SUPPLEMENTARY MATERIAL

CASE REPORTS

Case 1

Patient 1 is a 6-year-old female who was born at term after an uneventful pregnancy. Her parents were not consanguineous, and she had a healthy sister and brother. She started having seizures in the neonatal period, and electroencephalogram (EEG) performed at 3.5 months showed multifocal acute waves over the central region of the right cerebral hemisphere in the midline and the right temporal regions. During the study, a total of 4 episodes of proximal stiffening of the limbs with a decrease in EEG activity were recorded. These episodes were highly suggestive of infantile spasms. Later, seizures appeared mainly in the context of infections, and they were myoclonic, focal and generalized with a tendency to evolve into status epilepticus. At 17 months of age, a slow background was the only notable EEG finding. The patient had repetitive respiratory infections, otitis and pneumonia. She had a severe neurodevelopmental delay, and physical exams at 9 months and 4 years of age showed horizontal nystagmus and severe spastic-dystonic tetraparesis with brisk deep tendon reflexes, bilateral ankle clonus and the Babinski sign. She also had choreiform movements and was unable to walk and communicate.

Magnetic resonance imaging **(**MRI) performed at 2 and 5 years of age showed diffuse severe hypomyelination with a thin corpus callosum, white matter atrophy with posterior predominance, colpocephaly and brainstem and cerebellum hypoplasia. Spectroscopy showed a decreased N-acetylaspartate peak. Visual evoked potentials (VEPs) were normal. The results of metabolic analysis of plasma, urine and cerebrospinal fluid (CSF) were normal except for a mild decrease in 5-hydroxyindoleacetic acid levels. Array-CGH data were also normal.

Case 2

Patient 2 is a 4-year-old male who was born by caesarean section at 32 weeks of gestation because of intrauterine growth restriction, oligohydramnios and maternal preeclampsia with HELLP (haemolysis, elevated liver enzyme levels, and low platelet levels) syndrome. His

parents were not consanguineous. His birth weight was 1260 gr, and his Apgar scores were 9/9/9. He had extreme neonatal hypotonia with feeding difficulties and bilateral cryptorchidism. He was diagnosed with neonatal anaemia that required transfusion of three red blood cell concentrates. He started having seizures during the second week of life, which occurred daily during the first 2 months despite several antiepileptic drug combinations. Afterwards, seizures usually appeared during febrile illness and could evolve into status epilepticus. The seizures involved facial congestion, tachycardia and upper limb and palpebral clonic movements. The patient was treated with levetiracetam, and clobazam was added during febrile episodes. He suffered recurrent bronchitis episodes and pneumococcal bacteremia at 2 years of age. Physical exam at 3 years of age showed acquired microcephaly (head circumference: 46.2 cm; -3.3 SD), failure to thrive, poor eye contact, pendular nystagmus, severe hypotonic tetraparesis with areflexia and a startle response to acoustic stimuli. Neurodevelopment was seriously delayed, with the patient exhibiting severe motor delay, no language acquisition and profound cognitive impairment.

Repeated EEG showed multifocal epileptic abnormalities with slowed background but were normal from ten months of age. A nerve conduction study performed at the age of 11 months showed axonal neuropathy. MRI at the age of 11 months showed severe diffuse hypomyelination with a thin corpus callosum, white matter atrophy and colpocephaly. The latencies of auditory evoked potentials (BAEPs) and VEPs were prolonged. The results of plasma and urine metabolic studies were all normal.

Case 3

Patient 3, a 13-year-old female, was born at 35 weeks and 5 days and had a birth weight of 2740 gr and a length of 44 cm. Her mother had been diagnosed with systemic lupus erythaematosus, had suffered a previous spontaneous miscarriage, and underwent treatment with acetylsalicylic acid during pregnancy. Delivery was normal (Apgar scores 10/10), but the patient developed transient neonatal hypocalcaemia. During the postnatal period, gastroesophageal reflux disease, as well as an atrial septal defect, was diagnosed. Axial hypotonia, bilateral nystagmus, and early episodes of eye deflection were noted. The patient often cried during the first months of life, and a developmental delay became apparent at 6 months. At that time, she presented with status epilepticus with repeated tonic seizures

