




SHORT REPORT

Expanding the clinical spectrum associated with PACS2 mutations

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Whole exome sequencing (WES) has led to the understanding of the molecular events affecting neurodevelopment in an extremely diverse clinical context, including diseases with intellectual disability (ID) associated with variable central nervous system (CNS) malformations, and developmental and epileptic encephalopathies (DEEs). Recently, *PACS2* mutations have been causally linked to a DEE with cerebellar dysgenesis and facial dysmorphism. All known patients presented with a recurrent de novo missense mutation, c.625G>A (p.Glu209Lys). Here, we report on a 7-year-old boy with DEE, cerebellar dysgenesis, facial dysmorphism and postnatal growth delay, apparently not fitting with any recognized disorder. WES disclosed a de novo novel missense *PACS2* variant, c.631G>A (p.Glu211Lys), as the molecular cause of this complex phenotype. We provide a detailed clinical characterization of this patient, and analyse the available clinical data of individuals with *PACS2* mutations to delineate more accurately the clinical spectrum associated with this recently described syndrome. Our study expands the clinical and molecular spectrum of *PACS2* mutations. Overview of the available clinical data allow to delineate the condition associated with *PACS2* mutations as a variable trait, in which the key features are represented by moderate to severe ID, cerebellar dysgenesis and other CNS malformations, reduced growth, and facial dysmorphism.

KEYWORDS

cerebellar dysgenesis, developmental and epileptic encephalopathy, facial dysmorphism, growth deficiency, *PACS2*

1 | INTRODUCTION

Latest advances in genome analysis have significantly enabled the discovery of molecular defects underlying neurodevelopmental disorders (NDDs), a clinically heterogeneous group of rare diseases presenting with central nervous system (CNS) malformations often associated with developmental and epileptic encephalopathies (DEEs).^{1–6} Among the genes that have recently been implicated in DEEs, *PACS1* (phosphofurin acid cluster sortin protein 1, OMIM 607492), encoding a *trans*-Golgi-membrane traffic regulator highly expressed during human embryonic brain development, has been implicated in Schuurs-Hoeijmakers syndrome (OMIM 615009), a dominantly inherited DEE

with recognizable gestalt and brain structural abnormalities.⁷ So far, all reported patients share de novo missense changes affecting the same amino acid (p.Arg203Trp; p.Arg203Gln).^{7–10} Arg203 is highly conserved among orthologs, as well as in the paralog, *PACS2* (OMIM 610423). Remarkably, a recurrent de novo heterozygous missense variant in *PACS2* was more recently found in 14 unrelated individuals presenting with an NDD partially overlapping with the *PACS1*-related phenotype.¹¹ All patients presented with a recurrent missense mutation, c.625G>A (p.Glu209Lys), and had early-onset epilepsy with focal and generalized seizures, global developmental delay and autistic features, cerebellar dysgenesis and dysmorphic facial appearance.

Here we report on a child with DEE, in whom WES analysis disclosed a novel de novo pathogenic missense variant of *PACS2* involving

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the same functional domain that was previously reported in other affected individuals. Our findings expand the phenotypic spectrum of this novel disease and confirm the occurrence of a mutational hot-spot in PACS2-associated DEE.

2 | CASE REPORT

The 7-year-old male was the only child of non-consanguineous parents. Family history was unremarkable. Pregnancy was uneventful until the 35th week, when an ultrasound scan disclosed bilateral ventriculomegaly. Delivery was at 38 weeks, by Caesarian section because of breech presentation. Birth weight was 3040 g (25-50th centile), length 49 cm (25th centile), and occipito-frontal circumference (OFC) 35.5 cm (75th centile). At 3 days of life, respiratory distress and epilepsy were recorded, with seizures characterized by impairment of consciousness and upward rolling of the eyes. An electroencephalogram (EEG) at onset showed epileptiform abnormalities in both central regions. Phenobarbital was started with seizures remission, but reduction of drug after 1 month of treatment caused reappearance of seizures characterized by left upper limb hypertonia and blank staring followed by inconsolable crying. Therefore, vigabatrin and valproic acid were started with good control of the seizures (only one episode at age 13 months). Since no other episodes were further referred, both drugs were gradually discontinued at 6 years. Ophthalmologic evaluation and echocardiogram were normal. Brain magnetic resonance imaging (MRI), at age 27 months and at age 3 years and 10 months (Figure 1D-G), revealed an abnormal cerebellar foliation pattern along the basal and posterior portions of the right cerebellum hemisphere, quantitative reduction of the parietal region white matter in the supratentorial region, hyperintensity of the centrum semiovale in the frontal region, bilateral posterior periventricular white matter reduction and lateral ventricles asymmetry with prevalence of the right posterior horn. Global motor skills were delayed. He walked unsupported at 2 years and at 7 years, verbal speech was limited to a few words and he required assistance in hygiene and dressing. He still presented problems with chewing and dysphagia. Cognitive assessment (WISC-IV scale) revealed a total IQ of 47 (processing speed, 50; working memory, 64; perceptual reasoning, 67; verbal comprehension, 58). Because of a reduced growth velocity, growth hormone (GH) stimulation testing with clonidine and arginine was performed at 6 years, which revealed a GH peak of 4.09 and 2.40 ng/mL, respectively. Thus, GH treatment was started at 6 years because of a reduced growth velocity (Saizen, 0.5 mg for 6 days/week). At age 7, endocrinology work up revealed thyroid stimulating hormone: 2.78 μ IU/mL (n.v.: 0.64-6.27), free T4: 1.12 ng/dL (n.v.: 0.89-1.76) and insulin-like growth factor 1: 63.4 ng/mL (n.v. 55-222). Brain MRI revealed no pituitary abnormalities and last EEG were normal.

