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Expanding the phenotypic spectrum associated with *OPHN1* mutations: Report of 17 individuals with intellectual disability but no cerebellar hypoplasia

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ABSTRACT

Mutations in the oligophrenin 1 gene (*OPHN1*) have been identified in patients with X-linked intellectual disability (XLID) associated with cerebellar hypoplasia and ventriculomegaly, suggesting it could be a recognizable syndromic intellectual disability (ID). Affected individuals share additional clinical features including speech delay, seizures, strabismus, behavioral difficulties, and slight facial dysmorphism. *OPHN1* is located in Xq12 and encodes a Rho-GTPase-activating protein involved in the regulation of the G-protein cycle. Rho protein members play an important role in dendritic growth and in plasticity of excitatory synapses.

Here we report on 17 individuals from four unrelated families affected by mild to severe intellectual disability due to *OPHN1* mutations without cerebellar anomaly on brain MRI. We describe clinical, genetic and neuroimaging data of affected patients. Among the identified *OPHN1* mutations, we report for the first time a missense mutation occurring in a mosaic state. We discuss the intrafamilial clinical variability of the disease and compare our patients with those previously reported. We emphasize the power of next generation techniques (X-exome sequencing, whole-exome sequencing and targeted multi-gene panel) to expand the phenotypic and mutational spectrum of *OPHN1*-related ID.

1. Introduction

The *OPHN1* gene (MIM 300127) located in Xq12 encodes for the oligophrenin 1, a Rho-GTPase-activating protein involved in the regulation of the G-protein cycle (Billuart et al., 1998). Rho protein members are important mediators required for dendritic spine morphogenesis and neuronal cell migration (Fauchereau et al., 2003; Govek et al., 2004; Khelfaoui et al., 2007). *OPHN1* is highly expressed in fetal and adult brain, in particular in olfactory bulb, hippocampus and cerebellum (Billuart et al., 1998; Fauchereau et al., 2003). At a cellular level, the protein oligophrenin 1 is present in neurons and glial cells. Localized in both pre- and post-synaptic axons, OPHN1 plays an essential role in maturation and plasticity of excitatory synapses (Nadif Kasri et al., 2009).

The importance of OPHN1 in brain development has been demonstrated in ophn1-defective mice, which display a reduction in newborn neurons number and show dendritic spine immaturity and alteration in synaptic function (Khelfaoui et al., 2007; Allegra et al., 2017). Knockout mice exhibit behavioral defects in spatial memory, impairment in social behavior, hyperactivity, and enlargement of ventricles. Further studies demonstrated that pharmacological inhibition of RhoA pathway restores synaptic plasticity in Ophn1 knockout mice (Khelfaoui et al., 2009). Moreover, chronic treatment of mice with fasudil, a Rho Kinase (ROCK) and Protein Kinase A (PKA) inhibitor, restores not only spine density but also enhances survival of adult-born neurons, counteracts hyperactivity, restores recognition memory and limits the brain ventricular dilatation observed in Ophn1 knock-out mice (Allegra et al., 2017; Meziane et al., 2016). In OPHN1-deficient human induced pluripotent stem cells (h-iPSCs), the levels of ROCK are also elevated and treatment with fasudil improves neuronal-like

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appearance of the cells (Compagnucci et al., 2016).

The association of *OPHN1* point mutations or deletions with X-linked intellectual disability (XLID) is now well recognized. Mutations in *OPHN1* are responsible for XLID with cerebellar hypoplasia predominant in the vermis and cerebral ventricular enlargement, suggesting the delineation of a recognizable phenotype. Additional clinical features include seizures, strabismus and facial dysmorphism. The severity of intellectual disability (ID) ranges from moderate to severe, with intrafamilial variability. *OPHN1* missense variants have also been reported in individuals with ID associated with variable features such as autism or childhood onset schizophrenia but little clinical information is available regarding these patients (Piton et al., 2011; Hu et al., 2016; Tarpey et al., 2009; Classen et al., 2013).

We describe here the clinical and molecular findings of a cohort of 17 patients from 4 unrelated families affected by XLID due to novel *OPHN1* mutations, without posterior fossa abnormalities. We thereby expand the clinical and mutational spectrum of the disease.

2. Clinical reports

All the individuals enrolled in this study gave their consent form for genetics studies. Pictures are published with permission from patients and/or their legal representatives.

2.1. Family A

The five-generations Belgian family comprises four affected males (V.1, V.5, III.2, III.4) and four affected females (III.1, III.3, IV.1, IV.2) (Fig. 1).

Patient V.1 was referred for developmental delay at the age of 6 to our Center of Human Genetics. The boy was born at term after an uneventful pregnancy and a spontaneous delivery. His birth parameters were normal. He sat and walked unaided at age 12 months and 2 years respectively. He had a severe speech delay and said his first words after the age of 26 months. At age 6, he presented with unsteady gait and multiple falls. He had fine motor difficulties and an expressive vocabulary of about 30 words. He also had behavioral problems including inappropriate tantrums and intolerance to frustration. Risperidone treatment was introduced. Evaluation of cognitive functions at age 6 revealed an IQ score of 60 (WISC-IV), indicating a mild ID. He was admitted in a special education school. No epilepsy was reported.

