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Expansion of clinical and variant spectrum of *EEF2*-related neurodevelopmental disorder: Report of two additional cases

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Abstract

Eukaryotic translation elongation factor 2 (eEF2), encoded by the gene *EEF2,* is an essential factor involved in the elongation phase of protein translation. A specific heterozygous missense variant (p.P596H) in *EEF2* was originally identified in association with autosomal dominant adult-onset spinocerebellar ataxia-26 (SCA26). More recently, additional heterozygous missense variants in this gene have been described to cause a novel, childhood-onset neurodevelopmental disorder with benign external hydrocephalus. Herein, we report two unrelated individuals with a similar gene-disease correlation to support this latter observation. Patient 1 is a 7-year-old male with a previously reported, *de* novo missense variant (p.V28M) who has motor and speech delay, autism spectrum disorder, failure to thrive with relative macrocephaly, unilateral microphthalmia with coloboma and eczema. Patient 2 is a 4-year-old female with a novel *de* novo nonsense variant (p.Q145X) with motor and speech delay, hypotonia, macrocephaly with benign ventricular enlargement, and keratosis pilaris. These additional cases help to further expand the genotypic and phenotypic spectrum of this newly described *EEF2*-related neurodevelopmental syndrome.

Keywords

EEF2; neurodevelopmental delay; macrocephaly; hydrocephalus; ventriculomegaly

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Author Contributions

Rose Guo, Alyssa Rippert, Edward B Cook, Cesar Augusto P Alves and Lynne M Bird provided clinical data and wrote the initial manuscript. Kosuke Izumi conceptualized the study, provided clinical data, and edited the manuscript. All authors approved the final manuscript.

Introduction

Intracellular protein synthesis is the last step of the central dogma and plays an essential role in maintaining cellular homeostasis. Protein translation consists of four phases including initiation, elongation, termination, and ribosome recycling [Blanchet and Ranjan 2022]. Translation initiation is the first step of protein synthesis whereby the ribosome becomes engaged with initiation codon of messenger RNA (mRNA). During elongation, amino acids are added to the carboxy terminus of a nascent polypeptide chain. Upon recognition of a stop codon, the synthesized polypeptide is released from the ribosome. These four steps are orchestrated by eukaryotic initiation factors (eIF), eukaryotic elongation factors (eEF), eukaryotic termination or release factors (eRF), and recycling factors, respectively [Blanchet and Ranjan 2022]. While translation is used ubiquitously across all cell types, neuronal cells are particularly sensitive to translational defects, partly due to their large cellular size and requirement of mRNA translation in axons and dendrites [Glock et al. 2021; Job and Eberwine 2001]. Because regulation of gene expression in neuronal cells relies heavily on local translation, one can speculate that pathogenic variants in genes encoding translational regulatory molecules are likely to cause neurological disorders. Supporting this notion, variants in *EIF2B1*, *EIF2B2*, *EIF2B3*, *EIF2B4*, *EIF2B5*, *EIF2S3*, *EIF3F, EIF4A2*, *EIF4G1,* and *EIF5A* encoding eIF proteins, have been linked to neurological disorders [Borck et al. 2012; Chartier-Harlin et al. 2011; Faundes et al. 2021; Leegwater et al. 2001; Martin et al. 2018; Moortgat et al. 2016; Paul et al. 2022; Skopkova et al. 2017; van der Knaap et al. 2002].

In addition to the genes encoding eIF proteins, variants in the *EEF2* gene, which encodes eukaryotic translation elongation factor 2 (eEF2), one of the eEF proteins, have been reported in a limited number of individuals with neurological manifestations. A missense variant in *EEF2*, p.P596H, causes autosomal dominant adult-onset spinocerebellar ataxia 26 (SCA26) [Hekman et al. 2012]. Recently, Nabais Sá et al. identified three individuals with developmental delay with benign external hydrocephalus due to heterozygous, *de novo* missense variants in *EEF2* [Nabais Sa et al. 2021]. However, given the limited number of reported cases, the clinical and variant spectrum of a genetic disorder due to *EEF2* variants remain largely unknown. Here, we report two additional individuals with *EEF2* variants expanding the genotypic and phenotypic description of *EEF2*-related human disease (Fig 1).