At the last evaluation (7 years), growth parameters disclosed short stature (105 cm, -3.2 SD), low weight (18.5 kg, -1.9 SD), and relative macrocephaly (OFC: 51.5 cm, 50-75th centile). Physical examination disclosed a distinctive facial gestalt with features and signs that remained unchanged over the time, including synophrys, highly arched and sparse broad eyebrows, long eyelashes, low-set

and posteriorly rotated ears with uplifted lobe, broad nasal tip, smooth philtrum, thin and everted upper lip vermilion, downturned corners of the mouth, widely spaced teeth, and chin with horizontal crease (Figure 1).

Metabolic screening, including urine determination of glycosaminoglycans and screening for inborn errors of amino acid metabolism, were normal. Genetic analyses, including karyotype, high resolution CGH-array (44K; Agilent Technologies, Waldbronn, Germany), and epilepsy- and cohesinopathy-related gene panels testing (Supporting Information File S1) were negative. WES workflow and analysis were performed as detailed reported in the supplemental methods.

3 | RESULTS

Written informed consents for the use of photographs and research findings were obtained from the family.

Data analysis, variant filtering and prioritization allowed to identify the de novo missense variant, c.631G>A (p.Glu211Lys) in exon 6 of PACS2 (NM_001100913.2) as the molecular event underlying the condition. De novo occurrence of this missense change was confirmed by Sanger sequencing (Figure 2). The variant was private, being not reported in ExAC, ClinVar, and *in-house* databases. The affected residue, Glu211, is conserved in PACS2 orthologs and its paralog, PACS1 (Figure 2).

4 | DISCUSSION

Recently, a recurrent de novo missense variant, c.625G>A (p.Glu209Lys) in PACS2 was reported in 14 unrelated individuals with epilepsy, developmental delay, cerebellar dysgenesis and dysmorphisms. Here, we report on an additional patient with a different de novo PACS2 mutation, and revise the phenotype associated with this PACS2 mutation (Table 1).

Differential diagnosis of cerebellar dysgenesis includes disorders associated with OPHN1 and GNAO1 mutations (MIM #300486 and MIM #617493). PACS2 is a multifunctional protein mainly expressed in the brain, including cerebellum (GTEx, <https://gtexportal.org/home/>, and FANTOM5, <http://fantom.gsc.riken.jp/5/index.html>), that acts as a traffic modulator by controlling the endoplasmic reticulum-mitochondrial communication, with nuclear gene expression.¹² Olson et al suggested that the disease-causing p.Glu209Lys substitution alters the ability of the autoregulatory domain to modulate the interaction of PACS2 with client proteins participating in processes related to neuronal development and function.¹¹ Of note, the Glu211 is located within the autoregulatory domain of PACS2 and is close to the residue that was previously reported to be invariantly affected in the disorder.¹¹ Our finding further confirms the relevance of this region in the control of PACS2 function (Figure 2).

The clinical characteristics of the affected individuals carrying the recurrent p.Glu209Lys mutation, included neonatal/early-infantile onset epilepsy, global developmental delay with or without autism, cerebellar dysgenesis and facial dysmorphisms. In the majority of these individuals, facial features suggested no specific diagnosis prior to WES. Major craniofacial findings included

TABLE 1 Clinical features of individuals with PACS2 p.Glu209Lys and our proband with a novel de novo missense mutation p.Glu211Lys