The initial physical examination at age 6 showed height and weight in normal range (0.5 SD) but head circumference was less than −2 SD (48 cm). He had mild facial dysmorphism (Fig. 2) with large and thin eyebrows, asymmetric palpebral fissures, a short nose with anteverted nostrils, a short philtrum, and a tended upper lip with a high arched palate. In addition, small joint hyperlaxity of hands and bilateral sandal gap of feet were present. Neurological examination showed a bilateral convergent strabismus and a mild horizontal gaze nystagmus, an ataxic gait, with dysdiadochokinesia and hand dysmetria. He had brisk osteotendinous reflexes with bilateral clonus and slight rigidity of feet. Bucco-linguo-facial dyspraxia with open mouth was noted.

At the last follow up at age 13, behavioral difficulties were still important. Persistance of unstable gait and unsteady walk with a toe walking were present. Spasticity of lower limbs was decreased. IQ testing was performed using WISC-IV and showed a total score of 48 (moderate ID), with a result of 45 on verbal subtests, lower than performance IQ. EEG recordings showed a normal background activity. Cerebral MRI were performed twice (at the age of 5 and at the age of 11 years) and described as completely normal (Fig. 3).

Patient V.5 was followed in our Center since the age of 7 months because of developmental delay. He had a normal birth. He was described as an abnormally quiet baby with poor visual contact. Hypotonia was noted at the age of 7 months. Walking was acquired after the age of 3 years. The first words were pronounced at around 4 years and association of 2 words after the age of 5. Since the age of 4, he developed behavioral anomalies with stereotypies. No seizure was noted. He had surgery for right cryptorchidism. At age 11, the patient presented with unsteady gait and frequent falls. He had speech difficulties with poor language and pronunciation difficulties. Evaluation of cognitive functions (WISC-IV) revealed an IQ score of 41 (moderate ID). Clinical examination revealed normal growth parameters and head circumference, and some facial dysmorphic features including synophrys and thick eyebrows, deep-set eyes, a short nose and a slight pointed chin (Fig. 2). He displayed bilateral ptosis and divergent strabismus without nystagmus. Bilateral sandal gap of feet were present. Neurological examination showed an ataxic gait and dysmetria. Osteotendinous reflexes were normal and he had no spasticity of the lower limbs. EEG recording was normal. Cerebral MRI performed at 8 months and at 13 years did not reveal any anomalies, in particular in the

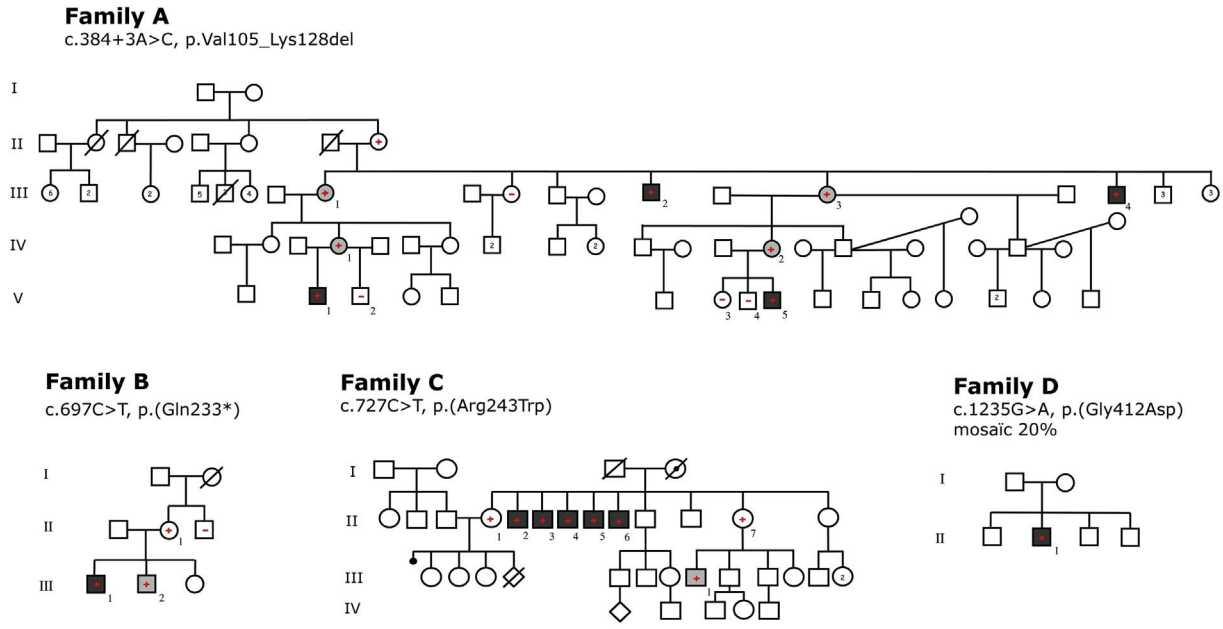

Fig. 1. Pedigrees of four families with *OPHN1*-related disorders.
Light gray: learning difficulties/mild intellectual disability; dark gray: moderate to severe intellectual disability; +: presence of the *OPHN1* mutation; -: absence of the *OPHN1* mutation.

