

Supplementary Material

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CASES

Case 2.

The child is a 9-year-old African American male with global developmental delay, hypotonia, ataxia, dysarthria, nystagmus, and intellectual disability. By six months of age, he developed nystagmus and paroxysmal episodes of forced upward eye gaze lasting up to two hours and resulting in an initial diagnosis of paroxysmal tonic upgaze (PTU). Spells occurred upon awakening in the morning and after naps, occasionally with fever. During the PTU events, he tilted his head downward; he is unable to feed himself and is unable to walk due to poor balance. Later in the day he displays nystagmus and gait ataxia but is able to walk unassisted and eat independently. His paroxysmal events of tonic upgaze and ataxia have remained stable over time. Brain MRI initially showed an incidental pineal cyst which resolved on follow up imaging. EEGs were normal. Genomic sequencing revealed a *de novo* frameshift variant in the *DAGLA* gene NM_006133.2:c.2484delC, p.(E829RfsX6).[SupplementalVideo2]

Case 3.

The child is a 4-year-old Caucasian male, the 37-week product of a twin gestation with global developmental delay, hypotonia, ataxia and dysarthria. Delivery was by planned caesarian section followed by NICU observation for 22 days monitoring due to apneic spells and feeding difficulty. The child displayed paroxysmal events including periods of head flexion with upward eye deviation without alteration in consciousness. MRI brain scan at one year 11 months showed an oval T2 hyperintense lesion in the right superior cerebellum, decreased volume of the left hippocampal formation and anterior left temporal lobe, and probable mild inferior vermal hypoplasia. Exome sequencing revealed a *de novo* variant in the *DAGLA* gene NM_006133.2:c.2551C>T, p.(Q851X).

Case 4.

The child is a 5-year-old Caucasian male with global developmental delay, hypotonia, ataxia, dysarthria, nystagmus and low average intelligence. Downbeat nystagmus with chin down anterocollis and avoidance of downgaze, presented at 7 months with possible worsening at the end of the day. The severity of ataxic motor movements and unsteady gait varied without a clear paroxysmal pattern. Brain MRI was normal. Exome sequencing revealed a *de novo* variant in the *DAGLA* gene NM_006133.2:c.2456_2457delAC, p.(H819PfsX5).[SupplementalVideo3]

Case 5.

The child is a 15-year-old Caucasian male with global developmental delay, hypotonia, ataxia, dysarthria, nystagmus, and intellectual disability. Hypotonia and vertical nystagmus was noted at 9 months and associated with abnormal head posture characterized by neck extension when he looks upwards and occasionally downbeat nystagmus with chin down position. Hypotonia and nystagmus were continual but with paroxysmal worsening upon waking, after naps or with fever. Duration of paroxysmal episodes decreased with age. Genomic sequencing identified a heterozygous frame-shift apparently *de novo* variant in the *DAGLA* gene NM_006133.2:c.2484delC, p.(E829RfsX6). The variant was absent in both parents but was also detected in the affected sister (case 6), suggesting parental gonadal mosaicism.

Case 6. (Sibling of Case 5)

The child is a 12-year-old Caucasian female with global developmental delay, hypotonia, ataxia, dysarthria, congenital nystagmus, and intellectual disability. Vertical nystagmus was noted during the first weeks of life. Abnormal eye movements included abnormal saccades, vertical nystagmus associated with downward eye deviation with upward head tilt or down beat nystagmus with a chin down torticollis position. Paroxysmal worsening of hypotonia, ataxia, and eye movements were noted within an hour of waking, after naps or

with fever. Over time, dysarthria and nystagmus decreased in severity. Brain MRI and EEG were normal. Genomic sequencing identified the same *DAGLA* variant as in her brother (case 5).

Case 7.

The child is an 11-year-old Caucasian male with global developmental delay, hypotonia, ataxia, dysarthria, nystagmus, and intellectual disability with moderate cerebellar features, including ataxia, dysarthria, dysmetria, dysdiadochokinesia, abnormal pursuits and saccades as well as nystagmus. The nystagmus is worse when he wakes up from sleep in the morning and when he is tired. Brain MRI at age 13 months was within normal limits while a repeat brain MRI at age 23 months showed nonspecific white matter increased T2 and FLAIR signal in the occipital regions compatible with terminal zones of myelination. EEG was normal. Extensive investigations were not contributory except for an EMG revealing mild myopathic changes in some muscles early in the disease course. Exome sequencing identified a *de novo* variant in the *DAGLA* gene NM_006133.3:c.2370C>G, p.(Y790X).

Case 8.

The child is a 4-year-old Caucasian male with motor delay, hypotonia, ataxia, and nystagmus. Since the first months of life, he displayed nystagmus with delayed achievement of motor milestones. Language acquisition and overall cognitive development are normal. Within the first year of life the child started to manifest paroxysmal events when awakening from a nocturnal sleep or a daytime nap. The parents define the awakening episodes as worsening of nystagmus, tremor and ataxic behavior accompanied by fearful expression. Episodic nystagmus was mostly upbeat but sometimes downbeat regardless of gaze position and was accompanied by chin-up or chin-down movements. This paroxysm lasts for about 1 hour and then the child returns to his baseline status with less severe ataxia and nystagmus. Brain MRI at age 3 years showed only mild inferior cerebellar vermian hypoplasia. Symptoms have been stable over time. Exome sequencing identified a *de novo* variant in the *DAGLA* gene NM_006133.3:c.2485G>T, p.Glu829X.[SupplementalVideo4]

Case 9.

