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## **A novel truncating variant p.(Arg297\*) in the *GRM1* gene causing autosomal-recessive cerebellar ataxia with juvenile-onset**

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## **Abstract**

*GRM1* gene, that is located on 6q24.3, encodes the metabotropic glutamate receptor type 1 (mGluR1), a transmembrane protein highly expressed in cerebellar Purkinje cells. Pathogenic variants in *GRM1* have been reported only three times in humans, causing autosomal-recessive cerebellar ataxia with early-onset and intellectual disability or dominant forms of cerebellar ataxia with less severe phenotype in adults. We report a six-year-old boy, born to inbred parents, with an early-onset cerebellar syndrome due to a homozygous autosomal-recessive *GRM1* pathogenic variant. In addition to cerebellar ataxia, axial hypotonia and oculomotor signs, he showed a severe and global developmental delay with lack of walking and speech and slight facial dysmorphic features. Brain MRI, performed at 1 year and at 5 years, showed a slowly progressive cerebellar atrophy. A novel homozygous truncating variant in the second exon of *GRM1* gene (c.889C>T, p.(Arg297\*)), inherited from the heterozygous healthy parents, was found by exome sequencing. Our observation not only emphasizes the central role of mGluR1-mediated signaling in cerebellar function and neurodevelopment but also provides valuable insights into the early clinical signs of recessive ataxia due to *GRM1* pathogenic variants that were not reported previously. The difficulties of clinical differential diagnosis between this disease and other forms of congenital ataxia and the unspecific cerebellar atrophy on MRI highlight the importance of large-scale genetic investigations.

**Keywords:** cerebellar ataxia; spinocerebellar ataxia autosomal recessive, 13;  
metabotropic glutamate receptor type 1; GRM1

## Introduction

The chronic cerebellar ataxias group together various genetic and acquired entities which can be clinically distinguished by the age of onset of clinical signs (congenital or degenerative forms) and their evolution (progressive or non-progressive forms). Inherited ataxia include multiple forms for the half of which the causative gene has not yet been identified and can be divided according to the mode of transmission that is either autosomal-dominant, autosomal-recessive, X-linked or mitochondrial. Among the autosomal-recessive forms, Friedreich ataxia (OMIM #229300) and ataxia-telangiectasia (OMIM #208900) are the two most frequent and are due to pathogenic variants in the *FXN* and *ATM* genes, respectively (Anheim et al., 2012). Autosomal-recessive cerebellar ataxia due to variant in the *GRM1* gene (OMIM #604473) is a very rare disease and has only been reported in two publications since 2012 (Guergueltcheva et al., 2012; Davarniya et al., 2015). In 2012, Guergueltcheva et al. first reported two biallelic variants in the *GRM1* gene in individuals from five Roma families with autosomal-recessive cerebellar ataxia (OMIM #614831, SCAR13) ranging from 6 to 57 years of age. Then, in 2015, Davarniya et al. reported another biallelic variant in *GRM1* gene in an Iranian family with individuals ranging from 28 to 40 years of age at examination (Davarniya et al., 2015). All had cerebellar ataxia and mild to severe intellectual disability. Pathogenic heterozygous variants in *GRM1* have been reported once in autosomal-dominant cerebellar ataxia with age of onset in adulthood between 20 and 50 (Watson et al., 2017). The phenotype was less severe than autosomal-recessive forms since they were able to walk and the intellectual deficit was absent or moderate.

Here, we report a novel truncating variant in the *GRM1* gene, p.(Arg297\*), in a proband with autosomal-recessive cerebellar ataxia with juvenile-onset and review

the literature of cerebellar ataxia due to *GRM1* variants. Early diagnosis allowed us to detail the first clinical and imaging data of the disease that were not reported previously.

## **Clinical report**

### Clinical data

A six-year-old boy, born to first-cousin parents of Tunisian origin, was first referred to our center at the age of 9 months for global developmental delay and abnormal movements. He was the first child of the couple and the parents were 29 and 36 years old (mother and father respectively) at time of birth (Fig. 1A). Appropriate parental consent for this report has been obtained. Both parents presented a normal neurological examination. There was no family history except isolated strabismus in two maternal aunts.

He was born at term after an uneventful pregnancy and the Apgar score was at ten at one minute. At birth, head circumference (34.5 cm, -0.5 standard deviation), height (52 cm, +0.5 standard deviation) and weight (3800 g, +0.5 standard deviation) were in the normal range.