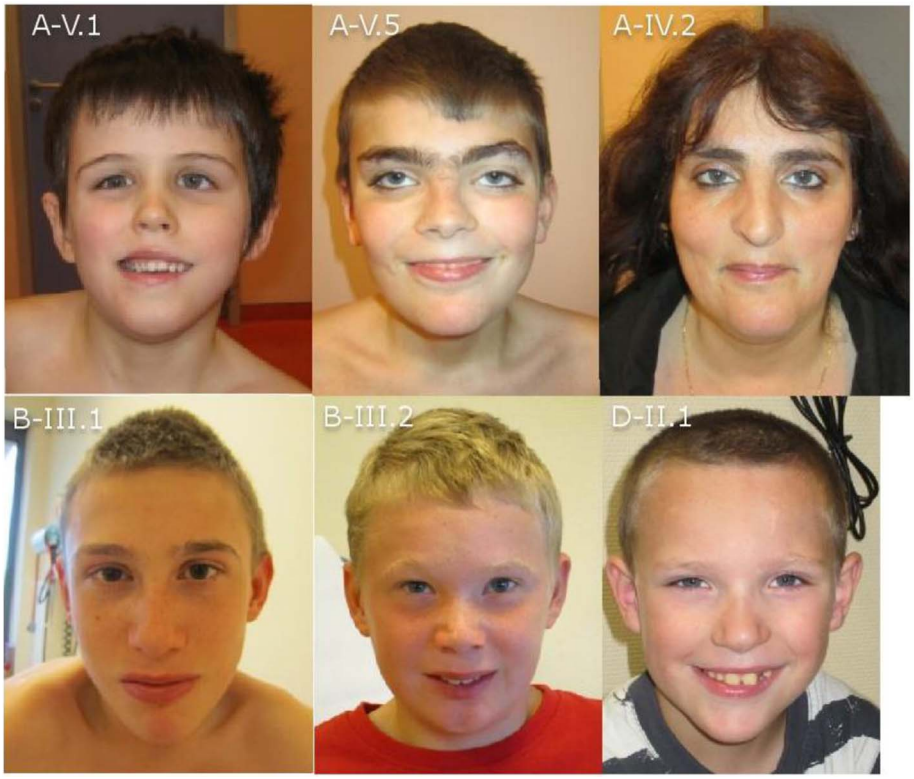

Fig. 2. Facial features of individuals with *OPHN1* mutation in families A, B and D.
A-V.1: patient at age 7. Note large eyebrows, bilateral strabismus, asymmetric and downslanting palpebral fissures, short nose with anteverted nostrils, short philtrum, and tended upper lip.
A-V.5: patient at age 13. Note synophris, thick eyebrows, deep-set eyes, bilateral ptosis, divergent strabismus, short nose and slightly pointed chin.
A-IV.2: mother of patient V.5. Note mild ptosis and pointed chin.
B-III.1: patient at age 15. Note long face, enophtalmia, long nose and pointed chin.
B-III.2: patient at age 11. Note short, asymmetric and upslanting palpebral fissures.
D-II.1: patient at age 8. Note asymetric palpebral fissures.

cerebellum or in supratentorial layers (Fig. 3).

Patients III.2 and III.4 are respectively 67 and 64-year-old males, with mild to moderate intellectual disability and speech difficulties including impairment of articulation and phonological processes. They never developed epilepsy. They are not able to neither read nor write and they required educational support. They work in a sheltered environment and are institutionalized. No neuroimaging studies could be performed. At clinical exam, they had mild facial dysmorphism including a long face with a long nose and a pointed chin. Osteotendinous reflexes were normal and they had no spasticity of the lower limbs.

Patients III.1, III.3, IV.1 and IV.2 are four affected females presenting similar clinical features with mild cognitive dysfunction and speech difficulties. All required educational support at school. They can read and write, but with difficulties. They are autonomous in daily tasks. They are not dysmorphic. In patient IV.2, mother of patient V.5, we noted a slight ptosis with asymmetric palpebral fissures and a pointed chin (Fig. 2).

2.2. Family B

In this second Belgian family, two affected males, their asymptomatic mother and one unaffected maternal uncle were enrolled (Fig. 1).

Patient III.1 was born at term after an uneventful pregnancy, with normal birth parameters. Motor development was slightly delayed, with walking achieved at 19 months. He had speech delay. He required special need education. Evaluation of cognitive functions confirmed mild intellectual disability (IQ 60). He was treated with risperidone for behavioral problems consisting of inappropriate tantrums, some aggressiveness and a low frustration tolerance. He developed epilepsy at 21 months with generalized tonic-clonic seizures and absences. Around the age of 4, the anti-epileptic treatment was switched from valproate to lamotrigine and levetiracetam with positive results. At clinical examination at age 15, he showed mild dysmorphic features including a long face, enophthalmia, a long nose and a pointed chin (Fig. 2). He had microcephaly (head circumference < -2 SD), dysmetria, slight ataxia, synkinesis, and limited extension of elbows and knees. Osteotendinous reflexes were normal. Brain MRI revealed enlarged lateral ventricles predominantly in the frontal horns and hypoplasia of the head of the caudate nuclei (Fig. 3).

