Patient	Genomic Variant [a]	Interpretation [ACMG Criteria] [b]	In silico Predictions [b]	gnomAD Data [c]
I	chr8: g.145138116T>C	VUS [PM2, PP2, PP3]	Pathogenic: FATHMM-MKL, M-CAP, MutationAssessor, MutationTaster, PrimateAI, SIFT Benign: DANN, DEOGEN2, EIGEN, MVP, REVEL	Allele Frequency: 7.16373E-06 Allele Count = 2 (European Non-Finnish) Not seen in homozygous state
II	chr8: g.145139663T>G	Likely pathogenic [PS3, PM2, PP3]	Pathogenic: DANN, DEOGEN2, EIGEN, FATHMM-MKL, MutationAssessor, MutationTaster, PrimateAI, SIFT Benign: M-CAP, MVP and REVEL	Allele Frequency: 4.01207E-06 Allele Count = 1 (Latino) Not seen in homozygous state
III	chr8: g.145139419A>G	Likely pathogenic	Pathogenic: ANN, DEOGEN2, EIGEN, FATHMM-MKL, M-CAP,	Not seen in gnomAD

		[PS3, PM2, PP3]	MutationAssessor, MutationTaster, PrimateAI, REVEL, SIFT Benign: MVP	
	chr8: g.145140583T>G	Likely pathogenic [PS3, PM2, PP3]	Pathogenic: DANN, EIGEN, FATHMM-MKL, M-CAP, MutationAssessor, MutationTaster, PrimateAI, REVEL, SIFT Benign: DEOGEN2, MVP	Not seen in gnomAD
IV	chr8: g.145139449C>T	Likely pathogenic [PM2, PM3, PP2, PP3]	Pathogenic: DANN, DEOGEN2, EIGEN, FATHMM-MKL, M-CAP, MutationAssessor, MutationTaster, PrimateAI, REVEL, SIFT Benign: MVP	Allele Frequency: 4.01213E-06 Allele Count: 1 (Latino) Not seen in homozygous state
V	chr8: g.145139449C>T	Likely pathogenic	Pathogenic: DANN, DEOGEN2, EIGEN, FATHMM-MKL, M-CAP,	Allele Frequency: 4.01213E-06

		[PM2, PM3, PP2, PP3]	MutationAssessor, MutationTaster, PrimateAI, REVEL, SIFT Benign: MVP	Allele Count: 1 (Latino) Not seen in homozygous state
	chr8: g.145140014-145140020del	Likely pathogenic [PVS1, PM2]	Pathogenic: GERP Benign: none	Not seen in gnomAD
VI	chr8: g.145140501-145140502del	Pathogenic [PVS1, PM2, PP5]	Pathogenic: GERP Benign: none	Allele Frequency: 0.000142617 Allele Count: 40 (Variety of populations) Not seen in homozygous state
	chr8: g.145140993T>C	Likely pathogenic [PM2, PM3, PP2, PP3]	Pathogenic: DANN, EIGEN, FATHMM-MKL, M-CAP, MutationAssessor, MutationTaster, PrimateAI, REVEL, SIFT Benign: DEOGEN2, MVP	Allele Frequency: 4.01355E-06 Allele Count: 1 (Latino) Not seen in homozygous state

VII	chr8: g.145139039del	Pathogenic [PVS1, PS3, PM2, PP5]	Pathogenic: GERP Benign: none	Allele Frequency: 2.0062E-05 Allele Count: 5 (1 in Latino, 4 in European Non-Finnish) Not seen in homozygous state
	chr8: g.145138101T>A	Likely pathogenic [PS3, PM2, PP3]	Pathogenic: FATHMM-MKL, M-CAP, MutationTaster, PrimateAI, REVEL, SIFT Benign: DANN, DEOGEN2, EIGEN, MVP, MutationAssessor	Allele Frequency: 2.86219E-05 Allele Frequency: 8 (6 in European Non-Finnish, 2 in Other) Not seen in homozygous state