Psychomotor delay was first noticed at the age of 6 months with axial hypotonia: he was not able to hold his head. He started to turn on his belly at 12 months, to seat in a tripod at 18 months, to crawl at 2 years and to kneel with help at 4 years.

Cerebellar syndrome had been evoked at the age of 1 year because he presented a hands dysmetria. A nodding when sitting was noticed at 3 years. In addition, he exhibited involuntary dystonic and choreic movements.

Transient and moderate pyramidal signs were noted around 1 year of age with hypertonia and brisk tendon reflexes of the four limbs, no longer described at 4 years

old, age at which on the contrary he presented a tendency to joint hyperlaxity of the lower extremities, predominant on ankles, with weak reflexes. He had no skin hyperlaxity and no seizure. The clinical findings and ages of acquisitions are summarized in Table 1.

Ophthalmological examination performed at the age of 10 months revealed a permanent convergent strabismus, a vertical gaze-evoked nystagmus, a limited ocular abduction and a normal visual acuity.

Concerning cognitive function, intellectual disability was profound. Despite speech therapy and normal audiometry, he had been able to pronounce only two syllables since he was 20 months old.

Upon recent examination, height (116.5 cm, mean), weight (17.5 kg, -1 standard deviation) and head circumference (52 cm, mean) were within normal limits. He presented a plagiocephaly and slight facial dysmorphic features with a prominent forehead and large and coarse ears without other congenital malformation (Fig. 1B, 1C). He had no telangiectasia or tendon xanthoma. He presented autism spectrum disorder with a lack of eye contact, a tendency to isolation and stereotypes. He cannot stand up and sitting remains unstable. Urinary and fecal continence were not acquired. Vomiting was common because of hypernauseal reflex. Despite a severe handicap (lack of walking and speech), physiotherapy allowed some progress. He attended to a specialized school 3 hours a week. He did not have long-term medication.

Pelvic radiographs and cardiac and abdominal ultrasound exploration were normal. Nerve-conduction studies and needle electromyography performed in Tunisia were normal.

Routine blood counts and biochemistry (albumin, ammonia, creatine phosphokinase,



alpha-fetoprotein, lactate, pyruvate) were within reference limits. Blood metabolites (A and E vitamins, cholesterol, chromatography of plasma amino acids, very long chain fatty acids) and urine metabolites (chromatography of urinary organic acids, urinary succinylaminoimidazole-carboxamide riboside) were normal. He had no immune deficiency.

#### Imaging data

Brain MRI (1.5 tesla, 4 mm slices), first performed at one year of age, was normal. At five, brain MRI showed moderate enlargement of both primary and secondary cerebellar fissures (Fig. 1D, 1E), as well as more pronounced foliation of the culmen. The middle cerebellar peduncle and brainstem were normal.

#### Genetic investigations

To rule out chromosomal aberrations, karyotype analysis by G and R-banding and array-CGH were performed when he was 2 years old. The results were negative just like the selective sequencing of 450 genes previously reported in intellectual disabilities.

Exome sequencing was performed when he was 6 years old. Covaris shearing of DNA was used for library preparation (End Repair, A-Tailing, Adaptors ligation, PCR amplification) and captured using the SeqCap EZ MedExome kit (Roche, Madison, WI). The captured library was sequenced by 2X150-bp paired-end reads protocol using the NextSeq 500 (Illumina, San Diego, CA). Ninety six percent exons were covered by at least 30X. Sequence alignment to the human reference genome (UCSC hg19) and variants call and annotation were performed using an in-house bioinformatic pipeline. **Synonymous variant, strand bias, mosaic <10%, depth <10**

reads, or variants with frequency >1% in public database including the 1000 Genome Project and the 6500 Exome Sequencing Project were unselected. Only exonic and splicing variants were considered. Out of a total of 92,639 detected variants, 668 survived first filtering. After a trio analysis, 9 variants matched with an autosomal recessive transmission hypothesis (considered most likely given the two inbred and clinically healthy parents), 46 were *de novo* changes, 6 matched with an X-linked transmission hypothesis and 34 with composite heterozygosity. Only one of these variants was retained after interpretation of each variant according to the corresponding *in silico* prediction if missense, gnomAD data, and available data from the literature on the involved gene, on the function of the encoded protein, and its involvement in human pathology. Therefore, the homozygous nonsense variant chr6:g.146,480,672C>T (RefSeq accession number NM\_001278064.1), c.889C>T, p.(Arg297\*), located in exon 2 of *GRM1* gene (OMIM #604473), was considered pathogenic. The *GRM1* variant was absent from the gnomAD database of control individuals. Its presence in the homozygous state in the proband and its inheritance from two heterozygous parents were verified by Sanger sequencing (Fig. 2A). These data were submitted to the ClinVar public database (SCV000898479.1).