Patient III.2 is his young brother, born at term with normal birth parameters. He had normal psychomotor development. He walked at 15 months and pronounced his first words at 12 months. He developed some learning difficulties with normal-range IQ score (92). He started speech therapy at 4 because of phonological difficulties. He never had seizures and EEG recordings were normal. He had spatio-temporal difficulties. He presented with mild facial dysmorphism with short, asymmetric and upslanting palpebral fissures (Fig. 2), but not microcephaly (OFC, 0.5 SD). At neurological examination he had bilateral synkinesis but not ataxia. Brain MRI revealed exactly the same anomalies as his brother with enlarged lateral ventricles and a normal posterior fossa (Fig. 3).

Their mother (patient II.1) is a 40-year-old healthy woman. Brain MRI was performed because of recurrent headaches and revealed a triventricular dilatation (data not shown) with a normal cerebellum.

2.3. Family C

This French four-generation family comprises six affected males (II.2, II.3, II.4, II.5, II.6 and III.1) and two asymptomatic females (II.1, II.7) (Fig. 1). All affected males presented with mild to moderate intellectual disability. They all required educational support and work in a sheltered environment. They never developed seizures and neurological examination was normal in each of them. Brain MRI was only available for individual II.5 and showed ventriculomegaly and a normal cerebellum (Fig. 3).

2.4. Family D

The affected individual (II.1, Fig. 1) was born from healthy Belgian parents with unremarkable familial history. He was referred at the age of 6 for mild to moderate ID with speech delay. He developed behavioral disorders (attention deficiency, anxiety, emotional lability,

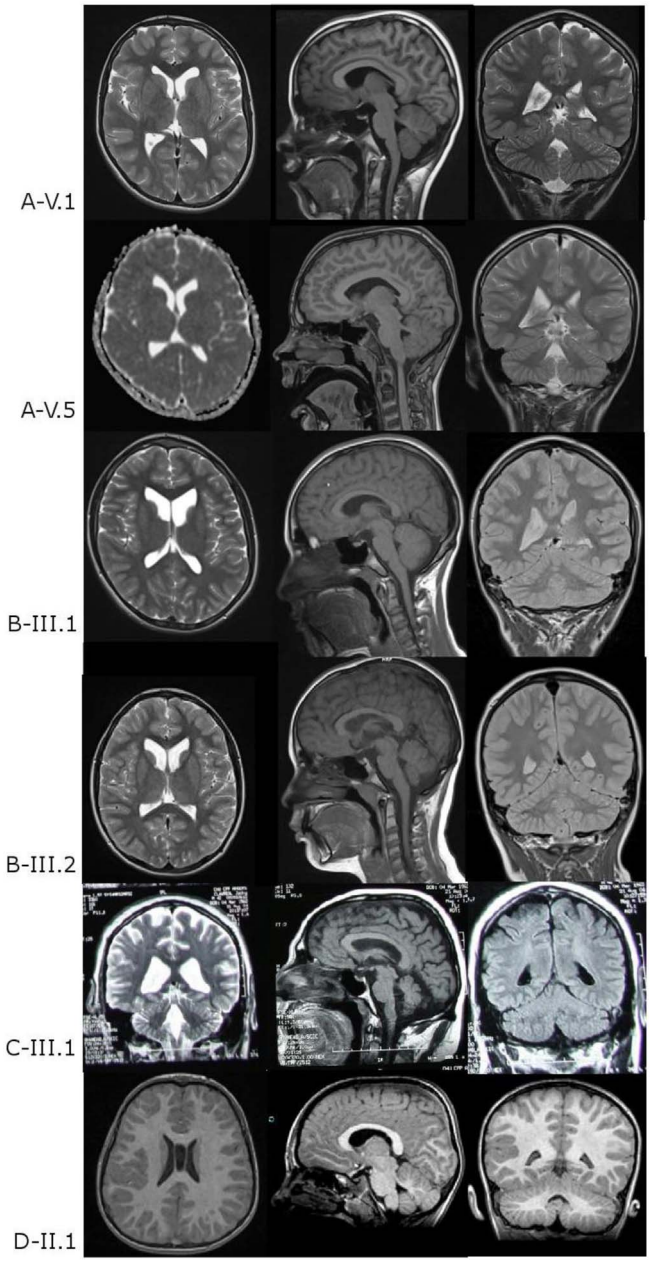

Fig. 3. Brain MRI of individuals from families A, B C and D with *OPHN1* mutation.
A-V.1 and V.5: axial (axial flair in V.5) and coronal T2, sagittal T1 weighted MRI of affected cousins from family A showing normal cerebellum and ventricles.
B-III.1 and III.2: axial T2, sagittal and coronal (inversion recovery) T2 weighted MRI of affected brothers from family B showing dilatation of lateral ventricles, prominent frontal horns, and hypoplasia of the caudate nuclei.
C-III.1: axial T2 and sagittal reconstruction T2 weighted MRI and coronal T1 showing dilatation of lateral ventricles. Note the absence of cerebellar hypoplasia.
D-II.1: axial flair, sagittal and coronal reconstrution T1 weighted MRI showing a normal cerebellum and no dilatation of lateral ventricules.