[a] UCSC Genome Browser hg19

[b] Interpretation adapted from varsome.com

[c] gnomAD v2.1.1 exome and genome data sets, accessed April 2020-

https://gnomad.broadinstitute.org/gene/ENSG00000197858?dataset=gnomad_r2_1

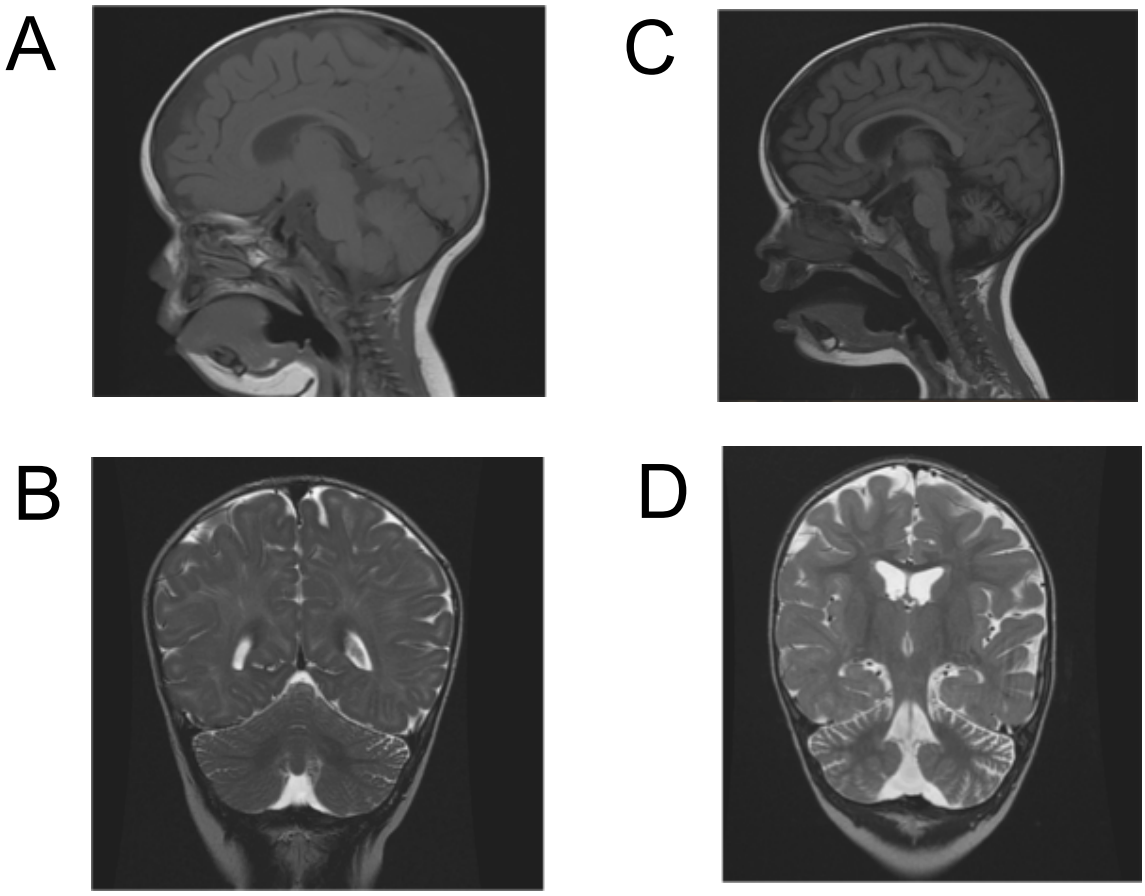

Figure e-1: MRI images from patient III. Initial sagittal T1 (panel A) and coronal T2 images (panel B) at 7 months of age (panel A) showed a normal cerebellum but presumed benign enlargement of the subarachnoid space of infancy. Follow-up sagittal T1 Flair (panel C) and coronal T2 (panel D) images at age 2 showed mild diffuse and symmetrical brain atrophy with progression in the cerebellum as compared to the prior scan.