## Discussion

*GRM1* gene is located on 6q24.3 and encodes the metabotropic glutamate receptor 1 (mGluR1, GenBank #AAI36281.1), a transmembrane protein highly expressed in cerebellar Purkinje cells (Shigemoto et al., 1992; Guergueltcheva et al., 2012; Watson et al., 2017). Metabotropic receptors form dimers of proteins composed of a large ligand-binding domain (Kunishima et al., 2000) followed by a cysteine-rich domain, a highly conserved seven transmembrane helical structure and an

intracellular C-terminal tail that coupled to G proteins (Pin et al., 1994; Kunishima et al., 2000; Guergueltcheva et al., 2012; Wu et al., 2014; Ohtani et al., 2014). In response to a ligand, they change their conformation (Kunishima et al., 2000) and produce a postsynaptic long-term depression by release of intracellular calcium (Pin et al., 1994; Shigemoto et al., 1994; Ichise et al., 2000; Fan, 2013). Long-term depression serves to selectively weaken specific synapses in order to make constructive and coordinated response to cerebellar long-term potentiation (Purves, 2008). Of the ligands that can bind to metabotropic receptors, glutamate is the most abundant excitatory neurotransmitter in the mammalian brain (Zhou and Danbolt, 2014). When binding to mGluR1, glutamate activates phospholipase C and produces diacylglycerol (DAG) and inositol triphosphate (IP3). These second messengers cause the release of intracellular calcium by targeting IP3-sensitive receptors and the activation of protein kinase C, jointly performed by calcium and DAG (Pin et al., 1994; Abdul-Ghani et al., 1996; Francesconi and Duvoisin, 2000; Kammermeier et al., 2000). The mGluR1 signaling is required for early postnatal development of the cerebellar cortex and plays a critical role in synaptic plasticity, memory formation and motor learning (Aiba et al., 1994b; Conquet et al., 1994; Gil-Sanz et al., 2008; Ohtani et al., 2014).

Support for the contribution of mGluR1 to human cerebellar ataxia has come from rare patients with paraneoplastic or primary autoimmune reactions against the receptor protein (Sillevis Smitt et al., 2000). The consequences of a loss of mGluR1 function are also demonstrated by results from knockout animal models showing a range of developmental and functional cerebellar deficits: early-onset ataxia, impaired synaptic plasticity (abnormal long-term potentiation) and abnormal spatial and associative learning (Aiba et al., 1994a; Aiba et al., 1994b; Ichise et al., 2000;

Gil-Sanz et al., 2008; Rossi et al., 2013).

Our observation confirms the severe and early-onset character of the recessive forms of cerebellar ataxia due to **biallelic** *GRM1* pathogenic variants in humans which has been reported only twice before. Early diagnosis and follow-up in pediatric neurology allowed us to detail the clinical evolution of the disease that was not reported previously. The first clinical signs that should make the clinician to evoke cerebellar ataxia appeared during the first year of life and consisted of dysmetria and nodding. Axial hypotonia may also be part of the cerebellar syndrome. Facial dysmorphism was poor and unspecific. **While the clinical course was rather marked by delay than regression, imaging data showed a progressive impairment.** Indeed, face to enlarged primary and secondary cerebellar fissures, a cerebellar atrophy was discussed on the brain MRI performed at five years, whereas it was normal at one year. Being a slight MRI feature at five years, the progressive cerebellar atrophy of our patient highlights that early imaging is useless for positive diagnosis in case of *GRM1* pathogenic variant. Consistent with the study of Guergueltcheva et al. (Guergueltcheva et al., 2012), there seems to be no correlation between clinical severity and neuroimaging findings which appear several years after clinical onset. Furthermore, both the unspecific delayed neuroimaging findings and the difficulties of clinical differential diagnosis with other forms of congenital ataxia highlight the importance of large-scale genetic investigations.