Case Report

Patient 1

Patient 1 is a 7-year-old male who was initially evaluated by the genetics service at 3 weeks of age for right-sided microphthalmia noted at birth. The prenatal course was unremarkable with normal routine ultrasounds. He was delivered at 37 weeks gestation with birth weight 2863 g (50^{th} centile) and length 49.5 cm (75^{th} centile). Head circumference is unknown. Ophthalmologic evaluation at 2 weeks of age demonstrated coloboma of the iris and retina in the right eye and a morning glory disc anomaly of the left eye.

Clinically, additional medical issues included gross motor and speech delay, failure to thrive, relative macrocephaly and eczema. Developmentally, he walked at 2 years old. At age 2,

his vocabulary consisted of only 6 words and at age 5, he spoke in short sentences with limited, repetitive words and perseverates on certain topics. He was subsequently diagnosed with autism spectrum disorder of moderate severity. At age 6 years, he was speaking in full sentences and was readily understood by unfamiliar listeners. In terms of growth, he remains gastrostomy tube-dependent for nutrition, initially due to oral feeding intolerance and episodic vomiting, and persistent oral motor dysfunction. Endocrinologic evaluation did not reveal an organic cause for his poor growth. He also had significant visual impairment in his right eye secondary to the coloboma. At 3 years and 5 months of age, his head circumference was 50.7 cm (49th centile) and his height was 87.8 cm (Z= −2.71).

At 7 years old, his weight is 18.2 kg (Z=−2.18), and height is 110.7 cm (Z= −2.43). Physical exam was notable for a prominent forehead, midface hypoplasia, right microphthalmia with ptosis, small palpebral fissures, deeply set eyes, low set ears, small upturned nose, long philtrum and thin upper lip (Fig 2). Brain MRI demonstrated mildly increased extra-axial fluid (Fig 2).

Patient 2

Patient 2 is a 15-month-old female who was initially evaluated by genetics for global developmental delay, macrocephaly and hypotonia. She was the product of a naturally conceived single gestation to a 35-year-old G3P2 mother and 36-year-old father. The prenatal history was notable for prominence of cerebral ventricles identified on routine ultrasound at 34 weeks gestation, confirmed by fetal MRI. There were no other anomalies identified and no other complications during pregnancy were reported. She was delivered at 37 weeks gestation with birth weight 3317 g (75thcentile), length 48.3 cm (50thcentile) and head circumference 35.1 cm (59th centile). Neonatal history was notable for difficulty with weight gain. She had frequent episodes of otitis media, requiring bilateral myringotomy tube placement at 15 months old. One week post-operatively, she was found to have a retropharyngeal abscess that required intravenous antibiotics. Developmentally, she had significant motor and mild speech delays. She sat at 11.5 months, crawled at 12 months, but was not yet walking at 15 months. Her first words were at 12 months and by 15 months, she had a 10-word vocabulary. Her social and fine motor milestones were age appropriate.

At 15 months, her weight was 9.6 kg (47th centile), height 78 cm (53th centile) and head circumference 50 cm (Z= 3.08). Her physical exam was notable for macrocephaly with frontal bossing, depressed nasal bridge, keratosis pilaris on the forehead, thin upper lip and axial and appendicular hypotonia (Fig 2). Brain MRI was performed given her macrocephaly and hypotonia and demonstrated benign enlargement of the ventricles and extra-axial spaces (Fig 2). She had a normal ophthalmologic exam without evidence of papilledema.

At 4 years old, her weight was 16.6 kg (62ndcentile), height 104.4 cm (77th centile) and head circumference 54.2 cm (Z= 3.02). Developmentally, she walked at 18 months and was working on climbing stairs and dressing herself. Her language development improved and was age appropriate. Her physical exam was notable for macrocephaly with frontal bossing, sparse eyebrows with thin terminal hair, broad nasal tip with low columella, dermatographia, and axial and appendicular hypotonia (Fig 2).

