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Supplemental Data

De Novo Mutations Affecting the Catalytic Cα Subunit of PP2A, *PPP2CA*, Cause Syndromic Intellectual Disability Resembling Other PP2A-Related Neurodevelopmental Disorders

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Supplemental data

Supplemental Case Reports of individuals with a *PPP2CA* mutation or partial deletion.

Individual 1

This 6-year-old boy was born at term after an uncomplicated pregnancy by caesarean section with Apgar scores of 9 and 10 after 1 and 5 minutes, respectively, and a birth weight of 3715 gram. He had laryngomalacia. His motor milestones were delayed: he could stand with support at the age of 12 months and could walk independently at the age of 20 months. At the age of 3 years he spoke several words. He attended special education and he is easily distracted and energetic. From 11 to 18 months he had atypical convulsions during fever. He had feeding difficulties.

Family history was unremarkable; he had 3 older, healthy, siblings.

Physical examination at the age of 6 years showed a height of 131 cm (+2 SD), weight of 26 kg (-0.2 SD) and head circumference of 56 cm (+2.5 SD). Target height was 175 cm (-1.2 SD). He had a broad forehead and frontal bossing, slight hypertelorism, prominent eyes, full nasal tip, low hanging columella, square shape of both ears, and short philtrum. Hands showed single palmar crease on the left side, long fingers and toes, and sandal gap on both sides. He had four small café-au-lait spots (< 0.5 cm). Brain MRI showed ventriculomegaly and enlargement of the subarchnoid space around the cerebrum without signs of increased cranial pressure. EEG, array-CGH and DNA analysis of *NSD1* (Sotos syndrome) and *PTEN* (PHTS) were normal. Whole exome sequencing revealed a *de novo* mutation in *PPP2CA* (c.572A>G; p.(His191Arg)).

Individual 2

This 7-year-old girl was born at term after an uncomplicated pregnancy and delivery and a birth weight of 3520 gram. After a few days she showed hypertonia and at the age of 4 months delayed development was noticed. At the age of 5.5 years she could make contact, sounds and was able to walk with foot casts and support.

From the age of 6 months she developed tonic-clonic convulsions, which changed into absences up to 50 times a day at the age of 5.5 years. She was diagnosed with epileptic encephalopathy and was treated with several medications including sodium valproate, levetiracetam, pyridoxine, clonazepam, rectal diazepam, alimemazine, multivitamins and had a ketogenic diet. She had severe sleeping problems and behavioural problems consisting of stereotypic behavior and automutilation. She had helmet therapy

because of plagiocephaly and was treated for malposition of the feet. She had feeding problems. She had normal vision, but hearing could not be tested.

Family history was unremarkable; she had one healthy brother.

Physical examination at the age of 6.5 years showed a height of 137 cm (+2.5 SD) and a weight of 30.2 kg (0 SD) and head circumference at the age of 1 year and 8 months was 48.3 cm (+0.5 SD). Physical examination at the age of 1 year and 8 months showed an open fontanel, plagiocephaly, some frontal bossing, broad nasal bridge, short philtrum, prominent upper lip, a sacral dimple, fetal finger pads and a single palmar crease on the left side. Skin showed no abnormalities. She had axial hypotonia and hypertonia of the legs with Babinski sign on both sides. Brain MRI showed central and peripheral ventriculomegaly, gracile corpus callosum and delayed myelination. MRS was normal.

Metabolic screening was normal and muscle biopsy showed no mitochondrial disease. DNA analysis of mtDNA, *UBE3A, MECP2, FOXG1, CDKL5* and analysis of methylation of the Angelman syndrome region and SNP-array were normal.

Whole exome sequencing revealed a *de novo* mutation in *PPP2CA* (c.882dup; p.(Arg295*)) and de novo variants of unknown significance in *ZNF687*; Chr1(GRCh37):g.151262240G>C; NM_020832.1:c.2721G>C (p.(Glu907Asp)), *MUC2*; Chr11(NCBI36):g.1088745T>C; NM_002457.2:c.7103T>C (p.(Leu2368Pro)), *FOXC2*; Chr16(GRCh37):g.86600973A>G; NM_005251.2:c.32A>G (p.(Asn11Ser)), *ARHGAP35*; Chr19(GRCh37):g.47423816A>G;NM_004491.4:c.1884A>G (p.Ile628Met)).

Individual 3

This 13-year-old boy was born at term after an uncomplicated pregnancy and delivery with a normal birth weight.