following a febrile episode. Treatment with clonazepam, phenytoin, and valproic acid was started. Afterwards, she suffered seizures during infectious diseases. At 16 months of age, she developed truncal ataxia with slight spasticity and nystagmus, which became more evident in the lateral right gaze. At 23 months of age, cerebellar syndrome was evident. She began to stand up with support and to say some syllables. At 3 years of age, she presented acute neurological deterioration during a respiratory illness, with cerebellar worsening and choreo-dystonic movement. She had several episodes of acute otitis media and pneumonia, exhibited hypogammaglobulinemia with a low CD19+ B-cell count, and was administered immunoglobulin. On the most recent neurological examination at 7 years of age, she did not walk alone, spastic paraparesis with increased tone in the lower limbs and brisk reflexes in all four limbs was evident, and ataxia and dysmetria had slightly improved. She had a moderate intellectual disability and exhibited smiling behaviour. Growth was delayed, with the patient having a weight of 39 kg (-1 SD) and a height of 141 cm (-2.7 SD).

Initial MRI brain scan at 6 months of age showed signal alteration (T2W and FLAIR) in the posterior fossa and cerebellar calcifications. By age 3.5 years of age, MRI demonstrated diffuse hypomyelination with dysplastic, a thin corpus callosum and medulla, and progressive cerebellar atrophy with vermian predominance and calcifications. MRI spectroscopy pattern was normal. Karyotype analysis was normal. Extensive metabolic testing revealed no abnormalities. Muscle biopsy did not show any relevant findings. Mitochondrial respiratory chain enzyme levels were normal. The candidate genes *CCM1/2/3*, *COL4A1, COL4A2, MECP2, CDKL5, SCA1,* and *PDHA1* were sequenced without abnormal findings.

Case 4

Patient 4 is a 19-year-old female of French descent. She was born at 36 weeks after an unremarkable pregnancy. A severe developmental delay became evident very early, with the patient exhibiting little spontaneous movement and no communicative intent. She developed spastic paraplegia and suffered focal seizures with impaired awareness. On examination, there were no dysmorphic features. She had lateral gaze nystagmus, truncal ataxia, severe spasticity of the upper and lower limbs with brisk reflexes, bilateral Babinski signs and bilateral hand tremors. Currently, she is dependent on others for daily life activities, walks

with support, is capable of saying two words but unable to say a whole sentence. MRI showed diffuse hypomyelination with more conspicuous T2W and FLAIR periventricular hyperintensity, thinning of the corpus callosum and brainstem and cerebellar atrophy. Extensive metabolic and genetic test results were normal.

Case 5

Patient 5 is a 10-year-old fraternal twin male born at 35.5 weeks from an unrelated-donor egg. The prenatal period was uneventful. The neonatal period was notable for hypoglycemia, hypotonia, torticollis and jaundice. Poor head control and delayed milestones were observed in the first 6 months of age; oral feeding difficulties and emesis marked the first 18 months of age. The family history is significant for paternal 3rd cousin with Crohn's disease; father with loose joints, strabismus, hypotonia and generalized fatigue in early childhood period.

Developmental milestones included the following: Rolling over at 9 months; sitting without support at 12 months; walking at 2 years, 9 months; and, speaking of first words at 24 months. Gait was abnormal due to generalized hypotonia, increased tone, and spasticity in bilateral lower extrimities. Independent walking and running, both with a wide-based gait, required the use of supra malleolar orthotics. Gait gradually worsened over time. At 6 years of age, some left-leg dragging began. Stair ascention was possible using a side rail. Increasing spasticity prompted surgical intervention with bilateral selective percutaneous myofascial lengthening to both achilles tendons. Spasticity has continued to progress since that time. Currently, bilateral ankle-foot orthotics allow ambulation over short distances using a walker. Assistance is needed for bed to chair and sitting to standing transitions. No support is needed for toileting.

Physical exam at age of 5 revealed short palpebral fissures, an upturned nose, overfolded upper ear helix with ear asymmetry, keratosis pilaris, and ligamentous laxity. He had generalized hypotonia and lower extremity spasticity in his hips and ankles. He has a marked alternating hair color and texture pattern.