Methods/Results

Patient 1 was identified during routine clinical care to likely have a genetic basis for his problems. His chromosomal microarray showed two copy number variants of uncertain significance: 329 kb loss at 17q25.3(chr17: 80,558,676-80,887,477 (GRCh37)) and 1,065 kb gain at 18q12.2(chr18: 33,748,334-34,813,244 (GRCh37)). 17q25.3 deletion includes seven genes and 18q12.2 duplication includes five genes. Subsequently, Patient 1 was enrolled in a research study at Rady Children's Institute for Genomic Medicine for rapid whole genome sequencing in acutely ill patients (WIRB #20171726). Whole genome sequencing was carried out (June 2019) according to methods reported in detail in Kingsmore et al. (2019). Initial results showed a single nucleotide variant (SNV) in *IGF2* (NM_001127598.2: c.663C>G, p.D221Q), a microdeletion of 17q25.3 (chr17:80,544,401-80,883,200 (GRCh37.1)) and a microduplication of 18q12.2 (chr18:33,748,944-34,817,330 (GRCh37.1)), both interpreted as variants of uncertain significance. Parental samples were then obtained, which revealed the *IGF2* SNV to be of maternal inheritance and the microdeletion to be of paternal inheritance. The 17q25.3 microduplication was found to be inherited from the mother. Additionally, a *de novo* variant in *EEF2* (NM_001961.3: c.82G>A, p.V28M) was found, initially interpreted as a variant of uncertain significance due to uncertain association of the gene with human disease, but later (2021) was revised to likely pathogenic when the same variant was published by Nabais Sá et al. (2021). V28 is an evolutionally conserved amino acid. The same variant has not been seen in a healthy population [Karczewski et al. 2020].

Patient 2 was identified during a routine inpatient consultation to have a suspected genetic basis for her constellation of medical diagnoses. Patient 2 had a clinical trio whole exome sequencing performed by GeneDx (August 2019) utilizing a proprietary capture system developed by GeneDx for next-generation sequencing with copy number variant (CNV) calling. Enriched targets were sequenced on an Illumina platform and analyzed using a custom-developed analysis tool [Retterer et al. 2015]. Initial results identified a *de novo* nonsense variant in *EEF2* (NM_001961.3: c.433 C>T, p.Gln145Ter) which was interpreted as a variant of uncertain significance classified as a candidate gene in accordance with ACMG guidelines [Richards et al. 2015]. p.Gln145Ter represents a novel variant and has not been seen in a healthy population [Karczewski et al. 2020].The variant was not previously published or observed in large population cohorts but predicted to result in protein truncation. Similar to Patient 1, the report was later (2022) revised to likely pathogenic variant after additional individuals with similar clinical features and missense variants in *EEF2* were reported by Nabais Sá et al.

Discussion

Here we describe two individuals with *de novo EEF2* variants presenting with neurodevelopmental phenotypes. Recently, Nabais Sá et al. reported three children with *EEF2* variants, and they proposed that *EEF2* variants cause a neurodevelopmental syndrome, termed *EEF2*-related disorder [Nabais Sá et al. 2021]. In addition, Zhao and Mata-Machado reported another patient with developmental delay, obesity and behavioral issues due to an *EEF2* missense variant [Zhao and Mata-Machado 2022]. Our report supports the notion that

heterozygous *EEF2* variants cause a neurodevelopmental syndrome, distinctively different from spinocerebellar ataxia syndrome (SCA26). Based on the common clinical features seen among the previously reported patients and those reported herein, the cardinal features of this syndrome are developmental delay, relative macrocephaly and facial dysmorphisms.

In addition to commonly seen clinical features, patient 1 demonstrated coloboma and microphthalmia. It remains unknown whether these ocular manifestations are a part of *EEF2*-related neurodevelopmental disorder or not. In the previous report by Nabais Sá et al. strabismus and myopia were reported as clinical features seen in individuals with *EEF2* pathogenic variants [Nabais Sa et al. 2021]. Further reports will be necessary to determine whether coloboma and microphthalmia are a part of the phenotypic spectrum of the *EEF2*-related neurodevelopmental disorder.