He had delayed development, could walk from the age of 2 years and started talking at the age of 4 years. His VIQ was 80 and PIQ 59, and he attended special education. He was diagnosed with PDD-NOS. His muscle tone was decreased. He had his first convulsion at the age of 10 years. He had focal epilepsy and convulsions mostly occurred at night. Electroencephalography demonstrated a left-temporo-occipital seizure focus, with an increase in focal epileptiform activity during sleep, fitting focal Electrical Status Epilepticus during Sleep (ESES). He was (previously) treated with levetiracetam, clobazam, methylprednisolone and lamotrigine.

He had feeding difficulties due to problems with chewing and (preparing the food for) swallowing, an open mouth posture, lack of facial expression, and articulation problems due to oral facial muscle

weakness. He was treated with vitamin B12 because of a vitamin B12 deficiency. He had no sleeping difficulties and normal vision and hearing.
His mother had one miscarriage and his sister died perinatally due to a coagulation defect of the mother. His father had two other children who were healthy.
Physical examination at the age of 11 years and 10 months showed a height of 164 cm (+1.3 SD), weight of 43.4 kg (-1.1 SD) and head circumference of 51.5 cm (-1.5 SD). He had periorbital fullness, restricted to the upper eyelids, long eyelashes and a full nasal tip. MRI of the brain, metabolic screening, array and DNA analysis of *PTEN* and *SCL2A1* showed no abnormalities.
Whole exome sequencing revealed a *de novo* mutation in *PPP2CA* (c.438del; p.(Phe146Leufs*29)).

Individual 4

This boy was the third child of healthy, non-consanguineous parents Family history was unremarkable. Pregnancy was complicated by diet-treated gestational diabetes mellitus. He was born full-term after normal delivery with Apgar scores of 9 and 10 after 1 and 2 minutes, respectively, and growth parameters in the normal range (birth weight 3450 gram, birth length 50 cm, OFC 36 cm). At birth he was noted to have bilateral adducted thumbs, unilateral camptodactyly of the third finger and umbilical hernia.
Development has been delayed. He could walk alone at the age of 19 months and had no spoken words at the age of 3.5 years. Physical examination at the age of 3 years and 10 months showed a height of 106 cm (+1.7 SD), weight of 16.7 kg (+0.7 SD) and head circumference of 46 cm (-3.9 SD). He had trigonocephaly, double hair whorl, short palpebral fissures, ptosis, low set and anteverted ears, high palate and retrognathia. Extremities showed a poorly developed flexure fold on the third finger of the right hand, an incomplete single transverse palmar crease and (reducible) adducted thumbs.
X-ray revealed ovoid appearance of vertebrae, coxa valga, slender distal phalanges and elevated right diaphragm. Abdominal ultrasound was normal. Cranial CT-scan ruled out craniosynostosis.
Brain MRI at the age of 2 years and 2 months showed posterior hypoplasia of corpus callosum, unmyelinated left temporal lobe and non-specific periventricular white matter hyperintensities.
Array-CGH and metabolic screening showed no abnormalities.
Whole exome sequencing revealed a *de novo* mutation in *PPP2CA* (c.922_924dup; p.(Phe308dup)).

Individual 5

This 19-year-old woman was born after a pregnancy complicated by oligohydramnios and delivery with a birth weight of 3742 gram. The baby suffered meconium aspiration but required no NICU stay. Development was delayed, and she had hypotonia. She could walk at the age of approximately 14 months, she spoke her first words prior to 2 years and had a language delay. She briefly stopped talking at the age of four years. She had a mild to moderate intellectual disability, and autism and ADHD. She had generalized tonic-clonic seizures and was diagnosed with Jeavons syndrome. She had constipation, a heart murmur, urinary urgency and recurrent ear and urinary tract infections.

Medical history showed wrist and foot fractures after minimal trauma. She had knee pain which limited daily activities and recently developed a burning pain from neck down through her left leg. She has had four periods of sudden vision loss in her left eye with blurry vision and pain. ERG was abnormal and additional studies are planned to further characterize an abnormal signal between her left and right eye. She had pigmented nevi and brownish waxy macules on the medial aspect left breast which might be caused by seborrhoeic keratosis.

Family history was unremarkable.

Physical examination at the age of 19 years showed a height of 162.8 cm (-0.2 SD), weight of 76.9 kg (>+2.5 SD) and BMI of 29. She did not have facial dysmorphisms but had crowded teeth. Extremities were unremarkable.

MRI at the age of 18 showed a T2 hyperintense focus within the left frontal lobe, likely a neural glial cyst. Array-CGH, DNA-analysis for Fragile X syndrome and lysosomal enzymes showed no abnormalities. Whole exome sequencing revealed a *de novo* mutation in *PPP2CA* (c.640 C>T; p.(Arg214*) and a mutation in *VWF* (NM_000552.3:c.2561G>A (p.Arg854Gln)) (Von Willebrand disorder).