The child is a 6-year-old Caucasian male with motor delay, hypotonia, ataxia, dysarthria, dyslalia, and abnormal eye movements. By 7 months he displayed paroxysmal upward eye deviation triggered in the morning and after sleep. An MRI of the brain, performed at 2 years of age, showed probable 7x8 mm heterotopia lateral to the corpus callosum. Exome sequencing revealed a *de novo* missense variant in the *DAGLA* gene: NM_006133.3:c.2613dup, p.(Ser872GlnfsX6).

METHODS

Genomic Sequencing

Case 1. This individual was sequenced three times:

- A. Trio Exome Sequencing: Detailed procedures for WES have been described previously.¹ In brief, whole blood samples were preserved using Paxgene DNA tubes (PreAnalytiX, Hombrechtikon, CH), and genomic DNA was extracted using the QIAamp system (Qiagen, Valencia, CA). Enriched exome libraries were captured using the Agilent SureSelect v7 systems according to the manufacturer's instructions (Agilent, Santa Clara, CA). Final libraries were generated using Illumina TruSeq sample preparation kits and underwent 100 bp paired-end sequencing on a HiSeq 2500 (Illumina, San Diego, CA).² Post-filtration, we identified: 3 de-novo mutations - one of which was excluded as a false positive due to repeated observations in other IDIOM subjects. The other non-DAGLA *de novo* variant was a predicted benign nonsynonymous variant in SRPR p.M410T 10 candidate homozygous variants 6 genes with candidate compound heterozygous variants. Candidate genes were evaluated manually, and the *de novo* DAGLA nonsense variant was Sanger validated.
- B. Trio Exome Sequencing was performed in a commercial laboratory (GeneDx; Gaithersburg, Maryland)
- C. Trio Genome sequencing and downstream analysis was performed as previously described^{3,4} All variants were prioritized by allele frequency, conservation, and predicted effect on protein function. The variants were confirmed by Sanger sequencing.

Case 2. Trio Exome Sequencing was performed in a commercial laboratory (GeneDx; Gaithersburg, Maryland)

Case 3. Trio Exome Sequencing was performed in a commercial laboratory (GeneDx; Gaithersburg, Maryland)

Case 4. Trio Exome Sequencing was performed in a commercial laboratory (GeneDx; Gaithersburg, Maryland)

Cases 5 and 6. Quad exome sequencing was performed at AP-HP. Sorbonne Université, Trousseau Hospital, in the quatuor with the following details: SeqCap EZ MedExome capture kit (Roche) and sequenced on Illumina NextSeq 500 with 151bp paired-end reads. The BaseSpace cloud computing platform (with BWA 2.1 and GATK Unified Genotyper 1.6) and the VariantStudio v.3.0 software provided by Illumina were used for analysis.

Case 7. Trio exome sequencing was performed using genomic DNA from the submitted sample. Exome libraries were prepared according to manufacturer's standard protocols using the Illumina TruSeq PCR-Free library preparation kit (Illumina, San Diego, CA) with 10 cycles of PCR, followed by enrichment with the IDT xGen Exome Research Panel v2, with additional spike-in oligos (Integrated DNA Technologies, Coralville, IA) to capture the mitochondrial genome and dispersed genomic regions for CNV detection. Samples were sequenced to a minimum of 7 Gb of 2x125 paired end reads for a mean of 80x average coverage or greater on the Illumina HiSeq 4000 or Illumina NovaSeq 6000. Bidirectional sequence was assembled, aligned to reference gene sequences based on human genome build GRCh37/UCSC hg19, and analyzed using custom-developed software, RUNES and VIKING (<https://www.childrensmercy.org/childrens-mercy-research-institute/research-areas/genomic-medicine-center/data-and-software-resources/>). The analysis was confined to the specific genes of interest for the individual and with MAF corresponding to disease incidence (*i.e.*, minor allele frequency <1%, gnomAD (<https://gnomad.broadinstitute.org/>)). Phenotype was recorded under clinical presentation. Variant interpretation was performed using the 2015 ACMG Standards and guidelines for the interpretation of sequence variants with systematic medical literature review. Variants are reported according to the Human Genome Variation Society (HGVS) nomenclature guidelines.

Case 8. Trio exome sequencing was performed at the Laboratory of Medical Genetics in Bambino Gesù Children's Hospital (Roma, Italy) on genomic DNA by using the Twist Human Core Exome Kit (Twist Bioscience, South San Francisco, CA, USA) according to the manufacturer's protocol on a NovaSeq6000 platform (Illumina, San Diego, CA, USA). The reads were aligned to human genome build GRCh37/UCSC hg19. The Dragen Enrichment application of BaseSpace (Illumina) and the TGen software (LifeMap Sciences, Inc.) were used for the variant calling and annotating variants, respectively. Sequence data were carefully analyzed and the presence of all suspected variants was checked in the public databases (dbSNP, Exome Aggregation Consortium (ExAC) and Genome Aggregation Database (gnomAD)). Putative disease-associated sequence variants were distinguished from polymorphisms using the following filtering criteria: an allele frequency below 1% in ExAC, species conservation of the underlying amino acid and a change in the protein's primary structure. The variants were evaluated by VarSome⁵ and categorized in accordance with the ACMG recommendations.⁶ Variants were examined for coverage, Qscore (minimum threshold of 30), and visualized by the Integrative Genome Viewer.⁵