Clinical exam at 10 years of age revealed weight 26.8 kg, 15%ile, Z score -1.14, Height of 133.6 cm 24%ile, Z score - 0.71 and of BMI 15 kg/m2 17% Z score -0.97. The face is elongated with asymmetry, mid face hypoplasia, micrognathia, high arched palate, and overcrowded teeth. There is ear asymmetry with an overfolded upper ear helix. Neurological

exam revealed mild lateral asymmetry and lower extremities more affected than upper. Reflexes were brisk and complicated by bilateral hamstring contracture. Opthalmological exam was normal. Speech evaluation revealed moderate to severe speech impairment related to low tone and diminished respiratory support for speech production in a co-articulatory manner. Audiological evaluation showed normal hearing sensitivity but abnormal brainstem auditory evoked response function; Neurodevelopmental evaluation revealed borderline intellectual functioning and impaired adaptive functioning. Functioning has decreased in the past three years, suggestive of progression.

Brain MRI showed progressive cerebral atrophy and delayed myelination that never reached completion. Patchy, diffuse T2 hyperintensities were noted bilaterally. Electroencephalography detected interictal epileptiform discharges in the right occipital and bilateral frontal regions. Repetitive nerve stimulation and needle electromyogram studies were normal and not indicative of a neuromuscular disorder. A radiographic skeletal survey detected osteopenia, which was confirmed by decreased bone mineral density on a DEXA scan. Extensive biochemical and mitochondrial studies in CSF, serum and urine were normal except for borderline-low decrease in CSF serine (normal in plasma and urine). The CSF neuropteran level was normal.

Other medical history includes constipation, epistaxis and three episodis of impetigo, and at 9 years and 8 months, a diagnosis of juvenile idiopathic arthritis was made. Given the normal platelet count and history of epistaxis, a platelet electron transmission microscopy study was performed and showed normal numbers of dense granules.

Case 6

Patient 6 is a 6-year-old boy born to Vietnamese and Caucasian parents. He was delivered at term by caesarean section due to breech presentation after an unremarkable pregnancy and had a birth weight of 2.63 kg (-1.2 SD). Congenital axial hypotonia was evident from birth. He had feeding difficulties and required a nasogastric tube for the first 6 weeks of life. Dysmorphic features were present, such as bilateral epicanthal folds, long palpebral fissures, a wide nose with anteverted nares, a wide mouth, small widely spaced teeth, thin fingers, and a single palmar crease on the right hand. He was diagnosed with mild bilateral sensorineural

hearing loss. Bilateral cryptorchidism and minor cardiac and renal malformations were also present. Ophthalmological examination revealed bilateral iris and retinal coloboma and myopia. He had seizures 2 hours after birth that were unresponsive to treatment with phenobarbital and levetiracetam but relapsed after the first month of life when topiramate was added. He has not had any subsequent seizures. Anticonvulsant drugs were successfully withdrawn at 18 months of age, and he remained seizure-free. He had postnatal growth failure, and his development was severely delayed; he sat at 17 months of age and walked at 4.5 years of age, exhibiting a wide-based unsteady gait and frequent falls. He was diagnosed with a language disorder and did not learn to speak. He had a severe intellectual disability with no behavioural abnormalities except for hand flapping when excited. At the most recent examination at 6 years, he weighed 15.8 kg (-2.3 SD), was 105.8 cm tall (-2 SD), and had a head circumference of 47 cm (-2 to -3 SD). He had axial hypotonia and spastic tetraparesis with brisk reflexes and bilateral ankle clonus.

Initial brain MRI showed delayed myelination, although on his last MRI at 3 years of age, myelination was normal. This last study revealed a dysplastic corpus callosum and mild brainstem and cerebellar atrophy.

