipation, myopia, unsteady gait, inguinal hernia

M male, *F* female, *HT* height, *WT* weight, *OFC* occipital-frontal circumference, *‰* percentile, *Y* yes, *N* no, *N/A* not applicable, *DD* developmental delay, *SD* speech delay

and three had delayed bone age. Other skeletal abnormalities include stenosis or fusion of the spine, short limbs, and syndactyly. Four individuals had connective tissue abnormalities including high arched palate, joint hypermobility, and translucent or doughy skin consistency. Ophthalmologic findings in two individuals included myopia and iris coloboma. Unique epidermal features included brittle nails, poor hair growth, and neonatal teeth. In several of the patients, the initial differential diagnosis included Noonan syndrome, cardiofaciocutaneous syndrome, Costello syndrome, Ehlers–Danlos syndrome, Cat-eye-syndrome, Robinow syndrome, Aarskog syndrome, Opitz G/BBB syndrome, Rubenstein–Taybi syndrome, Floating–Harbor syndrome, hidrotic ectodermal dysplasia, and a variety of skeletal dysplasias. Prior to WES, genetic testing, including microarray, chromosome analysis, and specific single gene, and multi-gene panels specific to the above conditions was non-diagnostic.

Discussion

We identified five de novo missense variants in *PPP1CB* in eight independent individuals with a common clinical phenotype of developmental delay and/or ID, congenital heart disease, macrocephaly, short stature, and dysmorphic features. The gene is highly intolerable to variation, and the frequency of missense variants in *PPP1CB* in ExAC is 8.5e-06. Heterozygous predicted pathogenic variants in *PPP1CB* account for approximately 0.2 % of individuals referred for WES with ID or developmental delay.

PPP1CB is one of the three isoforms of catalytic subunits of PP1: PP1α (PPP1CA), PP1β (PPP1CB), and PP1γ (PPP1CC). There is conservation of these proteins throughout evolution (Cohen and Cohen 1989), and the isoforms of PP1 are >85 % identical and expressed in every tissue examined (Cohen and Cohen 1989). PPP1CB protein contains a calcineurin-like phosphoesterase domain, a metallophosphatase domain, and a serine/threonine protein phosphatase domain, where all five de novo variants identified in this study are located. Four unrelated individuals all have the recurrent Pro49Arg missense variant, and two variants replaced Glu183 with a small neutral amino acid, suggesting that amino acids Pro49 and Glu183 may play an important role in PPP1CB function. Pro49 is highly conserved among proteins in PPP1C family (PPP1CA, PPP1CB, and PPP1CC). Recently, Gripp et al. identified the de novo *PPP1CB* missense variant Pro49R in three unrelated individuals. In comparing patients who carry Pro49Arg in our current study with those previously reported, all individuals had dysmorphic features and developmental delay, but there is some phenotypic variation in cardiac and skeletal anomalies and growth. Functional studies suggest that one

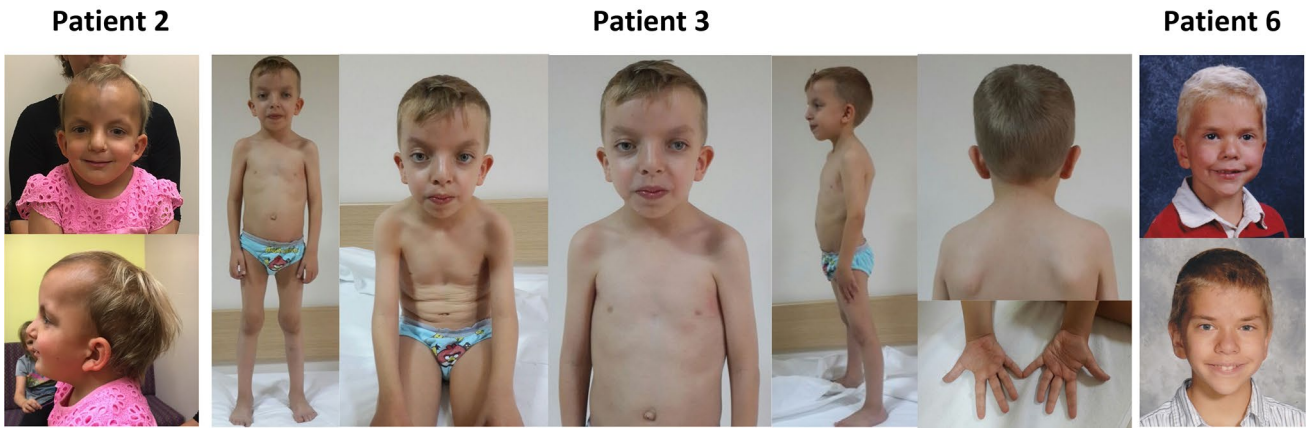

Fig. 3 Facial features of individuals with the Pro49Arg (Patients 2 and 3) and Glu183Val *PPP1CB* (Patient 6) de novo variants. Patient 6—at age 7 and 12 years. Facial features include broad nose, full cheeks, pointed chin, and wide mouth. Note the distinct change in hair coloring with age (color figure online)