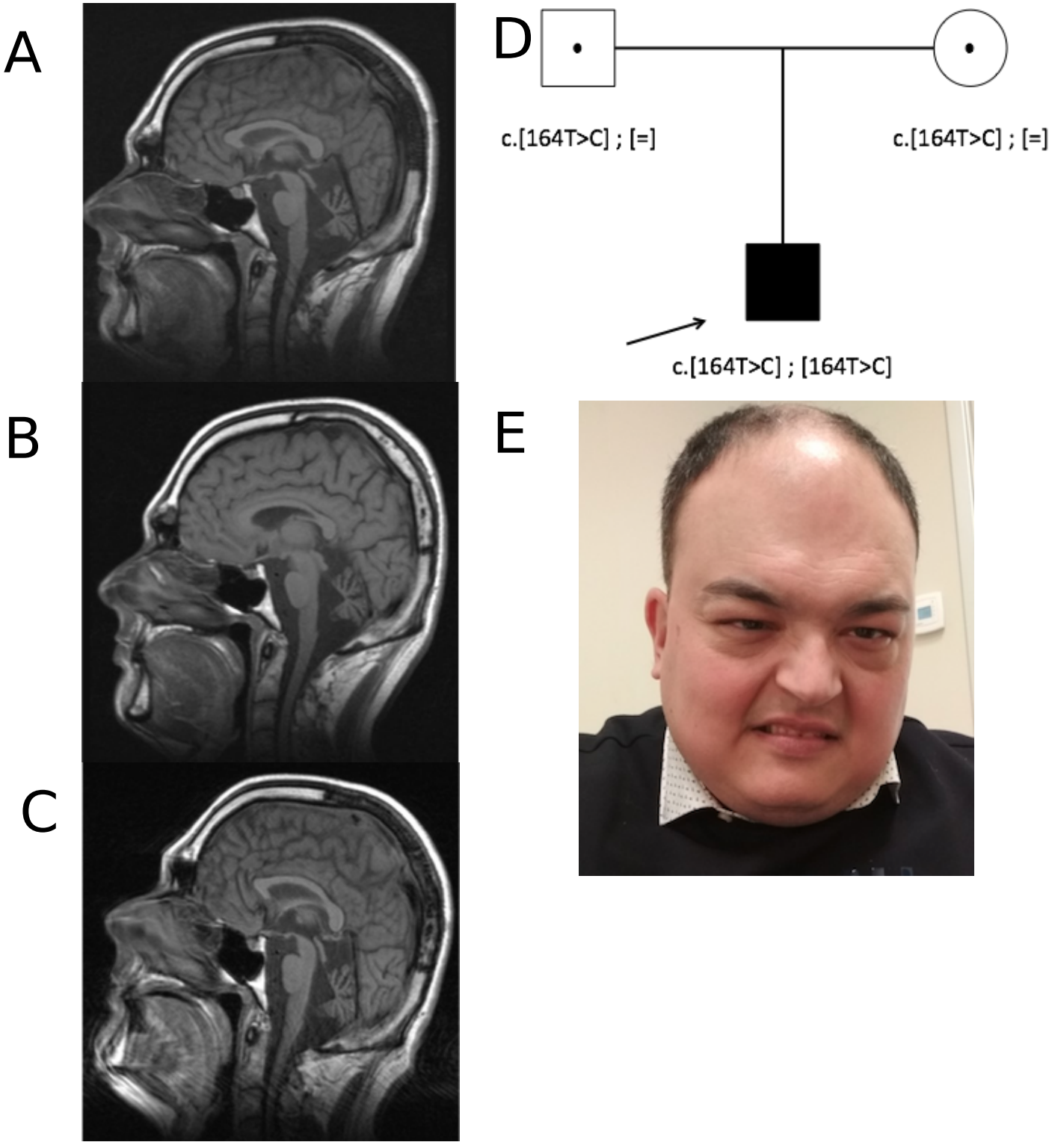

Figure e-2: MRI images of patient I from 2003 (A), 2007 (B), and 2010 (C), demonstrating stable cerebellar atrophy in this patient. Panel D shows the pedigree for this patient. Panel E demonstrates this patient's prominent forehead and apparent hypertelorism.