Case 7

Patient 7 is an 11-year-old girl, the only child of unrelated parents with unremarkable familiar history. She was born by caesarean section because of maternal hypertension at 37 weeks and 4 days after an uneventful pregnancy, and ultrasound and prenatal screening tests were normal. At birth, she had a weight of 3590 gr (1.7 SD), a length of 51 cm (1.48 SD), a head circumference of 36.5 cm (1.8 SD), and Apgar scores 9/10. She exhibited regular sleep with few nocturnal awakenings; otherwise, the neonatal period was normal. At 3 months, she started having episodes of redness of her face, fixed eyes, breathing cessation, hypertonia of the arms and legs, and unconsciousness for 30 seconds. Epilepsy was reasonably excluded by normal EEG. She was diagnosed with gastroesophageal reflux with a partial treatment response. Polysomnography showed hypoxemic events during sleep. Neurodevelopment was normal for the first months, and she sat at 7 months of age. A global delay became evident after that; she did not start to walk before 28 months of age, said her first words after three years of age, has difficulties with relationships, exhibits discontinuous eye contact and

requires speech therapy and physiotherapy support. At the most recent examination at 11 years of age, she presented stereotypic movement of the mouth, tongue and hands (opening-closing) and head (lateral rotation with lateral deviation of the gaze) and bruxism. Physical examination revealed plagiocephaly, a squared face, a high forehead, hypertelorism, epicanthal folds, a thin upper lip, a wide nasal root and anteverted nostrils with incisurae alae nasi. Microcephaly became evident, with the patient presenting a head circumference of 52.3 cm (-1.9 SD), weight of 40 kg (-0.44 SD) and length of 148 cm (-0.67 SD). Ophthalmological evaluation indicated ocular dyspraxia and strabismus. She presented axial hypotonia with spastic paraparesis, brisk deep tendon reflexes and bilateral Babinski signs. Currently, she is able to move a spoon to her mouth but she is not able to fill it. She is using a tablet with pictures for alternative communication. She has not achieved urinary or faecal continence.

Brain MRI at 12 months of age showed external hydrocephalus with remission in successive controls. MRI at 2 years of age showed hyperintensity of the white matter in the temporal and posterior regions and mild cerebellar atrophy (predominantly in the inferior lobe). PEVs revealed a slowing of the conduction. The candidate genes *FGFR2, FGFR3, TWIST, MECP2, CDKl5, FOXG1* and *MEF2C* were sequenced by Sanger sequencing with normal results.

Case 8

Patient 8 is a 5-year-old male patient who was born at 36 weeks of gestational age after an uneventful pregnancy. His birth weight was 2860 gr. His parents were first-degree cousins of Turkish origin. The mother reported one previous ectopic pregnancy and one previous abortion due to unspecified causes. A neurodevelopmental delay was evident since the first months, and he had severe neonatal hypotonia with suction problems in the first days of life, requiring a nasogastric tube for feeding. Bilateral cryptorchidism was noted. He started having seizures in the first week of life, and initial EEG showed symmetric delta theta activity with bilateral frontal focus. He had spontaneous cramping with disorganized motor skills. Ocular tracking and fixation were deficient. There was no grip and no hand-eye coordination.

At the age of 4 years, he is a watchful child who does not engage with gaze. He has predominantly axial hypotonus with appendicular spasticity mainly in the right limbs, and he does not maintain control of his head. Normal and symmetrical osteotendinous reflexes are

present. EEG at the age of 12 months showed poorly differentiated diffuse and symmetrical theta activity and unrecognizable background rhythm. He received levetiracetam and phenobarbital, and after the addition of clobazam at the age of 3 years, seizures stopped, but EEG continued exhibiting a 5 Hz background rhythm mixed with theta dysrhythmic activity and recurring spikes and spike-wave spread without physiological elements during sleep, during which spike-wave figures became more frequent with left predominance. The results of metabolic analysis of plasma, urine and cerebrospinal fluid were normal. MRI performed at 1 month of age showed bilateral perisylvian polymicrogyria. Metabolic studies performed in plasma, urine and cerebrospinal fluid (CSF) were normal

He was admitted several times due to non-hemolytic anaemia, requiring RBC transfusions. Recurrent vomiting and gastroesophageal reflux ended in hypovolemic shock requiring admission, and at two years of age, percutaneous endoscopic gastrostomy was performed. He had recurrent respiratory infections.