intolerance to frustration), treated with risperidone. Epilepsy started at age 6 and was treated with valproate. At age 8, he was able to name all the colours, to write his name and to count up to 10. He has difficulties in fine motor skills. Wechsler Non Verbal Scale of Ability revealed a low range score (72) and psychologic evaluation indicated attention deficiency and emotional immaturity. On clinical examination, the patient showed a right strabismus with asymmetric palpebral fissures and slight ataxia, but no facial dysmorphism (Fig. 2). Brain MRI showed normal cerebellum and posterior fossa (Fig. 3).

3. Molecular analysis

3.1. Methods

Blood samples were collected from affected individuals to perform X-chromosome Exome Sequencing (XES) in family A, whole exome sequencing (WES) in family C, and for epileptic encephalopathy multigene panel (150 genes) in families B and D. The molecular methods and bioinformatic pipeline for WES and XES have been previously described (Moortgat et al., 2016; Isidor et al., 2016). All variants in *OPHN1* (NM_002547.2) were validated using Sanger sequencing and cosegregated with the disease in all familial cases. Variants were submitted to Decipher data base (https://decipher.sanger.ac.uk/). Decipher accession numbers are listed in Supplementary Table S1.

In family A, total RNA was isolated from blood samples obtained from patient V.1 and controls, by using Maxwell 16 cell LEV total RNA purification Kit, following the manufacturer's protocol. cDNA was then amplified from total RNA by RT-PCR. Specific primers located respectively in exon 4 and 6 of the *OPHN1* gene, have been designed (sequences available on request). Polymerase chain reaction (PCR) amplification and sequencing reactions were performed by standard methods.

The X chromosome inactivation (XCI) assay relies on the polymorphic CAG_n repeat within the human *AR* gene. Methylation-sensitive restriction enzyme digestion is used to assess the relative methylation status of both chromosomes. X-inactivation was considered skewed (non random) if the ratio of the two alleles exceeded 80:20. XCI profiles were performed in peripheral blood leucocytes from patients IV.1, IV.2, III.1, III.3 in family A, from patient II.1 in family B and from patients II.1 and II.7 in family C (Table 1).

4. Results

We identified a total of four novel *OPHN1* variants, including a splice site variant (c.384 + 3A > C, p.Val105_Lys128del) in family A, a nonsense variant (c.697C > T, p.(Gln233*)) in family B and two missense variants (c.727C > T, p.(Arg243Trp) and c.1235G > A, p.(Gly412Asp)) in family C and D respectively. None of these variants were reported in public databases including dbSNP138, 1000 Genomes, Exome Variant Server (EVS) and Exome Aggregation Consortium (ExAC) Browser. In family A, the variant occurred in a splice-donor site in intron 5. cDNA analysis from blood of patient V.1 confirmed that the variant resulted in an abnormal but expressed RNA-product with skipping of exon 5, leading to an in-frame deletion of 24 amino acids (Supplementary Fig. 1; Table 1). In families C and D, we considered the two missense variants as likely deleterious. They affect amino acid residues that are highly conserved in the *OPHN1* orthologs of all vertebrate species and *Drosophila*. Physico-chemical properties between wild-type and mutated amino-acids are very different (high Grantham score, Supplementary Table S1). They are predicted to be « probably damaging » by different prediction algorithms (SIFT, MutationTaster, and PolyPhen-2) (Supplementary Table S1). In family C, the c.727 C > T, p.(Arg243Trp) variant was found in the 6 affected male members and co-segregated with the disease. In family D, the c.1235G > A, p.(Gly412Asp) variant is located in the Rho GTPase-activating domain, one of the three functional domain of the protein, and is present in a mosaic state with a constant level of mosaicism of 20% in blood, urine and buccal swab of the affected individual (Supplementary Table S1).

Unaffected heterozygous carrier females tested for XCI (patient II.1 in family B, and patients II.1 and II.7 in family C) presented with a random pattern of the X chromosome (Table 1). Mildly affected female patients in family A showed a skewed inactivation of the X chromosome for one (patient IV.1) and a random XCI for the other three (patient IV.2, III.1, III.3) (Table 1).

Table 1
Clinical and molecular features of patients with mutations in *OPHN1*, in our series and in previously reported patients.