Individual 6

This 2-year 4-month-old girl was born after a pregnancy complicated by a single umbilical artery on prenatal ultrasound. She was born at a gestational age of 39 weeks and 6 days with a normal birth weight. She received physiotherapy from the age of 6 weeks because of plagiocephaly. She refused breast feeding and after two weeks bottle feeding was started.

She was able to roll over from the age of 6 months and started to walk from the age of 2 years and 2 months. At the age of 2 years and 4 months she was babbling, did not use words, but did understand more than she could explain in words. At the age of 17 months, her development corresponded to that of a child of 13 months. BSID-II results showed a score of 61.

She had a fever-related tonic clonic insult twice. She had megalocornea, moderate excavated papillae and mild hypermetropia on both sides (+2).
She had a healthy older brother and a maternal aunt had attended special education.
Physical examination at the age of 1 year and 5 months showed a height of 80 cm (0 SD) and head circumference of 46.7 cm (-0.2 SD). She had plagiocephaly, prominent eyes, epicanthal folds, diastasis recti and mild shortened fifth digits.
Brain MRI showed a mildly underdeveloped pons and mesencephalon, mildly dilated lateral and third ventricles.
Metabolic screening and array-CGH showed no abnormalities.
Whole exome sequencing revealed a *de novo* mutation in *PPP2CA* (c.572A>G; p.(His191Arg)).

Individual 7

This 23-year-old female was born after an uncomplicated pregnancy and had a birth weight of ~2500 gram.
She could walk around the age of 12 months, had good fine motor skills, and possibly some mild hypotonia. Her language development was delayed: she spoke her first words at the age of two years, first sentences at age of four years and had speech therapy. At the age of seven years she had an IQ of ~130. At the age of 10 years ASD was diagnosed and her IQ was ~115. At the age of 16 years WISC-III showed a VIQ of 115 and a PIQ of 92. At the age of 17 years she had her first psychotic episode after which she had progressive cognitive dysfunction, with worsening of executive functions, and progressive behavioral problems defined by aggressive outbursts and episodes of confusion. At the age of 23 years she had a total IQ of ~100.
She had frequent airway infections as a young child.
Family history was unremarkable.
Physical examination at the age of 22 years showed a height of 175 cm (+0.7 SD) and head circumference of 60 cm (+2.8 SD). She had deep set eyes, small palpebral fissures, broad nasal tip, broad forehead and freckling around the mouth. Extremities showed no abnormalities.
Brain MRI, EEGs and metabolic screening were normal.
Array-CGH showed a paternally inherited duplication of ~223 kb at 4p15.2 and a maternally inherited duplication of ~1,68 Mb at Xp22.31.
Whole exome sequencing revealed a *de novo* mutation in *PPP2CA* (c. 668A>T; p.(Asp223Val)).

Individual 8

This 3-year-old girl was born after an uncomplicated pregnancy and delivery with Apgar scores of 9 and 10 after 1 and 2 minutes, respectively, and a birth weight of 3500 gram. Postnatally a clavicle fracture was diagnosed with torticollis to the right side. She had a muscular ventricular septal defect, which closed spontaneously.

Motor development was delayed because of hypotonia and hypermobility. Walking was achieved at the age of 35 months. Since she did not develop vocal language until the age of two, speech therapy was initiated. At the age of three years she could speak three-word sentences.

She had strabismus.

Family history was unremarkable.

Physical examination at the age of 2 years and 1 month showed a height of 83.5 cm (-1.3 SD), weight of 10.9 kg (-0.6 SD) and head circumference of 47 cm (-0.8 SD). She had plagiocephaly, megalocorneae (corneal diameter 13 mm), arched eyebrows, asymmetry of the nostrils, bifid nasal tip and hyperlaxity of the joints. Neurological examination revealed hypotonia with low symmetrical reflexes and a broad-based gait with hyperlordosis.

MRI at the age of 22 months showed a remarkably reduced amount of white matter, both supratentorial and infratentorial, some global atrophy of the brain (predominantly in the pontine and callosal area) and bilateral plexus choroideus cysts.

SNP array showed no abnormalities. Whole exome sequencing revealed a *de novo* mutation in *PPP2CA* (c. 373C>T; p.(Gln125*)). She also had a *de novo* variant of unknown clinical significance in *NIPBL* (NM_133433.3:c.3007_3009del; p.(Val1003del)), but was clinically not diagnosed with Cornelia de Lange syndrome.