Case 9. Trio whole-exome sequencing was performed following manufacturer's instructions and SOPs. Details: Library preparation was performed using the Nextera DNA Flex Pre-Enrichment LibraryPrep and Indexes (Illumina, San Diego, CA, USA). Target enrichment was achieved by using the Human Core Exome hybridization probes from Twist Bioscience (TwistBiosciences, South San Francisco, CA, USA). Paired-end sequencing of 150bp (300 cycles) was then performed on a NovaSeq 6000 Instrument using an S4 Reagent Kit (Illumina). Raw data analysis was performed using the software Varfeed (Limbus, Rostock, Germany) for both SNVs and CNVs. The variants were annotated and prioritized using Varvis (Limbus). For the routine reporting, we evaluated the morbid genes (i.e. genes with clinical relevance that is supported by literature, see also <https://morbidgenes.org/>). Potential protein-influencing variants were evaluated and prioritized based on their potential pathogenicity and clinical relevance. When analysis was negative, the data was analysed in a research setting including all enriched genes, and candidate variants were prioritized using AutoCaSc (<https://autocasc.uni-leipzig.de/>) and uploaded to GeneMatcher.

Western Blot Analysis

Proteins were then transferred to PVDF membrane in Towbin buffer. The membrane was blocked in 5% nonfat dry milk (w/v) in Tris-buffered saline with Tween 20 (TBST) for 1 h at room temperature, incubated with anti-FLAG monoclonal antibody (1:10,000 dilution in 5% nonfat dry milk in TBST) overnight at 4 °C. Blots were washed (3 × 5 min) with TBST, incubated with IRDye 800CW anti-mouse secondary antibody (1:10,000 in 5% nonfat dry milk in TBST) for 2 h at room temperature, washed (3 × 5 min) with TBST and scanned with a LI-COR Odyssey scanner.

Recombinant expression of enzymes by transient transfection of HEK293T cells for DAGLA activity evaluation

Full-length *H. sapien* DAGLA cDNA was cloned into expression vector pcDNA3.1 with a N-terminal FLAG-tag as previously described.⁸ For mutant DAGLA, a stop codon was introduced at E814 using QuikChange site-directed mutagenesis, following manufacturer's procedures. Plasmids were purified for transfection in HEK293T cells. One day prior to transfection, HEK293T cells were seeded to 6-cm dishes (1.1 × 10⁶ cells per dish). Polyethyleneimine (PEI) (7.5 µg/dish) and plasmid DNA (2.5 µg/dish) was mixed in serum free DMEM and incubated for 30 min at room temperature. The transfection mix was then added slowly to the cells. After 72 h, medium was aspirated, and cells were harvested by resuspension in PBS. Cells were pelleted by centrifugation (3 min, 1,400 g) and the pellet was washed with PBS. The PBS wash was repeated and cell pellets were frozen in liquid nitrogen and stored at -80 °C until sample preparation.

Transient transfection of HEK293T for Immunofluorescence Experiments

Transient transfection of HEK293T cells with DAGLA constructs were conducted using (JetOPTIMUS-11701) as per manufacturer's instructions. 2µg of wild type or mutant DAGLA DNA constructs were delivered. Cell were plated for experiment 24 h after transfection. For immunofluorescence microscopy, 200,000 cells were plated on Poly-d-lysine-coated coverslips (neuViro GG-18-PDL) and incubated for 24 h. Cells were then washed with chilled 1x PBS and fixed in 4% PFA. Cells were stained with primary antibody against DAGLA (Invitrogen PA5-23765) in 1:200 dilution and incubated overnight. Cells were washed in 1x TBST and incubated with secondary antibody (Invitrogen A32732) in 1:1000 dilution and incubated for 40 mins. ProLong Gold antifade reagent with DAPI (Invitrogen P36935) was used to stain nuclei. Pictures were taken using Echo microscope.