		Our cohort							
		Family A				Family B			Family C
No. affected		1 (V.1)	1 (V.5)	2 (III.2, III.4)	4 (IV.1, IV.2, III.1, III.3)	1 (III.1)	1 (III.2)	1 (II.1)	6 (II.2, II.3, II.4, II.5,II.6, III.1)
Age (years)		13	9	62.66	31,36,55,57	15	10	40	50,49,48,45,42, 27
Gender		M	M	2 M	4 F	M	M	F	6 M
Mutation[1]		c.384+3A > C p.Val105_Lys128del				c.697C > T p.(Gln233*)			c.727C > T p.(Arg243Trp)
XCI in female patients/ carrier					skewed (1*, 81/19), random (3*)			(carrier: random (44/56))	
Facial dysmorphism		+	+	+	−	+	+	−	−
	long face					+			−
	deep set eyes	+	+	−	−	−	−	−	−
	prominent/pointed chin			+		+			−
ID									
	mild				4'+	+	dyspraxia, learning difficulties (IQ 92)	−	1−
	moderate	+	+	+	−	−	−	−	5+
	severe							−	
Neurological features									
	Speech difficulties	+	+	+	4 ±	+	+	−	−
	Strabismus	+	+	+		−	−	−	−
	Hypotonia	+	+			−	−	−	−
	Cerebellar signs (CS), dysmetria (D), ataxia (A), nystagmus (N), dysarthria (DA)	+ (DA, A, N)	+ (DA, A)	+		+	+	−	−
	Epilepsy	−	−	−	−	+	−	−	−
	Behavior trouble	+	+	+	−	+	+	−	−
Other	Hypogenitalism		+						
Brain MRI	Ventriculomegaly	−	−	NA	NA	+ (hypoplasia of caudate nuclei)	+	+	+
	Cerebellar hypoplasia	−	−	NA	NA	−	−	−	−

	Our cohort		Menten et al. (2007)	Zanni et al. (2005)				Bergmann et al. (2003)	
	Family C	Family D	A	A	B	C	D	A	
No. affected	2 (II.1, II.7)	1 (II.1)	1	1	6	4	3	5	2
Age (years)	50,49,48,45,42, 27	7	11	6	9,NA	27,4,4,NA	8,NA	21,20,13,5,2	42.15
Gender	2F	M	F	M	M	M	M	M	F
Mutation[1]	c.727C > T p.(Arg243Trp)	c.1235G > A; p.(Gly412Asp) mosaïc 20%	t(X;9)(q12;p13,3)	c.154+2T > C p.?	del Ex 16-17	c.556C > T p.(Gln186*)	c.1645C > T p.(Gln549*)	deletion of exon 19 (17.6kb)	
XCI in female patients/ carrier	(carrier: random (II.1, II.7))				(carrier: random)				NA
Facial dysmorphism	−	+	−	−	+	+	+	−	
									+
		+							

(*continued on next page*)

Table 1 (*continued*)

	Our cohort		Menten et al. (2007)	Zanni et al. (2005)				Bergmann et al. (2003)	
ID									
	−	+	+						− (IQ:75, learning difficulties)
	−			+	+	+	+	+	
	−				+	+	+	+	
Neurological features									
	−	+	+	+					
	−	+	+	+				+	+
	−	−	−	+				+	−
	−	−	+ (A)	+ (CS)	+ (5*) (D, N, DA)		+ (CS)	+ (A)	−
	−	+	+	−	+ (1*)	+ (3)	−	+	−
	−	+	−		+ (1*, ADHD)		+ (1: ADHD)		
Other						+ (2)		+	
Brain MRI	NA	−	+	+	+	NA	+	+	−
	NA	−	+	+	+	NA	+	+	−

	Tentler et al. (1999)	Bienvenu et al. (1997)	Billuart et al. (1998); des Portes et al. (2004)						Philip et al. (2003)
	A	A	A					A	B
No. affected	2	1	4	4	2	1	2	2	4
Age (years)	7.4	12	NA	18,14,4,NA	NA	13	22,NA	NA	NA
Gender	F	F	M	M	F	M	M	F	M
Mutation[1]	deletion of 1.1 Mb	t X;12)(q11;q15)	c.1579del p.(Ile527Serfs*6)					c.745_752dup p.(Lys251Asnfs*6)	c.184C > T p.(Gln62*)
XCI in female patients/ carrier	severely skewed (mother carrier: random)				random				
Facial dysmorphism		+	−					+	
				+	+	+	+ (1*)		+
				+	+	+			
				+	+	+	+		+
ID									
	+				+			+ (1*)	
	+	+	+	+ (IQ:46)					+ (IQ:50-60)
						+	+		
Neurological features									
	−	+	+	−	−	−	+ (1*)		+
	+	+		+			+ (1*)		
	+ (A, N)	+ (DA)					+ (1* A)		+ (2*, mild A)
	+	+	+ (3*)	−	−		+ (1*)		+ (2*)
						+			
Other			+ (1*)						+
Brain MRI	+	+	+	+	NA	NA	+		+
	+	−	+ (cerebellar dysgenesis)	+	NA	NA	+		+

	Chabrol et al. (2005)	Madrigal et al. (2008)	Al-Owain et al. (2011)	Froyen et al. (2007)	Tzschach et al. (2015)	Rocas et al. (2013)	Mignon-Ravix et al. (2014)	Pirozzi et al. (2011)	Santos-Reboucas et al. (2015)	Busa et al. (2017)
	A	A	A	A	A	A	A	A	A	A
No. affected	4	1	2	2	1	1	3	4	2	1