of the functions of PP1 is to dephosphorylate substrates required for the completion of mitosis, and the regulatory subunit(s) associated with this function of PP1 is SDS22, also called PPP1R7 (Ceulemans et al. 2002). SDS22 interacts with PP1 and is part of a complex with PP1. Essential SDS22-binding sites are in the region of residues 43–173 of PPP1CC, and Pro49 is one of the highly conserved amino acids in this region. The missense variant Pro49Arg may alter the interaction of PPP1CB and SDS22 to regulate PPP1CB function. The Glu274Lys mutation is in the beta12-beta13 loop, which has been proposed to confer inhibitor specificity in PP1 (Kelker et al. 2009). Beta12-beta13 loop is important for toxin binding, including microcystin and okadaic acid, and is in the active site of PP1 (Maynes et al. 2001). Amino acid 274 in PP1 participates in PP1: toxin interactions through PP1: toxin hydrogen-bonds (Kelker et al. 2009), and substitution of a Lys for Glu at amino acid 274 may affect the interaction of PPP1CB with toxin or other proteins.

PPP1CB maps to chromosome 2p23.2 (Barker et al. 1994; Saadat et al. 1994). A 4.8 Mb microdeletion of 2p23.2, which contains 29 genes, including *PPP1CB*, was found in one affected subject who had overlapping features with our patients, including dysmorphic features and developmental delay. A recurrent microduplication at 2p23.2 was found in ten individuals who had overlapping features with the patients included in our study (https://decipher.sanger.ac.uk), suggesting that dosage of *PPP1CB* or nearby genes is critical to brain function. PPP1CB forms heterodimers with various PP1 regulatory subunits (Cohen and Cohen 1989). In the cardiovascular system, PPP1CB affects angiogenesis and endothelial migration in an endothelial tube formation assay (Iacobazzi et al. 2015). Cardiac-specific deletion of Ppp1cb (PP1β) in mice resulted in concentric remodeling of the heart, interstitial fibrosis, and contractile dysregulation without congenital heart disease (Liu et al. 2015).

PPP1CB is involved in several biological processes, including cell adhesion, cell cycle, small GTPase-mediated signal transduction, protein dephosphorylation, negative regulation of transforming growth factor, regulation of glycogen catabolic process, and striated muscle tissue development (Gibbons et al. 2007; Flores-Delgado et al. 2007; Bianchi et al. 2005; Moorhead et al. 1998). PPP1CB also regulates protein kinase C and protein kinase B (AKT), which are implicated in the regulation of cell growth, proliferation, survival, differentiation, and cytoskeletal changes. In primates, *PPP1CB* is expressed in spines, dendrites, axon terminals, axons, and glia in the prefrontal cortex, and is enriched in dendrites (Bordelon et al. 2005). PPP1CB is abundant in the brain and regulates synaptic plasticity (Jouvenceau et al. 2006). In mice, partial inhibition of PPP1CB in forebrain neurons improved learning efficacy, suggesting that PPP1CB is important in brain function (Genoux et al. 2002; Gräff et al. 2010). Suppressor of clear, *C. elegans*, homolog of (SHOC2), also called RAS-binding protein SUR8, is a regulatory subunit of PP1c in the Ras pathway and muscle Ras viral oncogene homolog (M-Ras) interacts with a Shoc2-PP1c dimer to form a ternary complex (Rodriguez-Viciana et al. 2006). Several studies identified a missense pathogenic variant p.S2G in *SHOC2* in Noonan syndrome or a Noonan-like syndrome with loose anagen hair (Cordeddu et al. 2009; Hoban et al. 2012; Gripp et al. 2013). The phenotype in patients with heterozygous pathogenic variants in PPP1CB clinically resembles the Noonan syndrome (Gripp et al. 2016), suggesting that mechanisms of PPP1CB in disease could be related to SHOC2 gene function.

In summary, we identified five de novo heterozygous missense variants in *PPP1CB* in eight individuals, with the recurrent Pro49Arg variant in four of the individuals.

Missense variants in *PPP1CB* may define a novel genetic syndrome associated with dysmorphic features, developmental delay/intellectual disability, congenital heart disease, macrocephaly, and short stature. *PPP1CB* is one of a growing number of phosphatases associated with neurodevelopmental disorders.

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Compliance with ethical standards

Conflict of interest Heather McLaughlin, Megan Cho, Kyle Retterer, Rhonda Schnur, Ingrid Wentzensen, and Sherri Bale are employees of GeneDx. Wendy Chung is a former employee of GeneDx and a paid consultant for Regeneron Pharmaceuticals. JRL has stock ownership in 23andMe, is a paid consultant for Regeneron Pharmaceuticals, has stock options in Lasergen, Inc., serves on the Scientific Advisory Board of the Baylor Miraca Genetics Laboratory, and is a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases, and bacterial genomic fingerprinting. The other authors declare that they have no conflict of interest.

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