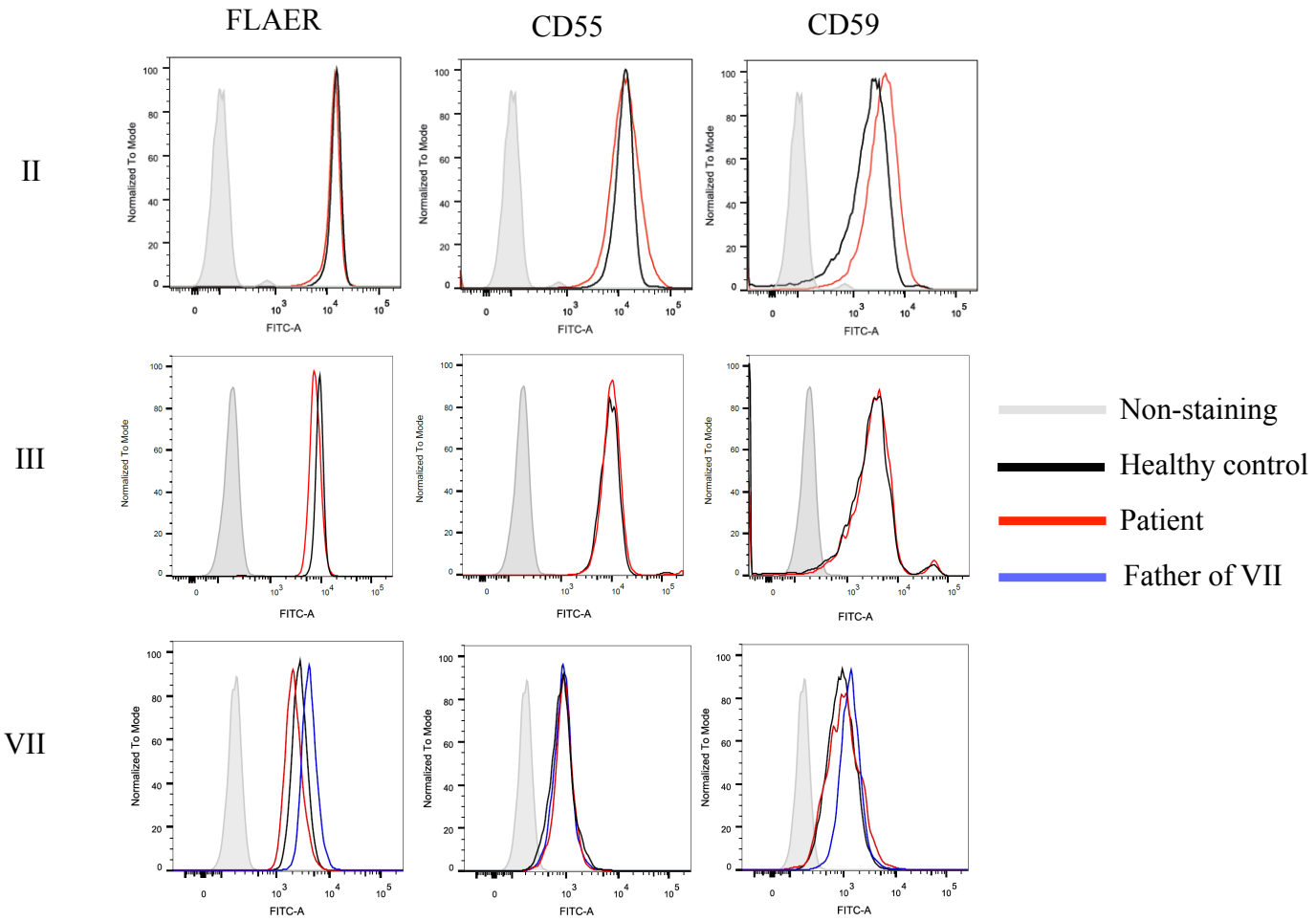

Figure e-3: Flow cytometry analysis of cell surface GPI-anchored protein (FLAER, CD55 and CD59) on granulocytes in patients II, III, and VII compared to a healthy control. The histograms are representatives of at least two separate experiments using three different controls. There was no significant reduction in GPI-anchored protein levels in affected individuals versus controls. Additionally, there was no reduction in GPI-anchored protein levels in the father of patient VII, who carries a frameshift variant. FITC-A: fluorescein isothiocyanate-area.