**The proband's mother was in the first trimester of a second pregnancy. Cerebellar atrophy appearing during childhood, antenatal imaging is useless for positive diagnosis in case of *GRM1* pathogenic variant. A targeted genetic screening was proposed and indicated that the fetus was not affected (heterozygous).**

Notably, the *GRM1* gene is also involved in dominant forms of cerebellar ataxia. In

2017, Watson et al. reported heterozygous pathogenic variants in *GRM1* in two families with degenerative cerebellar ataxia with onset between 20 and 50 years (Watson et al., 2017). Considering the twenty cases from nine reported families with recessive or dominant forms of ataxia due to *GRM1* variants, all had cerebellar syndrome (Davarniya et al., 2015; Guergueltcheva et al., 2012; Watson et al., 2017), only five cases (one recessive, four dominant) had no oculomotor signs. Speech was either absent (5 cases) or disturbed (12 cases) in most of the cases. Mild to profound intellectual disability was observed in all recessive cases but not in dominant case with adult-onset. Lack (2 cases) or delayed walking (4 cases) was observed in recessive forms with an average walking age at 4 years (for available data) but not in dominant forms with adult-onset. Among other inconstant clinical signs, pyramidal signs were observed in nine patients with recessive forms and in one with a dominant form. Five cases with seizures were reported in recessive forms with an average age of start at 13 months. No case of dysmorphic features had been reported. Short stature observed in nine literature cases (-3.8 SD on average) is debatable and should be compared to the expected target size (not available). The *GRM1* pathogenic variant characteristics, clinical findings and ages at examination in the present study and in literature are summarized in Table 1.

Differences in clinical severity between the recessive and dominant forms of ataxia due to *GRM1* variants were suggested to be related to the effect of the variant on the function of the protein. By studying mutant receptor activity in transfected cells with missense variants involved in dominant forms, Watson et al. observed gain-of-function (Watson et al., 2017). The reverse has been described by Guergueltcheva et al. in recessive forms of cerebellar ataxia with juvenile-onset that are due to loss-of-function (LoF) variants (Guergueltcheva et al., 2012). A case of frameshift

heterozygous mutation in the final *GRM1* exon causing an early-onset and as severe form of ataxia than autosomal-recessive forms was reported by Watson et al. in a five-year-old girl. The production of a truncated protein being confirmed by levels of expression in transfected cells, a negative dominant effect of the truncated protein on the wild-type was supposed to explain this observation (Watson et al., 2017). Such dual genotype/phenotype correlation depending on the functional effect of variants in a single gene has previously been reported, for example in the *GRIN1* gene (Rossi et al., 2017). In our case, the nonsense *GRM1* pathogenic variant occurs in exon 2, in the ligand-binding domain (Fig. 2B, 2C), suggesting a complete lack of functional mGluR1.

In summary, we report the third pathogenic variant in *GRM1* gene causing an autosomal-recessive form of cerebellar ataxia. Our observation not only emphasizes the central role of mGluR1-mediated signaling in cerebellar function but also provides valuable insights into the early clinical signs of recessive ataxia due to biallelic *GRM1* pathogenic variants. The difficulties of clinical differential diagnosis with other forms of congenital ataxia and the unspecific, late and slight cerebellar atrophy on MRI support the importance of large-scale genetic investigations (selected gene panel or exome sequencing) face to cerebellar ataxia.

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## Figures

-Figure 1:

(A) Genealogy of the consanguineous family.

The affected proband is homozygous for p.(Arg297\*) variant in the *GRM1* gene. Each of his parents is heterozygous for this variant. The proband's mother is pregnant with an unaffected fetus (heterozygous for this variant).

(B, C) Moderate dysmorphic features observed at the age of 2 years and a half.

The proband showed a prominent forehead, large and coarse ears, a permanent convergent strabismus and a positional plagiocephaly.

(D, E) Brain MRI (1.5 tesla, 4 mm slices).

Mid sagittal T2-weighted image performed at 1 year old (A) was normal. At 5 years old (B), MRI showed discrete cerebellar atrophy with subsequently moderate enlargement of both primary (arrow) and secondary (arrowhead) cerebellar fissures, as well as more pronounced foliation of the culmen (\*). The middle cerebellar peduncle and brainstem were normal.