Additionally, our report expands the mutational spectrum of *EEF2*-related neurodevelopmental disorder. The four previously reported cases all had missense variants (Val28Met, Cys388Tyr, Ala736Val, and His769Tyr) in exon 2, 9, 13, and 14 of the *EEF2* gene[Nabais Sa et al. 2021; Zhao and Mata-Machado 2022] (Fig 1). Patient 1 in our case report had the same missense variant, p.Val28Met as patient 1 in the report by Nabais Sá et al [Nabais Sa et al. 2021]. p.Val28Met represents a recurrent variant resulting in a neurodevelopmental phenotype. Patient 2 in our case report is the first patient to be reported in the literature with an *EEF2*-related neurodevelopmental disorder due to a nonsense variant. Based on the location of this nonsense variant which is located in exon 4 of 15 (Fig 1), it is anticipated that the mutant *EEF2* transcript undergoes nonsense mediated decay. *EEF2* loss-of-function variants are rarely identified in a healthy population (pLI of 1 and loss of function observed/expected upper bound fraction (LOEUF) of 0.172) [Karczewski et al. 2020], indicating that *EEF2* loss-of-function variants are detrimental to human development. Collectively, our finding suggests that *EEF2*-related neurodevelopmental disorder is due to loss-of-function of eEF2. Of note, fewer *EEF2* missense variants were observed than expected in the healthy population (observed / expected ratio = 0.42) [Karczewski et al. 2020]. Hence, in addition to four pathogenic missense variants (Table 1), there likely exist additional missense variants resulting in *EEF2*-related neurodevelopmental disorder.

Although eEF2 is a ubiquitously expressed protein involved in protein translation regulation, clinical symptoms of *EEF2*-related neurodevelopmental disorder and *EEF2*-related SCA indicate the particular importance of eEF2 in neuronal function. This observation comports with basic research findings suggesting the importance of eEF2 in regulating neurogenesis, synaptic function and social novelty behavior [Ma et al. 2022; Taha et al. 2020]. *EEF2*-related disorder serves as a valuable model to understand the role of eEF2 in regulating neurocognitive development in human.

In summary, we report two individuals with *EEF2* variant who manifested with developmental delay, relative macrocephaly and facial dysmorphism, providing further evidence that heterozygous pathogenic *EEF2* variants cause a child-onset disorder, *EEF2*-related neurodevelopmental disorder. Our data suggest that not only specific missense variants, but also loss-of-function variants cause *EEF2*-related neurodevelopmental disorder.

In the previous study evaluating the neurobehavioral phenotype of heterozygous loss-of-function *Eef2* mutant mice, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) potentiator, PF-4778574, corrected neurobehavioral phenotype of these mice [Ma et al. 2022]. Our demonstration that *EEF2* loss-of-function variants cause a neurodevelopmental phenotype in human, raising an interesting possibility that AMPAR potentiator may also ameliorate neurological symptoms of human patients with *EEF2*-related neurodevelopmental disorder. Towards the identification of such therapeutic strategies, further clinical cohort studies are warranted in order to delineate the clinical and mutational spectrum of *EEF2*-related neurodevelopmental disorder.