FIGURES AND TABLES

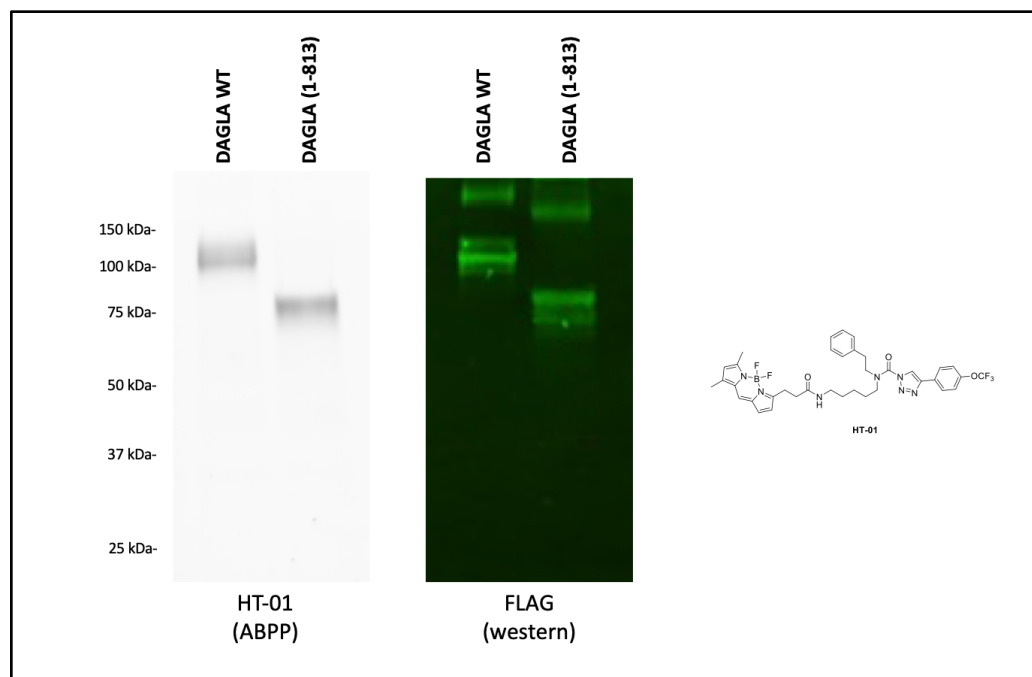


Figure S1: Activity of wild type DAGLA and mutant DAGLA (1-813) as measured by gel-based ABPP.

Lysate from HEK293T cells recombinantly expressing human DAGLA WT or MT were treated with activity-based probes, HT-01 or FP-Rh for 30 min at 37°C. The reactions were quenched by adding 20 μ L of 4X SDS-PAGE loading buffer. After separation by SDS-PAGE, samples were visualized by in-gel fluorescence scanning using the ChemiDoc MP system (Bio-Rad). Band intensities were quantified using the Image Lab (5.2.1) software (Bio-Rad).

Table S1: Extended Phenotype of Case Cohort M-Male; F-Female; y-years; nl-normal; Y-Yes; N-No; n/a-not available or not done; v-vertical; h-horizontal

CASE #	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9
GENETICS									
<i>DAGLA Variant</i>	NM_006133.2 c.2440G>T p.E814X	NM_006133.2 c.2484delC p.E829RfsX6	NM_006133.2, c.2551C>T p.Q851X	NM_006133.2:c.2 456_2457delAC p.H819PfsX5	NM_006133.2:c.2 484delC p.E829RfsX6	NM_006133.2:c.2 484delC p.E829RfsX6	NM_006133.3:c.2 370C>G p.Y790X	NM_006133.3:c.2 485G>T p.E829X	NM_006133.3:c.2 613dup p.S872QfsX6
Genomic Coordinates	chr11:61511272 G>T	chr11:61511312 TC>T	chr11:61511383 C>T	chr11:61511286 CCA>C	chr11:61511312 TC>T	chr11:61511312 TC>T	chr11:61511202 C>G	chr11:61511317 G>T	chr11:61511439 G>GC
Inheritance	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>	<i>de novo</i> (probable parental mosaicism)	<i>de novo</i> (probable parental mosaicism)	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>
AGE/GENDER									
Gender	M	M	M	M	M	F	M	M	M
Age (year of birth)	11y (2010)	9y (2012)	5y (2016)	7y (2014)	15y (2006)	12y (2009)	12y (2009)	4y (2017)	5y (2016)
PREGNANCY/ BIRTH									
Pregnancy Complications	Complicated by a small amount of first trimester bleeding and a fall in the 7th month	None	Fraternal twin gestation (twin sister); Twins conceived via IVF; Pregnancy complicated by cholestasis	Gestational diabetes and maternal hypothyroidism (taking Glyburide and Synthroid); Detection of left clubfoot at 26 weeks	Mother treated with Levothyroxine for Hashimoto's thyroiditis	Mother treated with Levothyroxine for Hashimoto's thyroiditis and Gestational diabetes	None	None	none
Birth	Full Term, Induced Vaginal, No Complications	Term, repeat C-section	37 weeks; planned C-section due to cholestasis and twin pregnancy	38 weeks, C-section due to size	38 weeks	36 weeks, neonatal Group B Streptococcal infection	Normal	Full term	Full term
Gestation (wks.)	40	40	37	38	38	36	40	40	40
Birth Weight (gms)	4423	3600	n/a	4139	2950	2770	3565	3000	3000
Birth Length (cm)	52	53	n/a	55.25	49	48	n/a	49	48
Birth Head Circumference	n/a	n/a	n/a	>90th percentile	34 cm	35cm	35 cm	33	34
GROWTH PARAMETERS									
Head Circumference (value/age and %ile and/or Z score)	52 cm (5yr 6 mo.) 67%ile and Z=0.43	53cm (9y5m) 42%ile Z=-0.22	52 cm (2y4m) 95th %centile (z=1.69)	54 cm (5y4mo) (96th percentile, z=1.76)	50.5 cm (3 yr.) 50th %ile; 53 cm (7y) 60th%ile	51cm(42 months); 52cm (10 y)			