Case 9

Patient 9, a 42-year-old man, was born to nonconsanguineous parents of Spanish descent. He was born at term after an unremarkable pregnancy and delivery. He has two older healthy siblings. While his development was considered normal during childhood, he was always considered a clumsy boy who fell frequently. At the age of 17 years, he started to experience difficulties walking. He complained of weakness and stiffness of the lower limbs that worsened over the years and urinary urge, with no other neurological findings. Spastic paraplegia became evident, and he was widely studied. Brain MRI at age 35 showed an arachnoid cyst of the posterior fossa with a mild mass effect on both cerebellar hemispheres and cervical spinal cord atrophy, which was otherwise normal. Visual evoked potentials were compatible with central bilateral involvement, and auditory evoked potentials showed long latencies, indicating bilateral conduction involvement. At age 21, he was diagnosed with ileocolonic Crohn's disease with a stenosing-inflammatory pattern and a steroid-dependent course. He underwent treatment with azathioprine, which was discontinued due to digestive intolerance. At age 27, mercaptopurine was started, and clinical and biological remission of the disease was achieved. Staged biopsies of the colon described fragments of the colonic

mucosa with moderate inflammatory infiltrate and the presence of eosinophils in the lamina propria.

On the most recent neurological examination at 40 years of age, he had lateral gaze exhaustible nystagmus. Strength of the lower limbs (MRC: 3/5) was moderately decreased, and strength of the upper limbs was normal except for the bilateral *first interosseous*, *abductor pollicis brevis*, and *abductor digiti minimi* muscles, which were slightly weak (MRC: 4/5). The tone was increased, and deep tendon reflexes were globally brisk in all four limbs, with bilateral Hoffman's sign, clonus, and bilateral extensor plantar responses. He had distal hypopallesthesia in the lower limbs and decreased pin-prick sensation in his feet. He has a paraparetic gait and needs crutches to walk. He worked as a carpenter until 27 years of age, after which he received a disability grant. He has two healthy daughters aged 3 and 12.