Individual 9

This 21-month-old boy was born after a pregnancy complicated by preeclampsia and delivery by C-section with Apgar scores of 5 and 6 after 1 and 5 minutes, respectively, and a birth weight of 3324 gram.

Development was significantly delayed. He was not able to walk independently, was nonverbal and was not using signs.

He had hypotonia and cortical visual impairment due to an optic nerve anomaly. He had dysphagia for which he had tube feedings, and he had mild bilateral sensorineural hearing loss.

Family history was unremarkable.

Physical examination at the age of 1 year and 6 months showed a height of 84 cm (0.43 SD), weight of 10.8 kg (-0.2 SD) and head circumference of 43.4 cm (-3 SD). He had a prominent metopic suture, bilateral epicanthal folds and mild micrognathia. Extremities showed bridged palmar crease, ulnar deviation of wrists and fingers bilaterally, and partially adducted thumbs, right greater than left.
MRI at the age of 8 months showed pontocerebellar hypoplasia and mild ventriculomegaly.
Array CGH, gene panel for cerebellar/pontocerebellar genes (*AMPD2, CASK, CDK5, CHMP1A, EXOSC3, OPHN1, RARS2, RELN, SEPSECS, TSEN2, TSEN34, TSEN54, TUBA1A, TUBA8, TUBB2B, TUBB3, VLDR, VRK1*) and metabolic screening were normal.
Whole exome sequencing revealed a *de novo* mutation in *PPP2CA* (c.380A>G; p.(Tyr127Cys)).

Individual 10

This 8-year-old girl was born at term after an uneventful pregnancy. The neonatal period was unremarkable.
She walked independently at the age of 15 months and spoke in full sentences after the age of 3 years. She had a developmental delay, learning difficulties and autism spectrum disorder. At the age of 8 years she had normal gross motor development, a delay in fine motor development and significant speech delay for which she had speech therapy.
She started having seizures when she was 3 years old. On average she had 1-3 seizures per week and she has never been seizure-free for more than a month. She has been treated with multiple anticonvulsants including lacosamide, levetiracetam, zonisamide, and valproic acid which caused increase in seizure frequency according to the mother. She also underwent a brief trial of cannabidiol (CBD) oil.
Physical examination at the age of 8.5 years showed a height of 119.4 cm (-2 SD), weight of 19.6 kg (-1.5 SD) and head circumference of 48 cm (-2.3 SD). She had no dysmorphic features. She had poor comprehension but can follow one-step commands. Cranial nerves were grossly intact. She had hypotonia, but strength was normal in all four extremities. She had normal symmetrical deep tendon reflexes and she had normal stance and gait. No abnormal movements were appreciated.
EEGs previously showed bicentral temporal interictal spike and wave activity and other times frequent generalized spike and slow-wave activity. MRI of the brain at the age of 7 years showed no malformation of cortical development. Nonspecific thinning of the corpus callosum and apparent, nonspecific, mild volume loss of the cerebellum were noted.
Whole exome sequencing revealed a *de novo* mutation in *PPP2CA* (c.794A>G; p.(Tyr265Cys)).

Individual 11

This 2-year 4-month-old boy was born after a pregnancy characterized by small placental abruption. At 5 months intra-uterine growth retardation was noted. He was born at 35.5 weeks by vaginal delivery with a weight of 1740 gram (-2.6 SD), length of 40.5 cm (-3.1 SD) and OFC of 30.5 cm (-1.5 SD). The Apgar score was 8/9/9. The placental abruption was considered too small to explain the growth retardation. His evolution was characterized by feeding difficulties, gastro-esophageal reflux, constipation and recurrent respiratory infections. He had umbilical and bilateral inguinal hernia.

He had on motor physiotherapy from birth until the age of 28 months. He acquired a stable sitting position at the age of 12 months, walked independently at the age of 18 months and spoke his first words at the age of 16 months. He had some pronunciation difficulties. Amelioration was noted once myringotomy tubes were placed. His vocabulary is rich and at the age of 28 months, he was able to make sentences. His comprehension was always considered very good.

Family history was unremarkable.

Physical examination at the age of 2 years and 4 months showed a height of 80 cm (-3 SD), weight of 9.7 kg (-3 SD) and head circumference of 46.5 cm (-2 SD). He had frontal bossing, upslanting palpebral fissures, full cheeks, thin upper lip and everted/prominent lower lip, retrognathia, and square configuration of the ear lobule (right>left). Extremities showed bilateral 5^{th} fingers clinodactyly.