-Figure 2:

(A) Checking the presence of the variant c.889C>T by sequencing the *GRM1* gene (NM\_001278064.1) in the proband and his parents.

The homozygous variant c.889C>T (p.(Arg297\*)) in *GRM1* was confirmed in the proband (individual IV.1), inherited from each of his two parents (individuals III.1 and III.2) who are heterozygous.

(B) Structure of *GRM1* gene.

Scheme of the *GRM1* gene (NM\_001278064.1), locating the pathogenic variant identified in the proband (p.(Arg297\*)), that affects exon 2, the three published by Guergueltcheva (Guergueltcheva et al., 2012) and Davarniya (Davarniya et al., 2015) in homozygous affected patients (top part of the figure) and the three

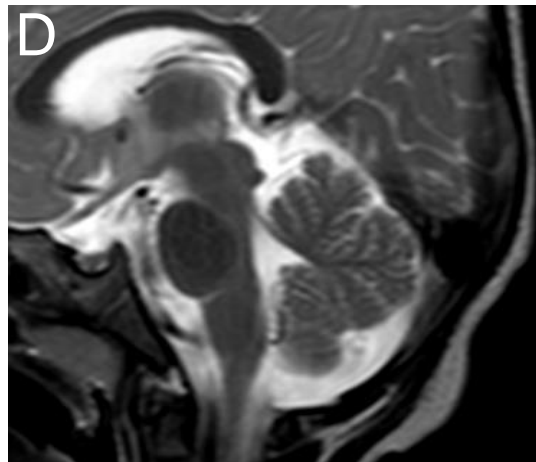
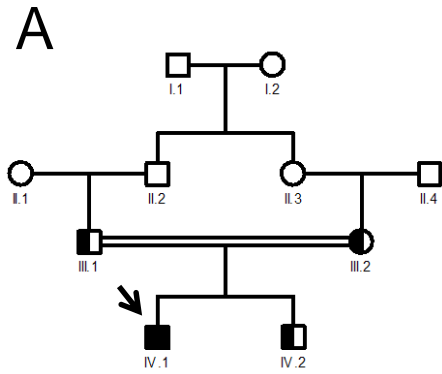
published by Watson (Watson et al.,2017) in heterozygous affected patients (lower part of the figure). Rectangles schematize the 8 exons.

(C) Structure of mGluR1 protein.

Three-dimensional representation (adapted from Neuroscience (Purves, 2008)) of the mGluR1 protein that counts 1194 amino acids. mGluR1 is composed of a large ligand-binding domain followed by a cysteine-rich domain, a highly conserved seven transmembrane helical structure and an intracellular C-terminal tail. Arrow indicates the mutated residue Arginine 297 that is located in the ligand-binding domain.

## **Table**

-Table 1: Summary of *GRM1* pathogenic variant characteristics and clinical observations in the present study and in literature.