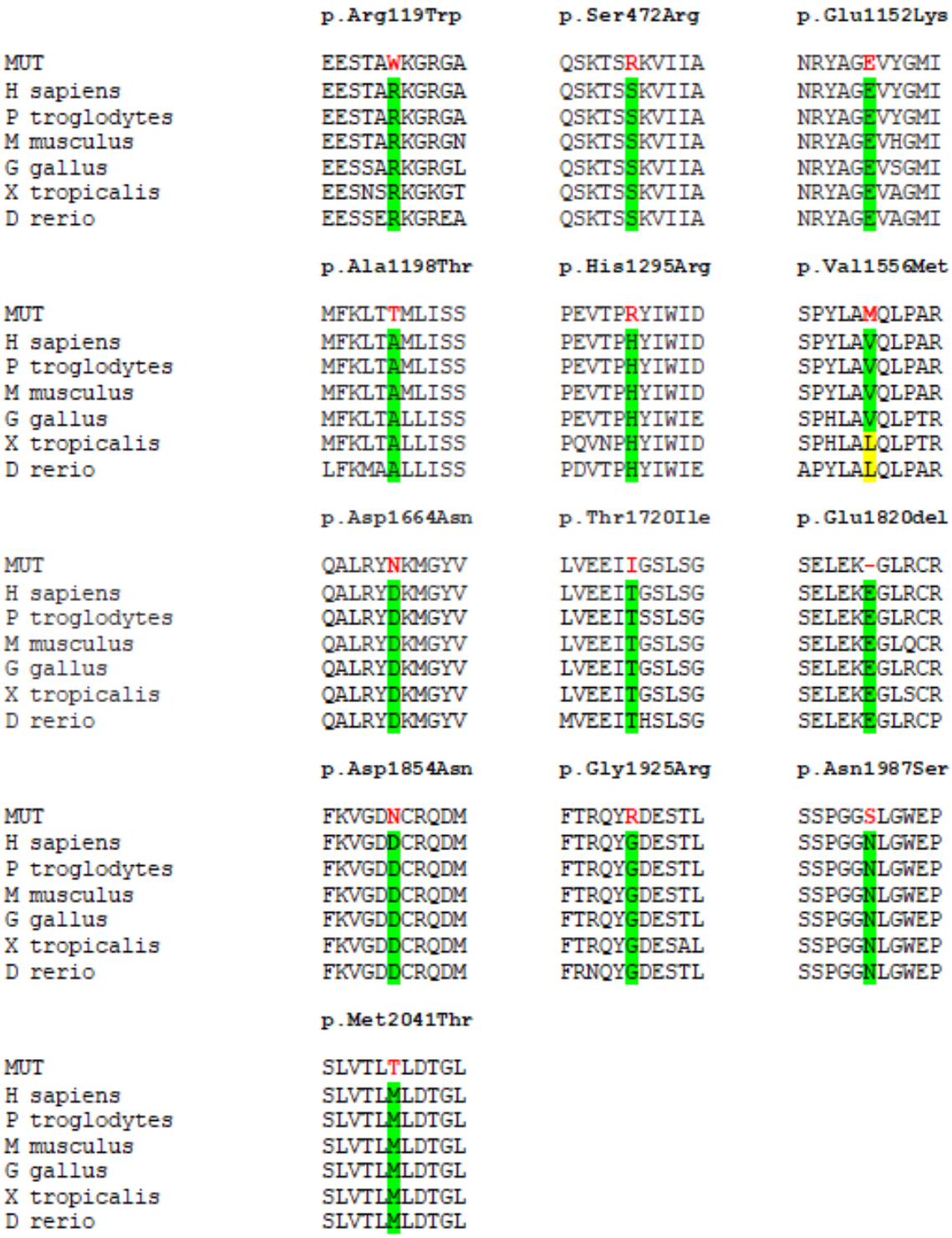
Case 10

Patient 10, an 18-year-old male adolescent, is the descendant of nonconsanguineous Latin American parents. Her mother had two older boys from a prior marriage. He was born at term after an unremarkable pregnancy. His development was considered normal during the first year, and he started to walk at 15 months of age, but his parents worried about frequent falls and a tiptoe gait and visited a neuro-paediatrician, who made the diagnosis of spastic paraparesis at 2 years of age. He received treatment with carbidopa/levodopa with no response and botulinum toxin with slight improvement. At 17 years of age, physical exploration showed mild progression of spasticity, with lower limb hypertonia, hyperreflexia, and bilateral ankle clonus. The upper limbs had normal tone but brisk reflexes. He had bilateral cavus feet and contractures of the hips, knees, and ankles. He has mild cognitive impairment and performs poorly in school, lagging behind his peers. Cranial MRI was normal apart from atrophy of the cervical spinal cord. The results of karyotype analysis and metabolic tests were normal.