Imaging of the brain was not performed. Metabolic screening showed no abnormalities. There was no methylation abnormality at the 11p15.5 locus and no maternal uniparental disomy for chromosome 7. SNP-array showed a *de novo* 120.36 kb deletion including the 5' region of *PPP2CA* and the 3' region of *CDKL3* (chr5:133,546,961-133,667,321) and a maternally inherited 353.74 kb deletion at chr18:63,620,373-63,974,115 in which no genes were located.

Individual 12

This 11-year-old boy, the first child to non-consanguineous parents, was born at 38 weeks' gestation after an uneventful pregnancy with a birth weight of 4140 gram. At birth he had poor suck and could not breastfeed well, so he was switched to special feeding at 2 weeks of age. Parents were concerned because of lack of milestone acquisition at 6 months of age, as by then he was still unable to hold his head or sit with support. By this point in time, he also didn't track well, and kept his hands in a clenched position.

At the age of 3 years and 10 months he was unable to sit, remained nonverbal and had minimal eye contact. Vineland Adaptive Behavior Scales (second edition) were administered at that point and

revealed a communication standard score of 42 (age equivalent approximately 1-2 months), daily living standard score of 48 (age equivalent approximately 0-2 months), socialization standard score of 53 (age equivalent approximately 0-4 months), and motor skills standard score of 28 (age equivalent approximately 1 month).

At 8 months of age he developed abnormal movements and subsequently experienced an afebrile tonic clonic seizure. He went on to have several seizure types, including staring spells, head drops, and tonic clonic seizures. At 10 months, an EEG was performed and revealed hypsarrhythmia (West syndrome). Despite treatment with multiple antiepileptic drugs (lamotrigine, levetiracetam, topiramate), he continued to have 1-2 seizures per day.

He developed chronic constipation starting at 1 year of age. He had an ASD (atrial septal defect) and low bone mineral density (Z-score -2.7 at spine L1-L4).

Physical examination at the age of 3 years and 10 months showed a height of 94.5 cm (-1.6 SD), weight of 12.5 kg (-2.1 SD) and head circumference of 48.5 cm (-1.1 SD). He had no dysmorphic features. His neurologic exam revealed truncal hypotonia, and adventitial movements consistent with choreiform activity in all four limbs.

Brain MRI showed no structural abnormalities, but slight accentuation of the contrast between gray matter and white matter. Brain MRS showed low NAA (N-acetylaspartate) and elevated choline at several locations studied. Extensive metabolic workup was unremarkable. Karyogram, chromosome microarray, DNA analysis for Fragile X syndrome*, MECP2, ARX, CLN1, CLN2, COX10, SCO2, SURF1, SCO1, POLG1* and mitochondrial DNA were normal.

Whole exome sequencing revealed a *de novo* mutation in *PPP2CA* (c.391G>C; p.(Asp131His)).

Individual 13

This 12-month-old boy was born at 37 weeks via Caesarean section for oligohydramnios and breech positioning. Apgar scores were 7 and 8 at 1 and 5 minutes, respectively. Prenatal ultrasound showed microcephaly. After birth, he was transferred to the NICU for respiratory distress, a heart murmur, and for small size. Measurements for birth weight, length, and head circumference were all greater than 2 standard deviations below the mean. He required CPAP for one day.

At 5 months of age, he had several episodes of sudden bilateral upper and lower extremity extension, with loss of tone and post-ictal perioral cyanosis and was started on levetiracetam. He continued to have breakthrough seizures on maximal dosing and was transitioned to oxcarbazepine, which has provided adequate seizure control.

He had global developmental delay. At 11 months of age, the Capute scales documented significant delays in language and cognition, with a CLAMS Developmental Quotient of 73 and a CAT Developmental Quotient of 21. Gross motor age equivalency was determined to be approximately 5 months through the Bayley Motor Scale. He had a trileaflet aortic valve. He had a normal neuro-ophthalmology exam apart from intermittent exotropia.

Family history was unremarkable.

Physical examination at the age of 12 months showed a height of 71.5 cm (-1.9 SD), weight of 7.9 kg (-1.9 SD) and head circumference of 40.2 cm (-4.6 SD). He had bilateral single palmar crease and adducted thumbs and several hyperpigmented macules, some with irregular borders. He has global hypotonia and hyperreflexia in the lower extremities.

His EEGs have captured clinical events with electrographic correlate arising from the left central and posterior regions with some multifocal sharps and spikes. There has not been evidence to date of an epileptic encephalopathy.

Brain imaging at 5 months demonstrated microcephaly, enlarged subarachnoid spaces, and additional nonspecific findings of cerebral underdevelopment.