(*continued on next page*)

Table 1 (*continued*)

	Chabrol et al. (2005)	Madrigal et al. (2008)	Al-Owain et al. (2011)	Froyen et al. (2007)	Tzschach et al. (2015)	Rocas et al. (2013)	Mignon-Ravix et al. (2014)	Pirozzi et al. (2011)	Santos-Reboucas et al. (2015)	Busa et al. (2017)
Age (years)	19,17,15,2	10	27.31	NA	fœtus	3	20,33,40	8,4,33,18	32, 51	7
Gender	M	F	M	M	F	M	M	M	F	M
Mutation[1]	c.644_645del p.(Val215Glyfs*35)	deletion of exons 21 + 22	deletion of exons 7 to 15 (68kb)	deletion of exons 5 to 25 (0,4Mb)	c.1489C > T, p.(Arg497*)	deletion of exons 14 to 25	deletion of exons 13 to 15 (4kb), pI370_F427delfsX24	c.597 + 2_597 + 3del p.?	c.781_891del; deletion of exon 7	deletion of exons 3 to 15 (123kb)
XCI in female patients/ carrier		random	(carrier mother: random, 67/32)		skewed (100/0)	(carrier mother: random)			random (71/29); NINF	
Facial dysmorphism				NA	−					
	+		+							
	+		+			+		+	+	
	+		+					+	+	
ID										
		+ (1*)		+			+ (1*)	− (borderline), '+	+	− (learning difficulties)
	+ (3*)		+	+		+	+ (1*)			
	+ (1*)		+				+ (1*)	+ (2*)	+	
Neurological features										
	+		+			+	+ (2*)	+ (3*)	+ (1*)	+
	+	"+	+	+			+	+ (3*)	+ (1*)	
	+					+		+ (3*)	+ (1*)	
	"−		+ (A)			+ (1*,A)	+ (3*, A)	+ (1*,A)		
	+	+				+	+	+ (2*)	−	−
	+ (3*,ADHD; 1*, autistic features)	+ (ADHD)	+ (1* autistic features)					+ (3*,autistic features; 1*, ADHD)	+ (1*,A; 2*, ADHD)	+
Other			+	+				(+ (1*)		
Brain MRI	+ (3*)		NA	+		+		+	−	+
	+	"+	NA	+	+	+		+	−	− (retrocerebellar arachnoid cyst)

Blank indicated not available, not applicable or not measured; [1] Mutations are reported according HGVS nomenclature, RefSeq *OPHN1:* NM_002547.2. *: the number of affected patients with the clinical feature.Abbreviations: ADHD, attention deficit, hyperactivity disorder; F, female; M, male; NA, not available; NINF, not informative XCI, X chromosome inactivation.

5. Discussion

Since 1998, *OPHN1* mutations have been identified in 49 male and 14 female patients from 16 unrelated families and 5 sporadic cases, with XLID or ID and cerebellar hypoplasia (Billuart et al., 1998; Santos-Reboucas et al., 2015; Busa et al., 2017; Pirozzi et al., 2011; Mignon-Ravix et al., 2014; Rocas et al., 2013; Tzschach et al., 2015; Froyen et al., 2007; Al-Owain et al., 2011; Madrigal et al., 2008; Chabrol et al., 2005; Philip et al., 2003; des Portes et al., 2004; Tentler et al., 1999; Bergmann et al., 2003; Zanni et al., 2005; Menten et al., 2007; Bienvenu et al., 1997) (Table 1). In this study, we report four novel mutations in four unrelated families with XLID or ID but without cerebellar anomaly.

All but one published male patients showed mild to severe intellectual disability with developmental and speech delay, also reported in all but one of our patients (Family B, II.2). Additional clinical features include seizures (42%) and behavioral disorders such as hyperactivity (40%), which are present in 2 and 6 out of 13 male individuals in our cohort respectively. Strabismus is present in 90% of previously reported patients and in 30% of affected individuals in our cohort. Ataxia is less frequent but unsteady gait, nystagmus and dysmetria are recurrent signs which could reflect a cerebellar dysfunction. Hypogenitalism including scrotal hypoplasia, cryptorchidism or micropenis are noted in 35% of patients whereas we observed cryptorchidism in only one of our patients (Family A, V.5). Dysmorphic features are often described in individuals with *OPHN1* mutation, consisting of a long face with deep-set eyes and marked infra-orbital creases, a long nose and a prominent chin (Santos-Reboucas et al., 2015; Pirozzi et al., 2011; Al-Owain et al., 2011). However, these characteristics cannot be related to a specifically recognizable clinical entity as it has been initially suggested, and absence of dysmorphism was previously reported (Billuart et al., 1998; Busa et al., 2017; Rocas et al., 2013; des Portes et al., 2004; Zanni et al., 2005; Menten et al., 2007). In our cohort, the more severely affected patients (family A) presented some of these reported facial features but affected individuals from family C, and family D did not (Fig. 2).