Weight (value/age and %ile or Z score)	33.8 kg (8yr 8mo) 86%ile (Z= 1.09)	25.3kg (6y10m) 75%ile and Z=0.67; 25.8kg (7y4mo) 68%ile and Z=0.47	15.2 kg (2y4m) 88%ile (z=1.21)	27.9 kg (5y4mo) (>99th percentile, z=2.74)	12 kg (3 yr.) 4th%ile (z= -1.75) 18.2 kg (7 yr.) 2.7 %ile (Z = -1.93)	1.1 %ile (Z = - 2.29) 13.4 kg (42 mo.) 16.1 %ile (Z = - 0.99) 23.1 kg (10 y) 1.6 %ile (Z = -2.5)	35 (11 yr.) 50 %ile	18 kg (3y 8 months) 50-75%ile	16 kg (4y 3m) 25-50 %ile
DEVELOPMENT									
Motor Development	Delayed Sit 12 months Walk 24 months	Delayed Sit 9.5 months Walk 17 months	Delayed Sit 10 months Walk 26 months	Delayed Sit 11 months Walk 3 years	Delayed Walk 33 months	Delayed Sit 10 months Walk with support 36 months	Delayed Sit 18 months Walk 2 years	Delayed Sit 14 months Walk 3 years	Delayed Walk 2 years
Language Development	Delayed: First words at 2-3 years	Delayed: at 23 months had 5-10 words	Delayed: babbling at 12 months; 25-50 words at age 3y	Delayed: First words at 2.5 years, sentences at 3.5 years	Delayed: at 36 months word association, at 4 years short sentences	Delayed: at 36 months had 8 words, at 42 months short sentences	Delayed: First words > 1 year; Speaks in short sentences	First words at 12 months. Full sentences at age 4.	Good vocabulary; Dyslalia
Social Development	nl	nl	nl	nl	nl	nl	nl	nl	nl
PHENOTYPE									
Age at Symptom Onset	Birth	6 months	Birth	Birth	9 months	First weeks	3 months	First month	7 months
First Symptom	Nystagmus (horizontal and vertical)	Nystagmus (horizontal and vertical)	Apneic spells; Feeding difficulty	Clubfoot	Vertical nystagmus	Nystagmus	Poor head control	Poor head control; Pendular nystagmus	Paroxysmal upward eye deviation
Regression	N	N	N	N	N	N	N	N	N
Seizures	N	N	N	N	N	N	N	N	N
Nystagmus (v/h)	Y (v,h)	Y (v,h)	N	Y (v)	Y (v)	Y (v)	Y (v,h)	Y (v)	N
Nystagmus Onset	Birth	6 months	None	7 months	9 months	First weeks	13 months (maybe earlier)	First weeks	None
Other Eye Movement Abnormality	Amblyopia; Saccadic Pursuits	N	Saccadic Pursuits	N	Saccadic Pursuits	Saccadic Pursuits	Saccadic Pursuits; Abnormal Saccades	Saccadic Pursuits	Saccadic Pursuits
Dysarthria	Y	Y	Y	Y	Y	Y	Y	N	Y
Hypotonia	Y	Y	Y	Y	Y	Y	Y	Y	Y
Ataxia	Y	Y	Y	Y	Y	Y	Y	Y	Y
Intellectual Disability	Y	Y	Too young to assess IQ	N - Low average cognitive skills	Y	Y	Y	N	N
Hearing	nl	nl	nl	nl	nl	nl	nl	nl	nl
PAROXYSMAL EVENTS									
Paroxysmal Nystagmus	Y	Y	N	Y	Y	Y	Y	Y	N
Paroxysmal Chin Down Posture	Y	Y	Y	Y	Y	Y	Y	Y	Y
Paroxysmal Chin Up Posture	Y	N	N	N	Y	Y	N	Y	N

Paroxysmal Upward Eye Deviation	Y	Y	Y	N	N	N	N	Y	Y
Paroxysmal Ataxia	Y	Y	N	N	Y	Y	N	Y	N
Other Intermittent Symptoms	N	N	N	Tremor; Startle when lying supine	Hypotonia	Hypotonia; Bradykinesia; Anxiety and Dizziness when lying supine	N	Hypotonia; Bradykinesia; Tremor	Intermittent left ptosis
Paroxysmal Event Triggers	Morning; Post sleep and Post nap	Morning; Post-nap; Fever; <i>night sleep duration correlates with spell length</i>	n/a	n/a	Morning; Post-nap, Fever.	Morning; Post-nap; Fever	Morning; Post sleep; Fatigue	Morning; Post sleep; Post nap	Morning; Post sleep
Relieving Factors	Eating sweets; Acetazolamide	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Reverse Diurnal Fluctuation	Y	Y	N	N - Possible Diurnal	Y	Y	Y	Y	N
Spell Trajectory Over Time	Improving	Slight improvements	Improving	Improving	Improving	Improving	Improving	Improving	Improving
OTHER									
Other	ADHD	Irritability	Torticollis; Plagiocephaly	Left clubfoot	Hyperactivity	Hyperactivity	n/a	n/a	Torticollis; Plagiocephaly
EXAM									
Age of Most Recent Exam	10	9y5m	3y4m	5 y	13 y	10 y	11y	3y8m	4y3m
Dysmorphism (Y/N)	N	N	wide nasal bridge and high arched palate	N	N	N	N	N	N
Eye Movements	Saccadic pursuits; gaze evoked nystagmus in all directions of gaze*	Difficulty initiating smooth pursuit; Horizontal and vertical nystagmus at end gaze; Normal saccades*	Saccadic Pursuits;	Downbeat nystagmus with chin down head position	Saccadic pursuits, rare nystagmus present but improved	Downbeat nystagmus with chin down head position	Saccadic pursuits; Hypo/hypermetric horizontal and vertical saccades; Slow vertical saccades; Nystagmus present, especially end gaze	Vertical (up and down beat) nystagmus in all gaze directions with chin-down or chin-up position respectively	Saccadic Pursuits
Speech	Dysarthria	Dysarthria	Dysarthria	Dysarthria	Dysarthria	Dysarthria	Dysarthria	nl	Dysarthria
Motor Tone	hypotonia	hypotonia	hypotonia	hypotonia	hypotonia	hypotonia	hypotonia	hypotonia	hypotonia
Motor Strength	nl	nl	nl	nl	nl	nl	nl	nl	nl
Finger to Nose	Mild dysmetria and tremor at endpoint	No dysmetria but with some overshooting on fast repetition	Mild dysmetria	Dysmetria and intention tremor	Mild dysmetria	Mild dysmetria	Moderate dysmetria and intention tremor	Dysmetria and intention tremor	Mild dysmetria