Chromosomal microarray identified a maternally inherited ~347 kb duplication of uncertain significance at chromosome 7q11.22. This duplication includes the terminal portion of one gene, *AUTS2*. Whole exome sequencing revealed a *de novo* mutation in *PPP2CA* (c.366G>A; p.(Gln122His)).

Individual 14

This 4-year-old girl was born at a gestational age of 39 weeks. She had hyperbilirubinemia on the fourth day of life requiring phototherapy until the eighth day of life. Primary microcephaly was noticed in the neonatal period. Tonic-clonic seizures and recurring vomiting started in the first year of life. Epilepsy was refractory to an initial therapy with topiramate and primidone as well as phenobarbital, but seizure frequency initially responded to a combination of levetiracetam and topiramate.

She had severe combined developmental delay and does not speak which could be partially explained by her being a refugee. She had no regression. She had severe truncal hypotonia.

Family history was unremarkable.

Physical examination at the age of 4 years showed a height of 99 cm (-0.8 SD), weight of 15 kg (-0.6 SD) and head circumference of 41 cm (< -2 SD). She presented with striking stereotypies. She had large, almond shaped eyes, small mouth, high palate, and an atypical single palmar crease on the left hand, pes planus and overlapping toes.

Continuous generalized deceleration was seen in EEG indicating diffuse brain dysfunction.
A suspicious craniocervical passage was reported in an MRI at the age of 6 months. An MRI at the age of 2 years showed slightly dilated external and internal subarachnoid spaces but no abnormalities of the craniocervical passage. Additionally, MRI showed diffuse but discrete atrophy, adequate myelination.
Whole exome sequencing revealed a *de novo* mutation in *PPP2CA* (c.263A>G; p.(Asp88Gly)).

Individual 15

This 3-year-old boy was born at term after an uneventful pregnancy and delivery with a birth weight of 3620 gram and Apgar scores of 10 and 10 at 1 and 5 minutes, respectively. In the first year of life, he cried frequently and was difficult to comfort and to handle. His psychomotor development was delayed; he was supported by physiotherapy and later by early support therapy. He started walking at the age of 18 months and spoke his first words at 18 months. At 2 years of age, his active vocabulary comprised 5 words, but he had a better speech comprehension. Upon clinical re-evaluation at age 3 years and 4 months, his development was delayed by 1-1.5 years. He showed behavioral anomalies with recurrent episodes of screaming and crying.
Family history was unremarkable.
Physical examination at the age of 3 years and 4 months showed a height of 99 cm (0 SD), weight of 17 kg (+1.2 SD) and head circumference of 48 cm (-2 SD). His craniofacial features were not dysmorphic; he had a small broad nasal tip with small alae nasi. He had a single palmar crease at his left hand and a sacral hemangioma.
EEG, cranial MRI scan, and audiometric testing were all normal.
Chromosome analysis by karyotyping was normal (46,XY), array analysis showed a paternally inherited 4q31.3 duplication of 398 kb in size, which was upon re-evaluation classified as benign CNV. Whole exome sequencing revealed a *de novo* mutation in *PPP2CA* (c.179G>T; p.(Gly60Val)).

Individual 16

This 7-year-old boy was born at term. At the age of 1 year he had his first generalized seizures and was successfully treated with valproate, but then suffered autistic regression and the treatment was changed to topiramate. After a few seizure free months, the topiramate was stopped. At the age of 6 years he started to have generalized seizures again and had a few status epilepticus events. He was treated with levetiracetam without success and could not tolerate lamotrigine. At the age of 7 years he was treated

with valproate, clobazam and topiramate. He had drug resistant epilepsy with generalized seizures on a daily basis, mostly upon awakening, and was about to start medical cannabis.

He was diagnosed with severe autistic spectrum disorder requiring permanent supervision and severe developmental delay with a Developmental Quotient lower than 50.

He had healthy parents. His brother had cerebral palsy due to perinatal stroke.