Supplemental Data: Additional clinical featu

Individual	I
Demographic info	
Country of Origin, ethnicity or origin	Italy
Patient year of birth	
Gender	Male
Age (years) - last observation	38 years
Molecular information	
Mutations	c.164T>C;p.(Met55Thr) homozygous
Method of testing (WES, ID panel, seizure panel, etc)	WES done at 38 years (ataxia gene panel done at 35 years - negative)
WES - research or clinical?	research
If clinical - what lab?	University of Pavia, Department of Molecular Medicine
If research - reference for sequencing protocol	(Sure Select QXT NSQ – Clinical Research Exome V2 protocol (Agilent Technologies) and ran on a Novaseq 6000 (Illumina) sequencer
Were the variants Sanger validated?	yes
Indication for testing (Ex- seizures, developmental delay, both, etc)	cerebellar ataxia
Family history	
Affected family member	no
Unaffected sibs	no
Consanguinity	no
Perinatal history	
Perinatal complications	none
At birth: duration gestation (weeks)	term
Apgars	9,9
- Weight, g	3300 g
- Length, cm	55 cm
- OFC, cm	N/A
Development	

Delay/ ID : +++ severe; ++ moderate; + mild (see also below)	++
How was ID/DD diagnosed (formal testing, Neurologist, Pediatrician, etc)	Pediatric neurologist
Sitting (months)	4 years
Walking independently (months)	6 years (with support)
Speech	Dysarthria
Speech - first words (months)	solo sillabe a 18 mesi mai linguaggio normale
Speech - decline at a later age	4-5 years
Feeding problems	no
Behavioural phenotype (Ex - impulsivity, ADHD, aggression, etc)	none
Intellectual disabillity	moderate ID
Current level of function/independence (living situation, activities able to perform independently, activities requiring support)	the patient is able to eat, play on the PC and undress independently, unable to stand, write or read, fine motors skills are impaired.

Neurology

Detailed neurologic examination	hypotonia, ataxia, dysmetria, dysarthria, intermittent head titubation
Hypotonia	Yes
Age of onset of hypotonia	4 months
Spasticity	no

Seizures	yes
Age of first seizure	14 months
Type of seizures	febrile, atypical absence, generalized tonic clonic
Seizure frequency	
Seizure outcome	controlled
Reaction on medication(s)	controlled on medication
Ictal/interictal EEG	slow background activity without epileptiform abnormalities
Current anti-epileptic drugs	valproate, phenobarbitol
anti-epileptic drugs ever tried	clobazam
- medications	only antiepileptics
History of Status Epilepticus	
History of febrile/complex febrile seizures	yes
Electroclinical syndrome classification	generalized epilepsy
Neuroradiology	Global cerebellar atrophy, stable at ages 21, 25, and 29 years without progression

MRI - magnet strength	1,5 Tesla
Hearing loss	no
Vision impaired	
if yes, cause	
Nystagmus?	no
abn eye exam	astigmatism, saccades
Dysarthria	yes
Dysmetria	yes
Ataxia	yes

Dysmorphological exam

Who performed the exam (Ex - geneticist, neurologist, pediatrician, etc)	Clinical Geneticist
Height	170 cm (17%, Z=-0.96)
Weight	78 kg (72%, Z=0.59)
OFC	59.5 cm (93%, Z=1.46)
Craniofacial features	yes
- Narrow forehead	prominent forehead
- Upturned nasal tip (anteverted nares)	
- Cleft palate / cleft uvula / submucous cleft	
- Gum hypertrophy	
- Malformed ears	
- Other dysmorphisms	hypertelorism
hand brachydactyly	no
Feet, toes abnl	no
- Scoliosis	no
- Nail anomalies	no
- Other Xray abn	
- Cardiac defect	no
- Renal anomalies	no
- Gastrointestinal abn	bilateral inguinal hernias, repaired as neonate
- Dentition	normal