Other Abnormal Movements	mild adventitious hand movements with arms outstretched	N	N	N	N	N	N	N	N
Reflexes	nl*	nl	nl	nl	nl	nl	nl	nl	nl
Gait	Normal base; Mildly unsteady. Can balance briefly on either foot; unable to tandem	wide based ataxic with hyperextension of back; difficulty standing with feet together	wide based	wide based unsteady	wide based unsteady; unable to balance on one foot	wide based unsteady; unable to balance on one foot	moderately ataxic; unstable standing feet together; unable to tandem; run ataxic and unsteady	moderately ataxic; unstable standing feet together; unable to tandem; run ataxic and unsteady	wide based
Other	*Exam varies- at times hyperreflexia and upgoing toes; gait varies - at times wide based; and/or dystonic postures, and/or truncal titubation; and/or toe walking; nystagmus severity and direction variable	* Eye movement findings vary over time	Intermittent mild left ptosis	n/a	n/a	n/a	Finger chase, toe-to-finger and heel-to-shin with moderate dysmetria; Bradykinesia and ; dysdiadochokinesia.	n/a	Intermittent mild left ptosis
TREATMENT TRIALS									
Medication	Acetazolamide	carbidopa/levodopa and dimenhydrinate with no benefit	n/a	n/a	n/a	n/a	n/a	n/a	levodopa
Dietary	Improved with sweets or night time cornstarch; ; Not improved with ketogenic diet	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
OTHER GENETIC TESTING									
Other Variants Identified by Exome/Genome	<i>HERC2</i> c.11698 G>A p.E3900K VUS from father	none	none	Compound heterozygous variants of uncertain significance in <i>SACS</i>	none	none	none	none	Compound heterozygous variants of uncertain significance in <i>ABCA2</i>
	<i>HERC2</i> c.11716 C>T p.R3906C VUS from mother								

	Unable to rule out contribution of bi-allelic <i>HERC2</i> variants Patient does not display blue eyes or colossal abnormalities previously associated with bi-allelic <i>HERC2</i> variants.			(c.12173G>A and c.7816A>C), but his phenotype was not felt to be consistent with spastic ataxia, Charlevoix-Saguenay type					(NM_001606.5:c.6059G>A and NM_001606.5:c.3674C>T), but his phenotype didn't match with Intellectual developmental disorder with poor growth and with or without seizures or ataxia (OMIM 618808);
Fragile X	nl	n/a	n/a	nl	nl	n/a	n/a	n/a	n/a
Chromosomal Microarray	nl	nl	nl	nl	n/a	n/a	nl	n/a	small deletion (23 kb): arr[GRCh38]16p13.3(7039053x2,7041953_7065289x1,7065462x2).
<i>SLC2A1</i> sequence and MLPA	nl	n/a	n/a	n/a	nl	nl	n/a	nl (MLPA not performed)	n/a
Mitochondrial Genome Sequencing	nl	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Other	n/a	<i>CACNA1A</i> gene testing nl	Prader Willi Angelman syndrome analysis via MS-MLPA nl	Angelman/Prader-Willi syndrome methylation assay, syndromic macrocephaly/overgrowth panel were normal.	Karyotype normal; Childhood Ataxia Panel (<i>ITPR1</i> , <i>SPTBN2</i> , <i>CACNA1A</i> , <i>COQ8A</i> , <i>CASK</i> , <i>SLC2A1</i> , etc.) normal.	Karyotype normal; Childhood Ataxia Panel (<i>ITPR1</i> , <i>SPTBN2</i> , <i>CACNA1A</i> , <i>COQ8A</i> , <i>CASK</i> , <i>SLC2A1</i> , etc.) normal.	Karyotype normal	<i>CACNA1A</i> gene MLPA testing nl; Ataxia Panel nl	<i>CACNA1A</i> testing normal
BIOCHEMICAL TESTING									
Lactate	nl x 3; slight elevation x 1	n/a	nl	nl	2.32	n/a	nl	n/a	nl
Pyruvate	1.67 mg/dl (0.3-1.5)	n/a	nl	n/a	nl	n/a	n/a	n/a	n/a
Plasma Amino Acids	nl x 2; branched chains (with normal allosioleucine) elevated x 2	n/a	nl	nl	slight hyperprolinemia	slight hyperprolinemia	nl	mild reduction asparagine 22.22 mmol/l (24-55)	nl
Acylcarnitine Profile	nl x 3	n/a	nl	nl	n/a	n/a	n/a	n/a	n/a
Ammonia	nl to slight elevation	n/a	nl	nl	nl	nl	nl	n/a	n/a
Carnitine	nl	n/a	nl	nl	nl	n/a	n/a	n/a	n/a
Urine Organic Acids	nl x 4	n/a	nl	nl	nl	n/a	nl	nl x 2	nl