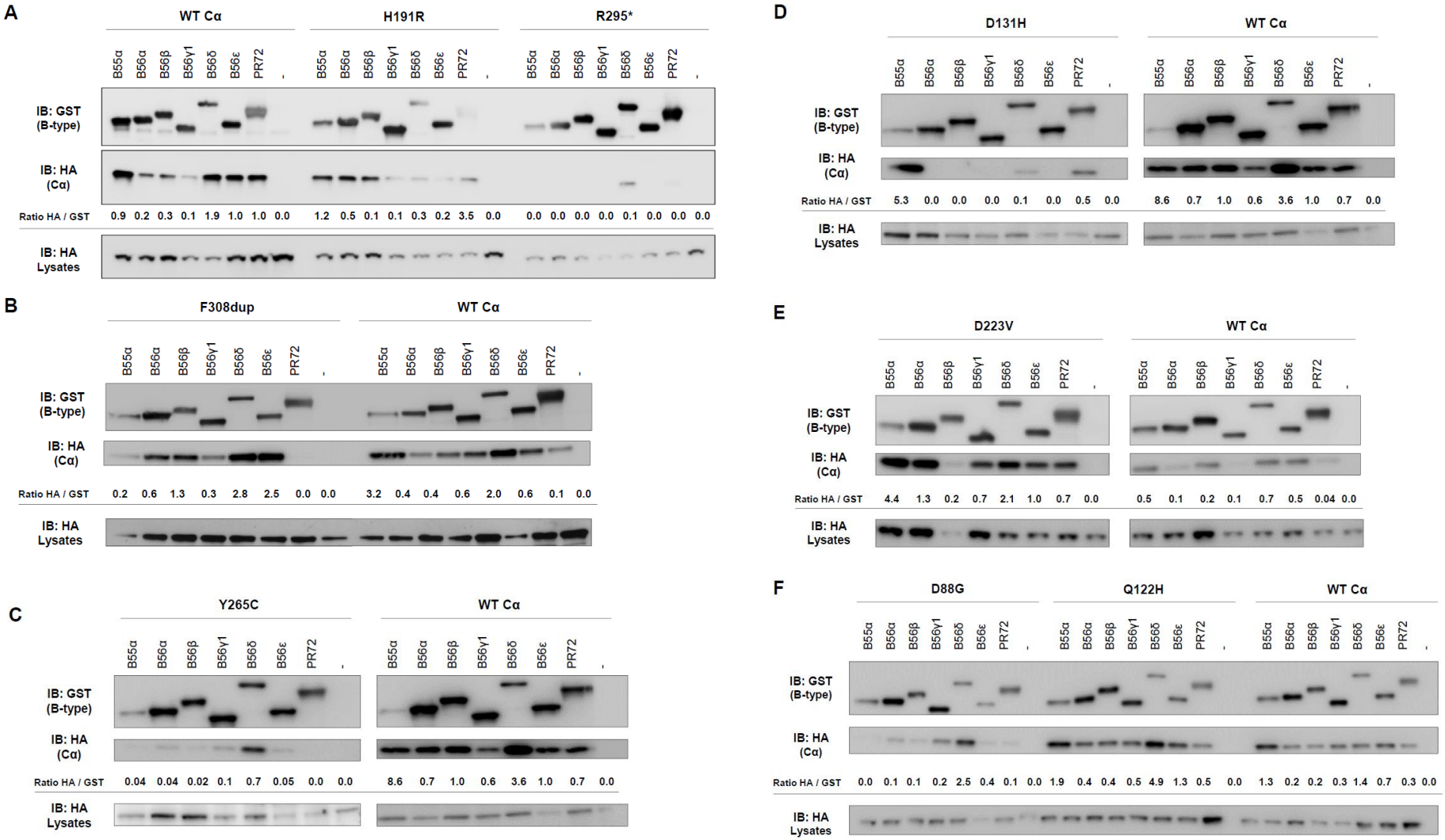
Physical examination at the age of 7 years showed a height of 113 cm (-2 SD), weight of 20 kg (-1.5 SD) and head circumference of 52.8 cm (0 SD).

Metabolic screening was normal. EEG at the age of 7 years showed general spike and wave activity corresponding with limb tremor and staring. Brain MRI at 1 year of age was normal, brain MRI at the age of 3 years showed mild dilatation of perivascular spaces and brain MRI at the age of 7 years showed more marked dilatation of perivascular spaces along the corona radiata bilateral.

Chromosomal microarray showed no abnormalities.

Whole exome sequencing revealed a *de novo* mutation in *PPP2CA* (c.667G>C; p.(Asp223His)).