Bone Scan

Bone scan performed?	yes
Age of bone scan	38 years

Ostepenia?	Z=--2.3

Biochemical features

- Urine organic acids	
- Plasma alkaline phosphatase (U/L)	normal
Age when measured	38 years
Was it measured serially? If yes, were all values similar?	no
-Plasma ferritin	
-Plasma transferrin	
Other biochemical studies	transient IgA deficiency, high plasma triglycerides

Other

Surgeries	bilateral inguinal hernias, repaired as neonate
Further information:	
Microarray performed	normal

ıres

II	III

USA	French Canadian
2018	2016
Female	Female
22 months	3 years 9 months

1.GPAA1 homozygous c.1049T>G p.Leu350Arg NM_003801.3 Biparental, 2.IL1RAPL2 heterozygous, c.663A>C, p.Lys221Asn, NM-017416.1, De Novo, VUS	c.917A>G, c.1559T>G
WES	Research reanalysis of ID panel on WES backbone
Clinical WES	Research
Greenwood Genetic Center	Fulgent
	PMID 30160830
Yes	yes
failure to thrive, developmental delay, anemia, and severe diarrhea	both

no	no
one	two
yes (parents first cousins)	no

none	none
39 weeks	39+5 weeks
9,9	not known
2693 g	not known
48.3 cm	not known
33.7 cm	not known

+ See last dev peds conclusion to the right	+++
Pediatrics, Dev peds, and neurology evaluation.	neurology
Delayed	3 years 9 months
Delayed	N/A
	none
	babbling at 3y8mo, no words
Oral aversion	no
	no
	no formal assessment yet
Delayed physical development skill acquisition, likely at least partially the cause of her delay in adaptive skills. Problem solving, receptive language, social-emotional functioning just slightly below age	

	alert and awake but nonverbal, no fixing and following, hypotonia with no antigravity movements, reflexes 2+ in upper and lower limbs
none	significant
	birth
No	yes, at elbows

none	yes
n/a	10 months or is it 8 mo?
n/a	Febrile, epileptic spasms, myoclonic jerks
n/a	5-10 spasms per day
n/a	not well controlled
n/a	partial control
never done	bursts of very high amplitude spike and slow wave generalized epileptiform discharged with frontal predominance, spike/polyspike and slow wave epileptiform discharges, slowing of background activity
none	ketogenic diet
n/a	vigabatrin, topiramate, prednisolone, clonazepam, B6, Keppra
Humira, ferrous sulfate, hydrocortisone cream	Multivitamin, selenium, lansoprazole, PEG
no	no
no	yes
n/a	generalized epilepsy
normal	MRI 7 months - prominence of cerebral sulci and anterior hemispheric fissure suggestive of benign enlargement of subarachnoid space of infancy; MRI 2 years - diffuse mild brain atrophy with progression in cerebellum, slightly delayed

	myelination, mild thinning of corpus callosum
unknown	
no	no, not formally tested
no	normal testing 2017
no	
No	no
right exotropia	paroxysmal upward gaze of infancy, resolved
None	non-verbal
None	no
No	no

Clinical Geneticist	Geneticist
75 cm (<1%, Z=-3.08)	88 cm (<1%, Z=-3.11)
10.1 kg (23%, Z=0.75)	12.1 kg (3%, Z=-1.92)
45.3 cm (13%, Z=-1.14)	46 cm (1%, Z=-2.24)
none	yes
no	prominent forehead
no	
no	no
no	no
no	no
no	deep set eyes, downslanting palpebral fissures, tented upper lip, small chin
no	no
no	no
no	no
no	no
no	N/A
no	no
no	N/A
inflammatory bowel disease, GJ-tube	N/A
normal	normal

No	no
NA	N/A

NA	N/A

normal	normal
220 (156-369)	98 (129-291 U/L) ;67 (N=145-324 U/L)
2 years 10 months	1.5 y and 3 y
All alk phos in the normal range. One was elevated (high)	yes
	previously normal
	N/A
amino acids normal, urine organic acids showed non-specific elevation of lactate	amino acids - normal; acylcarnitines - marked increased level of C2 acylcarnitine, most likely due to diet, treatment or fasting, small increased level of C4OH, several medium chain (C6, C8, C10) acylcarnitines and long chain (C14:1) acylcarnitines to be interpreted in context of fasting; biotinidase normal; MPS fractionation - normal; VLCFA/peroxisomal studies - normal; beta-hexosaminidase A - normal

bilateral myringotomy tubes	dermoid cyst removal
Main health concern is early onset inflammatory bowel disease. GPAA1 variants were incidental findings during work-up.	
normal	normal