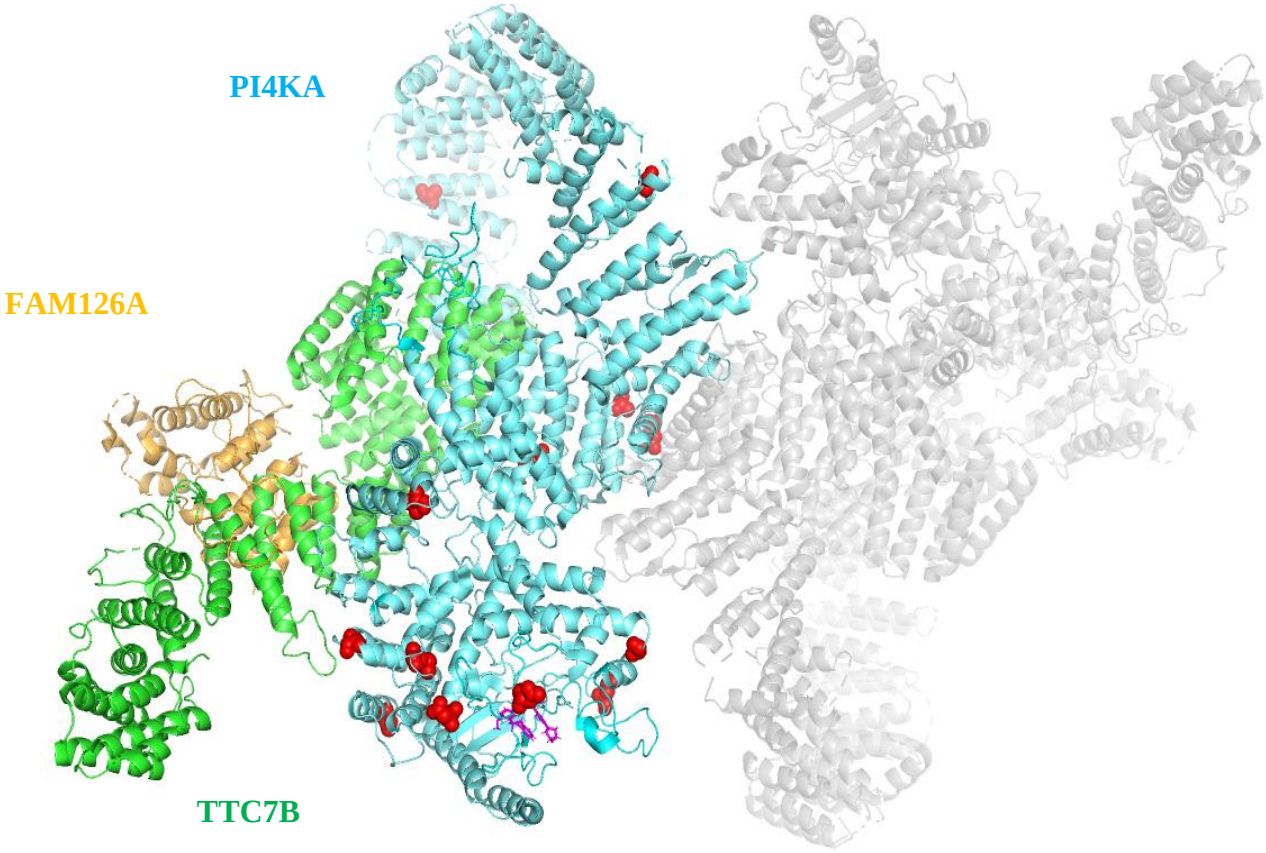
Patient	Genomic position (hg19)	cDNA change (NM_058004)	Protein change (NP_477352)	Frequency in gnomAD v2.1.1 (#/total alleles)	Frequency in gnomAD v3 (#/total alleles)	PolyPhen-2 (score)	M-CAP (score)	Mutation Taster	LRT Pred	GERP (score)	CADD score (Phred value)
P7	chr22:21188862 G>A	c.355 C>T (exon 3)	p.(Arg119Trp)	2.39e-5 (6/251348)	0 (no carriers)	Probably Damaging (0.997)	D (0.044)	D	NA	1.67	23.7
P7	chr22:21158635 T>G	c.1414 A>C (exon 12)	p.(Ser472Arg)	3.98e-6 (1/251456)	0 (no carriers)	Possibly Damaging (0.899)	T (0.020)	D	D	5.08	23.0
P5	chr22:21153533 G>A	c.1852 C>T (exon 16)	p.(Arg618Ter)	1.01e-4 (22/218722)	7.68e-5 (11/143266)	NA	NA	A	D	4.55	39
P1	chr22:21119189 dupG	c.2624dupC (exon 22)	p.(Pro876Ser fsTer36)	3.99e-6 (1/250576)	0 (no carriers)	NA	NA	NA	NA	NA	32
P1	chr22:21098918 C>T	c.3454 G>A (exon 30)	p.(Glu1152Lys)	3.98e-6 (1/251262)	6.98e-6 (1/143346)	Probably Damaging (0.988)	D (0.064)	D	D	5.84	32
P4	chr22:21096917 C>T	c.3592 G>A (exon 31)	p.(Ala1198Thr)	0 (no carriers)	0 (no carriers)	Probably Damaging (1.0)	D (0.087)	D	D	5.87	28.4
P3	chr22:21088699 T>C	c.3884 A>G (exon 33)	p.(His1295Arg)	0 (no carriers)	0 (no carriers)	Probably Damaging (1.0)	D (0.473)	D	D	5.17	25.7
P10	chr22:21083617 C>T	c.4666 G>A (exon 39)	p.(Val1556Met)	1.61e-4 (45/280184)	1.26e-4 (18/143320)	Possibly Damaging (0.743)	D (0.030)	D	D	5.15	23.7
P5	chr22:21080781 C>T	c.4990 G>A (exon 42)	p.(Asp1664Asn)	0 (no carriers)	0 (no carriers)	Probably Damaging (1.0)	D (0.459)	D	D	4.88	32
P6	chr22:21075585 C>T	c.5116+1G>A (intron 43)	NA	0 (no carriers)	0 (no carriers)	NA	NA	D	NA	5.12	34
P10	chr22:21073068 G>A	c.5159 C>T (exon 44)	p.(Thr1720Ile)	0 (no carriers)	0 (no carriers)	Benign (0.001)	D (0.045)	D	D	4.9	22.2
P9	chr22:21068746_ 21068748delCTT	c.5459_5461delAAG (exon 47)	p.(Glu1820del)	0 (no carriers)	6.98e-6 (1/143164)	NA	NA	NA	NA	NA	NA