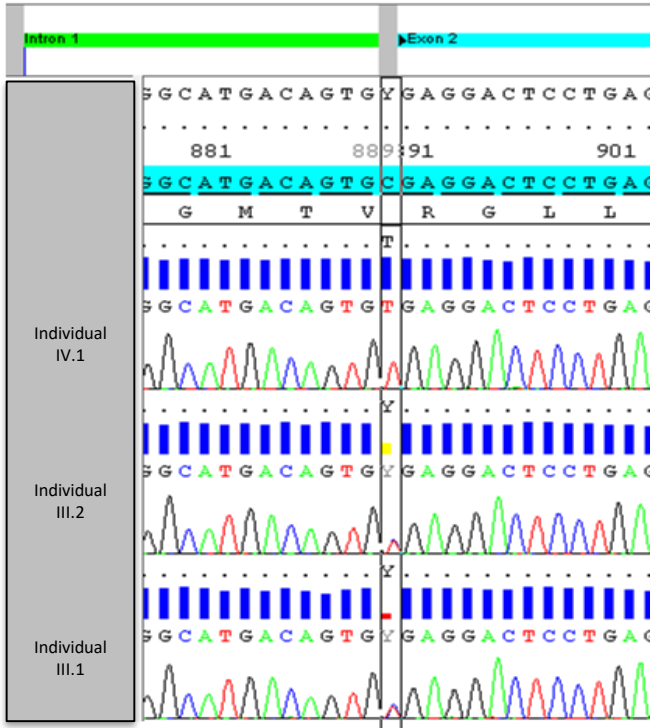
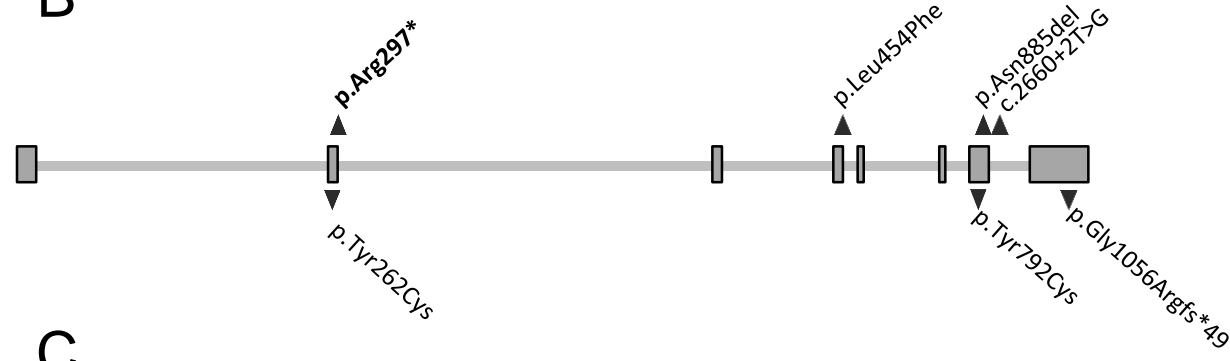
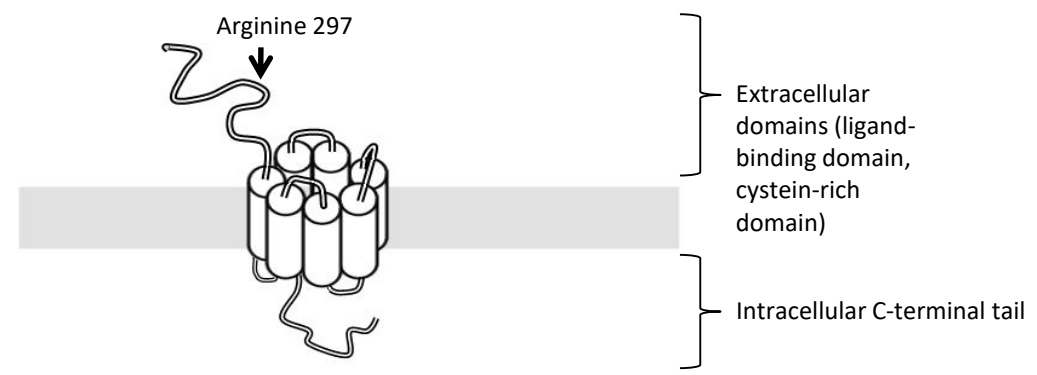
**A****B****C**

Table 1. Summary of *GRM1* pathogenic variant characteristics and clinical observations in the present study and in literature