Figure S1: Binding of mutant PP2A Cα subunits to B-type subunits



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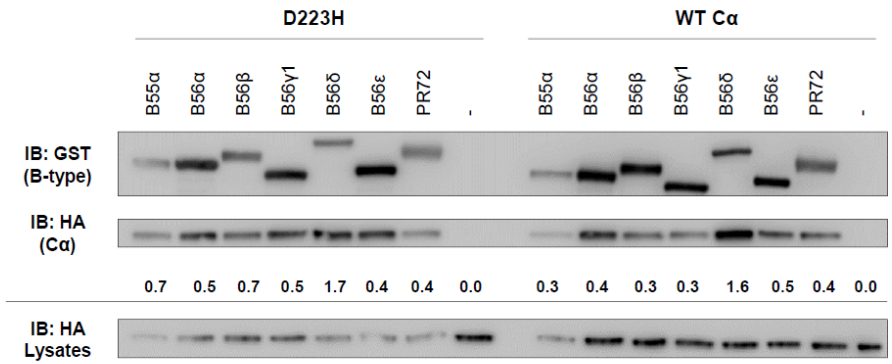

Legend Figure S1.

A-G. GST-tagged B-type subunits were co-expressed with HA-tagged WT or mutant Cα proteins (H191R, R295*, F308dup, Y265C, D131H, D223V, D88G, Q122H and D223H) in HEK293T cells. The presence of the PP2A C variants was subsequently assessed in GST pull downs by anti-HA immunoblotting. Each blot is a representative image of at least n=3.

Table S1. Oligonucleotides used for site-directed mutagenesis.

G60V Forward	5’-GTCTGTGGAGATGTGCATGTGCAATTTCATGATCTCATG-3’
G60V Reverse	5’-CATGAGATCATGAAATTGCACATGCACATCTCCACAGAC-3’
D88G Forward	5’-CTTGTTTATGGGAGATTATGTTGGCAGAGGATATTATTCAGTTGAAA-3’
D88G Reverse	5’-TTTCAACTGAATAATATCCTCTGCCAACATAATCTCCCATAAACAAG-3’
Q122H Forward	5’-GGAATCATGAGAGCAGACACATCACACAAGTTTATGGTT-3’
Q122H Reverse	5’-AACCATAAACTTGTGTGATGTGTCTGCTCTCATGATTCC-3’
Q125* Forward	5’-CATGAGAGCAGACAGATCACATAAGTTTATGGTTTCTATGATG-3’
Q125* Reverse	5’-CATCATAGAAACCATAAACTTATGTGATCTGTCTGCTCTCATG-3’
Y127C Forward	5’-GAGCAGACAGATCACACAAGTTTGTGGTTTCTATGATGAATG-3’
Y127C Reverse	5’-CATTCATCATAGAAACCACAAACTTGTGTGATCTGTCTGCTC-3’
D131H Forward	5’-CAGATCACACAAGTTTATGGTTTCTATCATGAATGTTTAAGAAAATATGGAAAT-3’
D131H Reverse	5’-ATTTCCATATTTTCTTAAACATTCATGATAGAAACCATAAACTTGTGTGATCTG-3’
F146fs Forward	5’-GTTTGGAAATATTTACAGATCTTTTTGAC-3’
F146fs Reverse	5’-GTCAAAAAGATCTGTAAATATTTCCAAAC-3’
H191R Forward	5’-GCCTACAAGAAGTTCCCCGTGAGGGTCCAATGTGTGACTTG-3’
H191R Reverse	5’-CAAGTCACACATTGGACCCTCACGGGGAACTTCTTGTAGGC-3’
R214* Forward	5’-GTTGGGGTATATCTCCTTGAGGAGCTGGTTACACC-3’
R214* Reverse	5’-GGTGTAACCAGCTCCTCAAGGAGATATACCCCAAC-3’
D223H Forward	5’-GCTGGTTACACCTTTGGGCAACATATTTCTGAGACATTTAATC-3’
D223H Reverse	5’-GATTAAATGTCTCAGAAATATGTTGCCCAAAGGTGTAACCAGC-3’
D223V Forward	5’-CTGGTTACACCTTTGGGCAAGTTATTTCTGAGACATTTAATCA-3’
D223V Reverse	5’-TGATTAAATGTCTCAGAAATAACTTGCCCAAAGGTGTAACCAG-3’
Y265C Forward	5’-GATTTTCAGTGCTCCAAACTGTTGTTATCGTTGTGGTAACC-3’
Y265C Reverse	5’-GGTTACCACAACGATAACAACAGTTTGGAGCACTGAAAATC-3’
R295* Forward	5’-GCAGTTTGACCCAGCACCTCGTTGAGGCGAGCCACATGTTACTC-3’
R295* Reverse	5’-GAGTAACATGTGGCTCGCCTCAACGAGGTGCTGGGTCAAACTGC-3’
F308dup Forward	5’-GATTACGCTTCTAGAATGGACGAGAAGGTGTTCACC-3’
F308dup Reverse	5’-ATCATGTCTGGATCCTTACAGAAAGAAGTAGTCTGGGGTACGACG-3’