P8	chr22:21067580C>T	c.5560G>A (exon 48)	p.(Asp1854Asn)	1.91e-5 (4/209116)	6.57e-6 (1/ 152230)	Probably Damaging (1.0)	D (0.529)	D	D	4.69	32
P2	chr22:21066803 C>G	c.5773 G>C (exon 50)	p.(Gly1925Arg)	4.35e-6 (1/229730)	6.98e-6 (1/143174)	Probably Damaging (1.0)	D (0.679)	D	D	4.49	28.3
P6	chr22:21065110 T>C	c.5960 A>G (exon 52)	p.(Asn1987Ser)	6.79e-5 (17/250476)	0 (no carriers)	Probably Damaging (0.999)	D (0.062)	D	D	5.01	24.1
P3	chr22:21064247 A>G	c.6122 T>C (exon 53)	p.(Met2041Thr)	0 (no carriers)	0 (no carriers)	Probably Damaging (1.0)	D (0.732)	D	D	5.26	27.2
P4, P9	chr22:21064210_ 21064213delTGTC	c.6156_6159del GACA (exon 53)	p.(Thr2053Ser fsTer4)	0 (no carriers)	0 (no carriers)	NA	NA	NA	NA	NA	NA

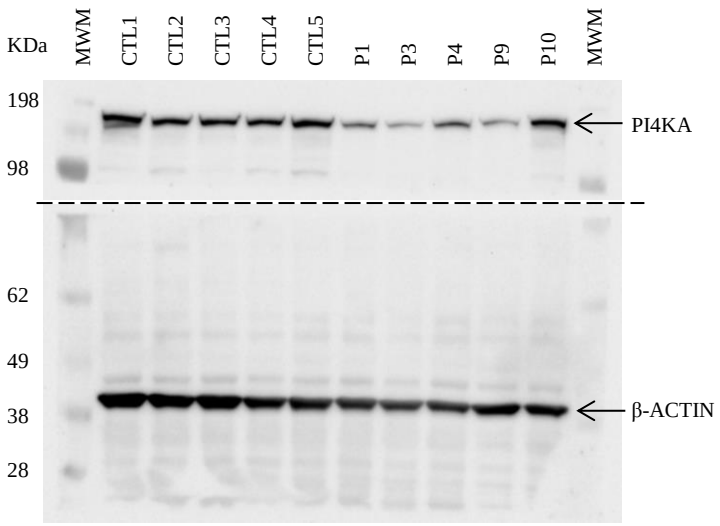
Supplemental Table 1: Features of PI4KA variantsfound in patients. NA: not assessed. Grey: Deleterious predictions.



Supplemental Figure 1: Amino acid sequence alignments of PI4KAprotein across several species.



Supplemental Figure 2: 3D representation of the PI4KA/TTC7B/FAM126A dimer. Blue: PI4KA; green: TTC7B; orange: FAM126A; grey: opposite PI4KA/TTC7B/FAM126A heterocomplex; Pink: A1, PI4KA inhibitor occupying ATP binding space in the catalytic domain. Red balls represent missense/in-frame variants found in our cohort.



Supplemental Figure 3: Unmodified full-length blot used to make the images in Figure 3. Patients' fibroblasts (n=5) and controls (CTL, n=5). MWM, Molecular Weight Marker. The following antibodies were used; anti-PI4KA (12411-1-AP Proteintech) for human cells as validated in www.antibodypedia.com (AB_2268237) and anti-β-ACTIN (A2228, Sigma).