Reference Patient	AD forms			AR forms													Total		
	F3 I-2, II-1, III-1	F2 II-1, II-2, III-1	F3 II-1	II-1	V III-1	V III-2	B IV-2	B IV-3	P II-1	P II-2	P II-3	M II-1	M II-2	Devarniya et al., 2015 IV-1	IV-2	IV-3	Present study IV-1	AD forms	AR forms
<b><i>GRM1</i> pathogenic variant characteristics</b>																			
nucleotide	c.2375A>G	c.785A>G	c.3165dup	c.2652_2654del and c.2660+2T>G	c.2652_2654del and c.2660+2T>G	c.2652_2654del and c.2660+2T>G	c.2652_2654del and c.2660+2T>G	c.2652_2654del and c.2660+2T>G	c.2652_2654del and c.2660+2T>G	c.2652_2654del and c.2660+2T>G	c.2652_2654del and c.2660+2T>G	c.2652_2654del and c.2660+2T>G	c.2652_2654del and c.2660+2T>G	c.1360C>T	c.1360C>T	c.1360C>T	c.889C>T	exonic variant 7	intronic variant (splice) 10
protein (if exon variant)	p.Tyr792Cys	p.Tyr262Cys	p.Gly1056Argfs*49	p.Asn885del	p.Asn885del	p.Asn885del	p.Asn885del	p.Asn885del	p.Asn885del	p.Asn885del	p.Asn885del	p.Asn885del	p.Asn885del	p.Leu454Phe	p.Leu454Phe	p.Leu454Phe	p.Arg297*	missense 6	exonic variant 14
functional effect on mGluR1	GoF	GoF	LoF	LoF	LoF	LoF	LoF	LoF	LoF	LoF	LoF	LoF	LoF	LoF	LoF	LoF	LoF	truncating variant 1	deletion 10
<b>Measurements</b>																			
head circumference (cm) / age	no data	no data	-1.5 SD (value not provided)	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	55 (+1.5 SD)/28y	54 (+0.5 SD)/37y	54 (+0.5 SD)/40y	34.5 (-0.5 SD)/birth	52 (0 SD)/6y	in the normal range
height (cm) / age	no data	no data	-1.5 SD (value not provided)	120 (+1 SD)/6y	137 (-1 SD)/11y	126 (-1 SD)/9y	147 (-3 SD)/26y	143 (-3.5 SD)/32y	151 (-4 SD)/37y	138 (-6 SD)/27y	150 (-2 SD)/42y	152 (-3.5 SD)/57y	154 (-3 SD)/47y	150 (-4 SD)/28y	150 (-4 SD)/37y	150 (-4 SD)/40y	52 (+0.5 SD)/birth	116.5 (0 SD)/6y	2.7 SD on average
weight (kg) / age	no data	no data	-1.5 SD (value not provided)	22 (+0.5 SD)/6y	42 (+0.5 SD)/11y	40 (+1 SD)/9y	42.5 (-2 SD)/26y	42.5 (-2 SD)/32y	50 (-2 SD)/37y	23 (-5 SD)/27y	60 (-0.5 SD)/42y	45.1 (-2.5 SD)/57y	47.1 (-2.5 SD)/47y	55 (-2.5 SD)28y	58 (-1.5 SD)/37y	60 (-1.2 SD)/40y	3.8 (+0.5 SD)/birth	17.5 (-1 SD)/6y	in the normal range
<b>Psychomotor development</b>																			
walking	yes	yes	at 21 months	at 4 years	at 5 years	at 4 years	childhood	at 1 year	no	no	no	no data	at 3 years	no data	no data	no data	no	absent 0	absent 3
<b>Oculomotor signs</b>																			
oculomotor movements	no	nystagmus (II-1, II-2)	nystagmus	limited ocular abduction	convergent strabismus	convergent strabismus	limited ocular abduction nystagmus	nystagmus	nystagmus	convergent strabismus limited ocular abduction	limited ocular abduction	convergent strabismus	no	convergent strabismus nystagmus	nystagmus	nystagmus	limited ocular abduction permanent convergent strabismus nystagmus	3	13
gaze-evoked nystagmus	no	yes (II-1, II-2)	yes	no	no	no	yes, horizontal	yes, horizontal	yes, horizontal	no	no	no	no	yes	yes	yes	yes, vertical, at 10 months	3	7
<b>Cognitive function</b>																			
mental retardation	no	no	moderate	mild	mild	mild	moderate	moderate	moderate	profound	severe	severe	severe	severe	severe	moderate	profound	no 6	mild 3
speech	disturbed (II-1)	disturbed	disturbed	disturbed simple sentences at 4y	disturbed simple sentences at 3y	disturbed simple sentences at 2y	disturbed	disturbed	no speech	no speech	no speech	no speech	no speech	disturbed simple sentences at 10y	disturbed simple sentences at 11y	disturbed simple sentences at 11y	no two syllables since 20 months	moderate 1	severe 5
<b>Others</b>																			
pyramidal signs	yes (III-1)	no	no data	yes	yes	yes	yes	yes	yes	no	yes	yes	yes	no data	no data	no data	moderate, transient hypertonia and sharp reflexes around 1y	1	10
seizure	no	no	no data	no	at 5 months	at 2 years	no	no	no	one abnormal EEG	no	no	no	at 1 year	at 6 months	at 1-2 years	no	0	5

Table 1 summarizes clinical signs for our proband and literature cases. The following abbreviations are used: AD, autosomal-recessive; AR, autosomal-recessive; LoF, loss-of-function; GoF, gain-of-function; y, year(s).