The phenotypic variability is also observed in female patients. Carrier females are usually described as asymptomatic, which could be attributed to protective skewed inactivation of their affected X chromosome. This hypothesis is strengthened by the fact that a random pattern of X chromosome inactivation is observed in most of the affected female patients, usually with mild ID as in our cohort (Al-Owain et al., 2011; Philip et al., 2003; Tentler et al., 1999; Zanni et al., 2005), but occasionally with severe symptoms (Santos-Reboucas et al., 2015) (Table 1). The large phenotypic variability in females might reflect that the inactivation patterns measured in the peripheral blood may not be representative for other tissues, in particular for the brain. Moreover, it is now well-known that about 10% of X-linked genes show variable patterns of inactivation and are expressed to different extents from some inactive X chromosomes (Carrel and Willard, 2005). Interestingly, we noted that 1 out of 14 previously reported female patients with a severe phenotype, including moderate to severe ID, dysmorphic features and cerebellar signs, showed an extremely skewed X-inactivation pattern (Tentler et al., 1999). We could hypothesize that this severely affected female expressed her X mutated allele. However, expression studies to identify the inactive X chromosome were lacking in this publication (Tentler et al., 1999).

On brain MRI, the most striking hallmark shared by reported patients is the cerebellar hypoplasia, reported in more than 95% of male patients and in 50% of female patients with *OPHN1* mutations. Different degrees of cerebellar dysgenesis are observed, predominantly in the lower vermis. Cerebellar hemispheres are variably affected from dilatation of the cisterna magna to retrocerebellar cyst (Busa et al., 2017; Chabrol et al., 2005; des Portes et al., 2004). Supratentorially, slight to severe dilatation of lateral ventricles was found in 90% of the reported male patients, as observed in family B and C of our cohort. Global reduction of the cerebral volume predominating in the frontal lobes and hypoplasia of the head of the caudate nuclei, as seen in family B, were occasionally reported (des Portes et al., 2004; Bergmann et al., 2003). Hippocampal alterations were recently described in one family (Santos-Reboucas et al., 2015). Up to now, cerebellar anomalies associated with ID led the clinician to *OPHN1* sequencing. The next generation technologies brought us to identify *OPHN1* mutations in patients with minimal or atypical symptoms, even without cerebellar anomalies as observed in our cohort. For families A, B and D, we cannot exclude a very slowly progressive cerebellar hypoplasia, not yet detectable during childhood.

Molecularly, since the initial balanced t(X; 12) translocation reported in 1998, 20 *OPHN1* mutations have been reported: 10 intragenic deletions, 4 nonsense mutations, 3 splice site mutations, 2 frameshift mutations and 1 balanced translocation t(X; 9) (Billuart et al., 1998; Santos-Reboucas et al., 2015; Busa et al., 2017; Pirozzi et al., 2011; Mignon-Ravix et al., 2014; Rocas et al., 2013; Tzschach et al., 2015; Froyen et al., 2007; Al-Owain et al., 2011; Madrigal et al., 2008; Chabrol et al., 2005; Philip et al., 2003; des Portes et al., 2004; Tentler et al., 1999; Bergmann et al., 2003; Zanni et al., 2005; Menten et al., 2007; Bienvenu et al., 1997). These mutations likely result in loss of oligophrenin 1 function. There is no obvious correlation between the severity of the disease and the type of mutation. Here, we report 4 novel mutations including one splice site mutation (c.384 + 3A > C, p.Val105_Lys128del) resulting in skipping of exon 5, one nonsense mutation (c.697C > T, p.(Gln233*)), and two missense mutations (c.1235G > A, p.(Gly412Asp); c.727 C > T, p.(Arg243Trp)). The two missense variants affect highly conserved amino acid residues and are predicted to be « probably damaging » by various prediction algorithms (Supplementary Table S1). Of note, from the ExAC database the number of missense variants in *OPHN1* is smaller (106) than expected (166), and they have calculated a z-score of 2.28, which is significant and suggest such missense variants could be deleterious. In the literature, non-recurrent missense variants in *OPHN1* were identified in patients with autism or schizophrenia (Piton et al., 2011) or in patients with ID but without accurate phenotypic information (Hu et al., 2016; Tarpey et al., 2009; Classen et al., 2013). Our report highlights that missense mutations in *OPHN1* can be observed in patients with mild to moderate ID associated with seizures, strabismus, cerebellar signs or ventriculomegaly (Families C and D). In patient III.1 of family D, the missense mutation was present in a mosaic state with a level of 20% in the various analyzed tissues (blood, urine and buccal swab). To our knowledge, this is the first report of a patient presenting with an *OPHN1* mosaic mutation. The mosaicism is not correlated with a milder phenotype as the patient presents with moderate ID, behavioral troubles, epilepsy and strabismus. However, the mosaic levels in peripheral tissues might not reflect the brain pattern.

In conclusion, we describe four novel *OPHN1* mutations in three unrelated families and one sporadic patient with ID, mild or no facial dysmorphism, and absence of cerebellar anomaly. The new technologies (whole or X-exome sequencing and target multigene panels) allowed us to expand the phenotypic spectrum of *OPHN1* mutation in males and females.

Conflicts of interest

All authors state that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmg.2018.03.002.

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