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Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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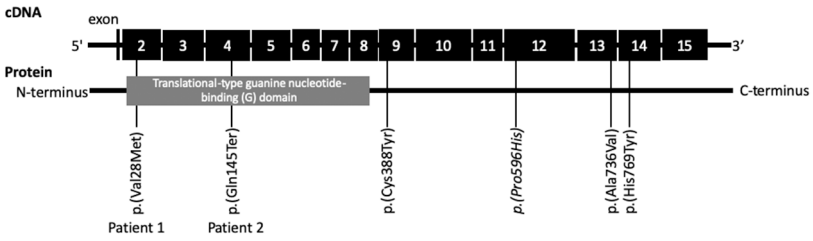
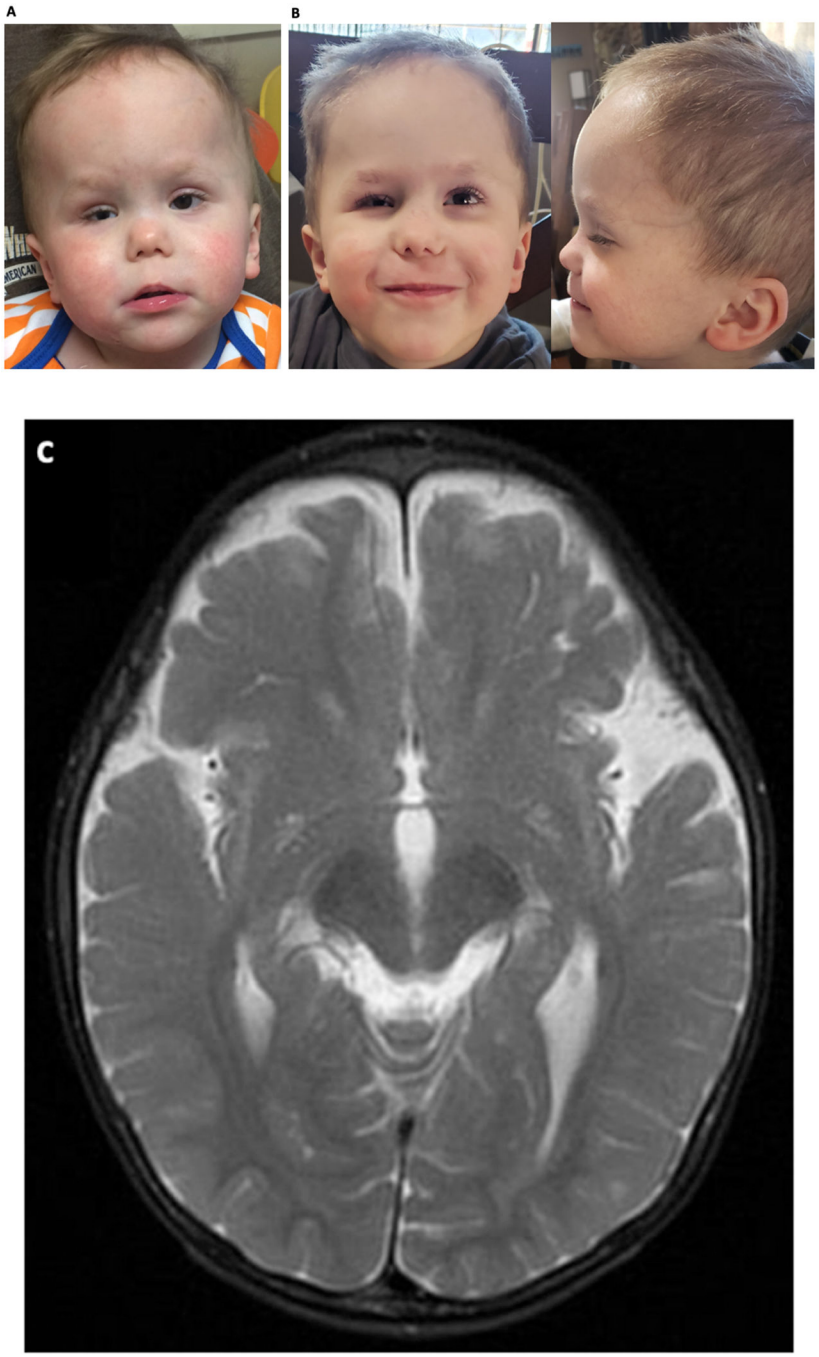


Fig 1.

Locations of identified variants in *EEF2* (NM_001961). Variants identified in the Patient 1 and Patient 2 as well as previously reported missense variants are depicted [Hekman et al. 2012; Nabais Sa et al. 2021; Zhao and Mata-Machado 2022]. The variant of the Patient 1 was identified in a different subject reported by Nabais Sa et al. 2021. Missense variant causing SCA26 is indicated in Italics [Hekman et al. 2012].