Urine Amino Acids	nl x 2	n/a	n/a	n/a	nl	n/a	nl	mild increase taurine 160.14 mmol/mmol Cre (15-146); cysteine 13.79 mmol/mmol Cre (2-10); lysine 103.00 mmol/mmol Cre (2-60)	nl
Other		n/a	n/a	n/a	n/a	n/a		urine oligosaccharides: nl	n/a
Creatine	nl	n/a	n/a	n/a	nl	n/a	n/a	n/a	nl
Guanidinoacetate	nl	n/a	n/a	n/a	nl	n/a	n/a	n/a	n/a
Biotinidase	nl	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Carbohydrate Deficient Transferrin	nl	n/a	n/a	nl	nl	n/a	n/a	n/a	n/a
Leukocyte Lysosomal Enzymes	nl	n/a	n/a	n/a	nl	nl	n/a	n/a	n/a
TPP1 and PPT1 Enzyme Activity	nl	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Urine Creatine; Guanidinoacetate; Ratio	nl	n/a	n/a	n/a	nl	n/a	nl	n/a	n/a
EM of Buffy Coat	nl (no ultrastructural inclusions)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Vitamin B12	n/a	n/a	n/a	nl	n/a	n/a	nl	n/a	n/a
Urine oligosaccharides	n/a	n/a	n/a	nl	n/a	n/a	nl	nl	n/a
Very Long Chain Fatty Acids	n/a	n/a	n/a	nl	n/a	n/a	nl	n/a	n/a
Other	n/a	n/a	n/a	urine Homocysteine - nl	n/a	n/a	homocysteine, purines and pyrimidines, phytanic acid, urine mucopolysaccharides normal	n/a	homocysteine nl
MISCELLANEOUS									
CBC	nl	n/a	n/a	n/a	n/a	n/a	nl	n/a	n/a
Chemistry	nl	n/a	n/a	nl	n/a	n/a	nl	nl	nl
Liver Function	nl	n/a	n/a	nl	nl	n/a	nl	n/a	nl
Hepatic Function	nl	n/a	n/a	n/a	nl	n/a	nl	nl	nl

CPK	nl to slight elevation	n/a	mild elevation	n/a	nl	nl	n/a	nl	nl
TSH	nl	n/a	nl	nl	n/a	n/a	nl	nl	nl
fT4	nl	n/a	nl	nl	n/a	n/a	n/a	nl	nl
AFP	nl	n/a	n/a	n/a	nl	nl	nl	n/a	n/a
Vitamin E	nl	n/a	n/a	nl	n/a	n/a	n/a	n/a	n/a
Lead	nl	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Other	n/a	n/a	n/a	Triiodothyronine (T3) - nl	n/a	n/a	folate nl	n/a	n/a
CSF									
Protein	12 mg/dl (15-25) (L)	n/a	n/a	12 mg/dl (15-50) (L)	nl	n/a	n/a	28 mg/dl (20-45 mg/dl)	nl
Glucose	45 mg/dl (40-70 mg/dl)	n/a	n/a	nl	2.4 mmol/L (2.8-4.4 mmol/L)	n/a	n/a	62 mg/dl	nl
CSF:Plasma Glucose Ratio	0.33 (L)	n/a	n/a	n/a	0.8	n/a	n/a	0.7	nl
WBC	nl	n/a	n/a	n/a	n/a	n/a	n/a	nl	nl
Lactate	nl	n/a	n/a	n/a	n/a	n/a	n/a	10 mg/dl	nl
Pyruvate	nl	n/a	n/a	nl	n/a	n/a	n/a	n/a	n/a
Amino acids	nl	n/a	n/a	non-specific low values (methionine, isoleucine, & ornithine).	nl	n/a	n/a	n/a	nl
Organic Acids	nl	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Neurotransmitter metabolites (5-hydroxyindoleacetic acid, Homovanillic acid, 3-O-methylidopa)	nl	n/a	n/a	nl	n/a	n/a	n/a	mild reductions in homovanillic acid and 3-Methoxy-4-hydroxyphenylglycol not clinically significant (HVA: 231 nmol/L; range 236-867 nmol/L and 3-Methoxy-4-hydroxyphenylglycol: 35 nmol/L; range 47-81 nmol/L).	nl
Pterins	nl	n/a	n/a	nl (Tetrahydrobiopterin and Neopterin Profile)	n/a	n/a	n/a	n/a	nl
5-methyltetrahydrofolate	nl	n/a	n/a	nl	n/a	n/a	n/a	n/a	nl
CSF:Plasma Glycine Ratio	n/a	n/a	n/a	nl	n/a	n/a	n/a	n/a	nl