IV	V	VI

Germany (Kurdish Turk)	Israel	France
2015	April,2016	
Female	Female	Male
5 years 3 months	4 years 8 months (last sen Dec 2020- updated)	5 years 5 months

c.947C>T; p.Ala316Val (homozygous)	Paternal: C.947C >T, P.Ala316Val Maternal: C.1233_1239del, P.Pro412TyrfsTer19	c.1477_1478del maternal , c.1831T>C paternal
Trio WES	WES	WES
clinical	clinical	clinical
Universitätsklinikum Tübingen, MVZ Fachgebiet Medizinische Genetik, Hoppe-Seyler-Str. 3, 72076 Tübingen, Germany	reanalysis-The Raphael Recanati Genetic Institute - Beilinson Hospital	Pitié-Salpêtrière, Paris, France
NA		Roche Medexome Prep and Illumina NextSeq 500
No	yes	yes
Developmental delay, hypotonia, megalocorneae	seizures, global developmental delay, cerebellar atrophy, vision impairment, failure to thrive	both

no	no	no
yes (1 female sibling, GPAA1 carrier)	two	
yes (parents first cousins)	no	no

none	Polyhydramnious, choroid plexus cyst	unknown
39 weeks	41 weeks	
10,10	9,10	
3420 g	3841 g	
53 cm	unknown	
33 cm	unknown	

+++	+++; regression and arrest around 6-8 months (around the time that seizure started) she had a regrression in her expressive skills (stoped cooing and babling)	Yes
pediatric neurologist (Dr. V. Degenhardt, Salzgitter, Germany)	DD diagnosed by a certified pediatric neurologist (specialist in pediatric neurology and child development)	
30 months	No independent sitting (she can sit on a special chair with axis support)	
?	No independent walking	
Severe delay	No words	poor language skills
?	No words	
?		
?	yes - since first months of life	
yes	DQ <55 (Developmental quotient)	

hypotonia (especially axial), restricted experience of pain	Increased deep tendon reflexes, hypotonia, extensor response, decreased facial mimics, joint hyperlaxity, increased tone in ankles	hypotonia, no spasticity, no nystagmus, no dysmetria, no ataxia. Dysarthria present
yes (especially trunk, back, shoulders)	yes	yes
4 months (Helios Klinik Salzgitter, Germany)	Neonatal	
No	yes - ankles	no

yes	yes	yes
~3 years	8 months	22 months
febrile	Febrile, startle/reflex (to touch, bright light), myoclonic, absence with eyelid myoclonia, generalized tonic-clonic	
last in 2019	daily basis (every day)	
well controlled, no current medications	not well controlled	
none required	partial control (with medications she does not have GTC seizures, and the frequency of myoclonic seizures was decreased. she still has brief myoclonic seizures on a daily basis: upon awakening and when falling asleep , and also reflex seizures (when someone touches her hand she may also have a brief myoclonic seizure)	
normal (2015 and 2019)	multifocal spike and wave, polyspike wave	
none	sultiam, clonazepam, Ethosuximide, ketogenic diet, B6 (no effect for B6), vit D , ciproheptadin (for appetite induction)	
none	levetiracetam, brivaracetam, valproic acid, topiramate, clobazam, cannabis oil	
none	melatonin, B6, Vit D, antiepileptics	
	yes	
yes	Yes	
normal EEG	Reflex epilepsy, Myoclonic encephalopathy in non progressive disorders (ILAE classification)	
normal MRI 2015	hypoplastic cerebellar hemispheres and vermis, superior peduncle, and corpus callosum	

	callosum	
unknown	3T	
no	no	
normal VEP 2015	no	
Yes	yes	no
megalocornea, strabismus concomitans convergens	no	
No	no	no (poor language)
NA	no	no
Yes, Ataxia of the trunk while standing	no (not walking)	no