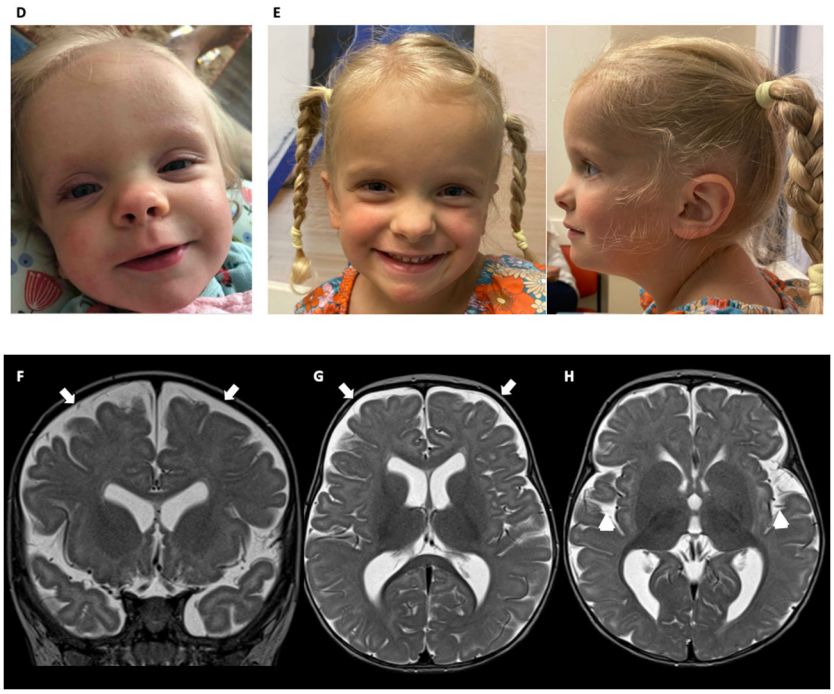


Fig 2.

Clinical features of *EEF2*-related neurodevelopmental disorder. (A-C) Facial features and neuroimaging finding of the Patient 1. (A) Patient 1 picture at 19 months. (B) Patient 1 pictures at 5 years. Note prominent forehead, midface hypoplasia, right microphthalmia with ptosis, small palpebral fissures, deeply set eyes, low set ears, small upturned nose, long philtrum and thin upper lip. (C) Brain MRI of the Patient 1 at 3 weeks of age. Mild prominence of the extra axial space is demonstrated. (D-H) Facial features and neuroimaging finding of the Patient 2. (D) Patient 2 pictures at different ages, 15 months, and (E) 4 years, show the evolution of facial appearance and dysmorphisms. (F-H) Brain MRI findings of the Patient 2 at 8 months old. There is prominence of the extra-axial subarachnoid CSF spaces in the frontoparietal regions (arrows, F and G), along with prominence of the lateral ventricles and under-opercularization of the insula bilaterally (arrows, H).

Table 1.