Other	n/a	n/a	n/a	n/a	n/a	n/a	n/a	no oligoclonal bands	n/a
MUSCLE									
Light Microscopy	Slightly prominent lipid droplets on oil-red-O staining, there were no ragged red fibers notable on trichrome stain but cytochrome oxidase staining showed increased sub-sarcolemmal staining.	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Electron Microscopy	Mitochondria were mildly increased in number, focally aggregated in the subsarcolemmal region, and increased in size but showed no inclusions or cristae abnormalities	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Electron Transport Chain Enzyme Analysis	Analysis of mitochondrial respiratory chain enzyme activity showed elevated citrate synthase activity consistent with increased mitochondrial content but reduced complex II-III activity. The tissue was embedded and fatty and thus not of high quality for analysis but all other enzymes assayed were normal and the results were felt to be valid especially in light of histochemistry and electron microscopy findings.	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

CoQ10	nl	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Mitochondrial DNA Sequencing and Deletion Testing	nl	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Mitochondrial Depletion Studies	nl	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
IMAGING									
MRI Brain	nl x 3	incidental pineal cyst (age 2y) resolved on repeat x 2	oval T2 hyperintense signal in R superior cerebellum; decreased volume of L hippocampal formation and anterior L temporal lobe; probable mild inferior vermian hypoplasia (age 2y)	nl x 3	nl x 2	nl x 2	nl (age 1y); FLAIR images - some increase white matter signal in occipital poles, possible normal variant (age 2y)	mild inferior vermian hypoplasia	probable 7x8 mm heterotopia lateral to the corpus callosum (age 2y)
MRS Brain	nl	n/a	n/a	n/a	n/a	n/a	n/a	nl	nl
Other	n/a	n/a	n/a	n/a	n/a	n/a	cardiac echo nl	n/a	n/a
ELECTROPHYSIOLOGY									
EEG	rare epileptiform discharges (bilateral frontal) in sleep	nl x 3	n/a	n/a	nl	nl	nl	nl	nl
Electromyogram (EMG)/Nerve Conduction Studies (NCS)	n/a	n/a	n/a	n/a	n/a	n/a	mild myopathic changes in the left tibialis anterior, deltoid and extensor digitorum communis muscles. There was a question of pseudo myotonic discharges also in the left tibialis anterior, but it didn't last, so it is of unclear significance. No evidence of a peripheral neuropathy	EMG:n/a; NCS: nl	n/a
Other	n/a	n/a	n/a	n/a	n/a	n/a	EKG nl	n/a	n/a

OPHTHALMOLOGIC - OTHER									
Visual Acuity Normal	N (20/80 OD and 20/125 OS)	Y	Y (astigmatism)	N -(20/50 and 20/40)	N - hyperopia; astigmatism	N	Y (moderate bilateral hyperopic correction because he has a small variable esotropia which could be partially improved with this optical correction)	N hypermetropia with need for corrective lenses (+4.00 bilateral)	Y
Fundus Exam	enlarged optic nerve cups and subtle parafoveal gray halo (previously visible OD as well)	nl	n/a	nl	nl	nl	nl	nl	nl
Slit Lamp Exam	nl	nl	n/a	nl	nl	nl	n/a	n/a	n/a
Optical Coherence Tomography	nl	n/a	n/a	nl	n/a	n/a	n/a	n/a	n/a
Electroretinogram	n/a	n/a	n/a	n/a	nl	nl	n/a	n/a	n/a
Visual Evoked Potentials	n/a	n/a	n/a	Present but non-repeatable responses were induced from both eyes.	nl	n/a	n/a	results uninterpretable. Patient not cooperative	n/a
Other	n/a	n/a	n/a	intermittent esotropia and intermittent left hypertropia	n/a	n/a	.n/a	n/a	n/a
OTHER									
							audiology nl	bulbar ultrasound: results uninterpretable. Patient not cooperative	

VIDEO LEGENDS

Supplemental Video1a: Case 1: In the first segment, the child displays sustained upward gaze and father describes how episodes' severity has changed over time. There is elliptical nystagmus with lateral gaze and downward nystagmus with down gaze. In the second segment, pursuit movements are full and nystagmus is again demonstrated.

Supplemental Video1b: Case 1: In the first segment the child displays mild dysmetria with tremor at end point with finger to nose testing. In the second segment, gait is minimally unsteady. The child has difficulty balancing on one foot, performing tandem gait, and displays slight dystonic hand postures.

Supplemental Video2: Case 2: In the first two segments the child displays upward gaze deviation with downbeat nystagmus on attempted downgaze without alteration in awareness; In the third segment, the child maintains a significant chin down posture which helps maintain his preferred upward gaze which is his null point; In the fourth segment, child again displays upward gaze deviation with downward nystagmus but at lesser severity than at a younger age. Gait is mildly unsteady.

Supplemental Video3: Case 4: The child displays wide base unsteady gait with mild dystonic hand postures

Supplemental Video4: Case 8: In the first segment, the child displays sustained upward gaze accompanied by downward chin posture without alteration in awareness. Vertical nystagmus is seen in downgaze by the movement of the eyelids. Truncal titubations are present though there is no obvious appendicular ataxia. In the second segment the child displays wide based unsteady gait. There is mild appendicular ataxia.

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