clinical geneticist	Geneticist and Neurologist	
111 cm (33%, Z=-0.43)	90 cm (1%, Z=-2.2)	105 cm (7%, Z=-1.5)
26 kg (96%, Z=1.70)	11 kg (<1%, Z=-3.11)	17.7 kg (31%, Z=-0.5)
45 cm (<1%, Z=-5.35)	46 cm (2%, Z=-2.1)	51.5 cm (69%, Z=0.5)
yes	yes	unknown
broad forehead	no	
	no	
no	no	
no	no	
protruding ear lobes (similar to father)	no	
laterally ascending eyes, epicanthus, high-arched and narrow palate, tent-shaped upper lip, brachycephaly, microcephaly	epicanthal folds, mild upslanting palpebral fissures, tented upper lip, accessory nipple	
short hands (3rd centile), short middle finger (<3rd centile)	no	
no	no	Single palmar crease
no	No	
no	nails and hair grow slowly	
no	No	
no	normal echo, 1-2/6 systolic murmur, on 2020 admission sinus arhythmia reported.	
no	no	
no	no	
delayed (14 months)	normal	

No	No	N/A
N/A	N/A	

N/A	no	

normal	normal	
155 (N <281)	114 (normal, 2019); 83 (low, normal:117-390) 29/12/20	
2 years (2017)	measured in 2020, what age was the patient? 3.9 years	
No	measured serialy : jan/20:59, May/20:84, Dec/20:83	
iron low	N/A	
	N/A	
metabolic parameters, karyotype, microarray, MECP2, Angelman, elevated AST and AFP, low creatinine. Additional WES findings include: NFASC (NM_001005388.2) - c.1894C>T; p.Arg632Trp (homozygous) (VUS) PKP2 (NM_004572.3) - c.2509del; p.Ser837Valfs*94 (heterozygous, maternal) ATAD3A (NM_018188.3) - c.1847A>T; p.Lys616Met (homozygous) (VUS) , c.439G>A; p.Ala147Thr (homozygous) (VUS) SCD5 (NM_001037582.3) - c.608C>T; p.Ile203Thr (homozygous)	normal VLCFA, urine organic acids, biotinidase, lactate, NH3, acylcarnitines, amino acids, ceruloplasmin, free and total carnitine	

none	N/A	
sialorrhea	hair and nails grow very slowly	
normal	prenatal array normal	

VII

USA
2017
Male
3 years

c.149T>A maternal , c.619delA paternal
WES
Clinical
GeneDX
Yes
DD, hypotonia

No
yes (1 full sister, one half-sister)
no

none
41 weeks 2 days
7,9
4479 kg
54.6 cm
36.2 cm

Global developmental delay. Bayley at 32 months was score of 70 (very low)
Developmental Pediatrics, Neurology, Pediatrician
11-12 months
unable
nonverbal
NA
NA
None
None, negative autism assessment (score of 8 on ADOS2), BASC-3 typical for age
Low cognitive score and adaptive skills scores on developmental assessment at 32 months

low appendicular tone, mild slippage on vertical suspension, hyporeflexia, ataxia, mild 6th CN palsy (L > R)
yes
4 months
None

yes
15 months
generalized tonic clonic
well controlled
controllled on medication
bifrontal sharp waves
Levetiracetam, Vitamin B6
none
vitamin B6, antiepileptics (unclear if B6 beneficial)
no focal lesions at 5 months

unknown
no
yes
no
high hyperopia requiring spectacles, esotropia
N/A (non-verbal)
No
yes

Genetics
97 cm (59%, Z=0.23)
15.3 kg (72%, Z=0.58)

49 cm (34%, Z=-0.42)

non-dysmorphic
None
None
None
None noted on last exam
SI overfolding superior helicies B with mild superior prominence on left
None
None
no
no
None
murmur, normal function
no
no
normal

DEXA -1.0SD age/sex matched at lumbar spine
3 years

None

NA
132 , 142 (both normal)
15 months, 3 years
NA
NA
normal lactate and ammonia

none
Likely benign 210kb duplication at 9q34.3