Genotype and phenotype of patients with *EEF2* variants

Patient	1	2	3 (Nabais Sá et al 2021)	4 (Nabais Sá et al 2021)	5 (Nabais Sá et al 2021)	6 (Zhao et al 2022)
Genotype						
cDNA change	NM_001961.3: c.82 G>A	NM_001961.3: c.433 C>T	NM_001961: c.82G>A	NM_001961: c.1163G>A	NM_001961: c.2305C>T	NM_001961: c. 2207 C>T
Protein change	p.V28M	p.Q145X	p.V28M	p.C388Y	p.H769Y	p. A736V
Inheritance	*de novo*	*de novo*	*de novo*	*de novo*	*de novo*	*de novo*
Gestational age at birth	37 weeks	37 weeks	39 weeks	37 weeks	38 weeks	Unknown
Birth weight (centile range)	2863g (50th)	3317g (75th)	3345g (25-50th)	3440g (unknown centile)	2470g (3-15th)	N/A
Birth length (centile range)	49.5cm (75th)	48.3cm (50th)	N/A	52cm (unknown centile)	N/A	N/A
Birth head circumference (centile range)	Unknown	35.1cm (59th)	N/A	35cm (unknown centile)	N/A	N/A
Prenatal complications	None	Ventriculomegaly at 34 weeks gestation	None	None	None	Maternal gestational diabetes and Decreased fetal movement end of pregnancy
Gender/Age at examination(s)	Male/7 years	Female/15 months/4 years	Male/3 years	Male/6 years/9 years	Male/6 years	Male/8 years
Weight (centile range)	18.2kg (1.5th)	9.6kg (47th)/16.6kg (62th)	18kg (85th)	19kg (25th)/26.4kg (30th)	15.5kg (−2.2 SD)	Obese (BMI 99.4th)
Height (centile range)	110.7cm (Z= −2.43)	78cm(53th)/104.4cm (77th)	101cm (50th)	111cm (20th)/130cm (30th)	102cm (−2.5 SD)	Unknown
Head circumference (centile range)	53 cm (50-75th)	50cm (Z= 3.08)/54.2cm (Z= 3.02)	52cm (85-97th)	52.7cm (80th)/53.4cm (75th)	52.3cm (75th)	Unknown
Developmental milestones						
Motor delay	+	+	+	+	+	+
Age at walking	2 years	18 months	22 months	24 months	26 months	18 months
Speech delay	+	+	+	+	+	+
Age at first words	~2 years	12 months	15 months	24 months	12 months	3 years
Intellectual disability	+	−	−	−	−	N/A

Patient	1	2	3 (Nabais Sá et al 2021)	4 (Nabais Sá et al 2021)	5 (Nabais Sá et al 2021)	6 (Zhao et al 2022)
Degree of intellectual disability	Mild-moderate	−	N/A	N/A	Mild	N/A
Neurologic abnormalities	−	+ (hypotonia)	+ (hypotonia, unsteady gait, high stepping)	+ (Poor motor coordination)	−	+ (balance issues, dysmetria, impaired tandem gait)
Brain abnormalities	− (mildly increased extra-axial fluid)	− (benign enlargement of ventricles and extra-axial spaces)	+ (Mild–moderate enlargement of lateral and third ventricles, diffuse thinning of CC, left temporo-occipital focal dysplasia)	+ (Mild–moderate enlargement of the lateral and third ventricles and external CSF spaces)	+ (Mild enlargement of the lateral ventricles and external CSF spaces)	+ (slightly prominent retrocerebellar fluid without mass effect)
Behavioral issues	+ (autism spectrum disorder – moderate severity)	−	−	+ (Autistic behavior)	−	+ (ADHD)
Craniofacial features	+ (prominent forehead, midface hypoplasia, right microphthalmia with ptosis, small palpebral fissures, deeply set eyes, borderline low-set ears, small upturned nose, long philtrum and thin upper lip)	+ (frontal bossing, depressed nasal bridge, broad nasal tip, low columella, thin upper lip, sparse eyebrows with thin terminal hair)	+	+	+	−
Other medical issues						
Ophthalmologic	+ (right eye: iris and retinal colobomata; left eye: morning glory disc anomaly)	−	+ (strabismus requiring surgery)	+ (Myopia)	−	−
Skin/hair/nails	+ (eczema)	+ (keratosis pilaris)	+ (Fine sparse scalp hair, sparse eyebrows, hypoplastic and dystrophic toenails, capillary malformations)	+ (Fine sparse scalp hair, fast growing and brittle toe nails)	+ (Fast growing hair and nails, hypoplastic nails of 5th toes)	+ (pruritis of distal extremities)
Growth abnormalities	+ (failure to thrive; requiring gastrostomy tube)	−	−	−	Short stature and failure to thrive	Tall stature and obesity

Abbreviations: g, grams; cm, centimeters; kg, kilograms; +, present; −, absent