Olson et al ¹¹															
Clinical features	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	Our patient
Sex	F	F	M	F	M	M	M	F	F	M	M	F	F	F	M
Gestational age (wk)	38	37	38	35	37	40	term	37	term	term	39.5	34	33	39	38
Birth parameters															
Weight (SD)	-0.3	Median	+2	+1.8	-1.7	+2.5SD	+1SD	-3SD	Median	NA	Median	+0.5	-0.5	+0.6	3040
Length (SD)	+0.6 SD	NA	NA	NA	-0.9 SD	+2 SD	+1SD	-1.5SD	-2SD	NA	-1SD	-1.5SD	Median	+0.9SD	49
OFC (SD)	+0.4 SD	NA	NA	NA	-2.3 SD	>3SD	median	-2.3 SD	median	NA	median	NA	median	-0.1SD	35.5
Growth at last evaluation/y	Normal/16 y	NR/4 y	Normal/15 y	Normal/8 y	Height: -1.8SD/19 mo	NR/8 y	Normal/16 mo	NR/5 y	NR/3 y	NR/7 y	Normal/12.5 y	NR/9 mo	NR/3.5 y	NR/5.5 y	-3.20 SD/7 y GHD
Facial appearance															
Synophrys	Yes	Yes	Yes	Yes	No	No	No	No	Yes	Mildly dysmorphic	No	No	No	No	Yes
Hypertelorism	Yes	Yes	Yes	Yes	No	No	No	Yes	No		No	Yes	Yes	No	Yes
Down slanting palpebral fissures	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No		No	No	Yes	No	Yes
Broad nasal root	No	Yes	No	Yes	Yes	No	Yes	Yes	Yes		Yes	Yes	Yes	Yes	Yes
Thin Vermilion	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes		Yes	Yes	Yes	Yes	Yes
Upper lip															
Wide mouth with downturned corners	Yes	Yes	Yes	Yes	Yes/No	No	No	Yes	No		Yes	Yes	Yes	No	Yes
Prominent incisors	Yes	No	Yes	Yes	NA	No	NA	No	No		No	NA	NA	No	No
Widely spaced teeth	Yes	No	Yes	No	NA	No	NA	No	No		Yes	NA	NA	No	Yes
Everted vermillion of lower lip	Yes	No	Yes	No	No	Yes	No	No	No		No	No	Yes	Yes	Yes
CHD		DC				VSD							ASD		No
Ocular	Myopia	No		SB	SB	SB, myopia									No
Genitourinary			Cryptorchidism	SB		Testis ectopia	Cryptorchidism				Micropenis, cryptorchidism, CPP				No
Skeletal/distal limb anomalies															
		No	2-3 toes syndactyly	No	V-clinodactyly, STPC	Finger pads	No	No	No	Bilateral STPC	V-finger brachy/clinodactyly	V-clinodactyly	No	STPC, V-clinodactyly	No
	Metatarsus varus, slender fingers			Anaemia	Anaemia	NA	Anaemia	NA	NA	NA	NA	NA	NA	NA	No
Developmental features															
Walking age	22 mo	NA	18 mo	18 mo	18 mo	22 mo	Not walking	27 mo	Not walking	24 mo	24 mo	NA	36 mo	36 mo	24 mo
Speech delay	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
DD/ID	Yes	Yes	Yes	Yes	Yes	Yes	Mild	Yes	Yes	Yes	Yes	Yes	Mild	Mild	Moderate

TABLE 1 (Continued)

Olson et al ¹¹															
Clinical features	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	Our patient
Neurological															
Hypotonia	Yes	NA	NA	NA	No	no	axial	No	diffuse	diffuse	No	Yes	axial	diffuse	No
Nystagmus	No	Yes	No	No	Yes	No	No	No	No	No	transient	No	No	No	No
Stereotypies	No	transient	No	No	No	Yes	No	No	Yes	Yes	No	transient	Yes	No	
Behavioural features	No	Yes	No	Mild autistic	No	COD	NO	Atypical social	Atypical social	Autism spectrum disorder	Autism spectrum disorder	No	No	Selective mutism	Transient hyperactivity
Epilepsy															
Age of onset (d)	6 d	4 d	4 d	7 d	2 d	2 d	2 d	2 wk	2 d	1-2 mo	1 d	3 d	2 wk	3 d	3 d
Seizure types	Focal	GTCs	NA	GTCs	Clonic and GTC	NA	Focal with tonic stiffening and autonomic features, later clonic	Focal, later tonic	Focal, tonic-clonic and myoclonic, later GTCs and generalized tonic	Clonic seizure with eye deviation, later GTC	Focal (stopped at 2 mo), later GTCs	Focal tonic-clonic status epilepticus	Focal tonic-clonic, later focal or generalized	Tonic, later tonic or GTCs	Focal neonatal epilepsy stopped at 13 mo
Age at last seizure	NA	6 mo	NA	2 y ^a	9 mo	3 mo	NA	2 y	2 y	2 y	NA	NA	NA	NA	6 y
EEG features	NA	Neonatal to 3.5 mo: normal background; 1 y: normal	NA	Neonatal excess multifocal sharp waves/generalized bursts of epileptic activity	4 mo: generalized slowing with MF sharp waves and frequent focal seizures	Neonatal: left temporal spikes; 3.5 year: left paroxysmal temporal rolandic spikes, generalized slowing	6-7 wk: MF epileptiform activity; 9 mo: normal	6 wk: subtle aberration; R fronto-central and L temporal	Neonatal: excess discontinuity, excess MF sharp waves; 2 y: intermittent generalized spikes, slowing, intermittent L temporal slowing	4 months: normal; 17 months: rare generalized spikes	Neonatal: epileptic discharges, L rolandic region	Neonatal: excess MF spikes and sharp waves; 2 mo: background; poorly organized; high amplitude; background, lack of state change, MF spikes	6 d: normal; neonatal/infantile: MF epileptiform activity, high amplitude slow spikes bilateral temporal; 17 mo: diphasic spikes at vertex	Neonatal: excessive L and R central and temporal waves; 3 y: mild generalized slowing, frequent Left temporal epileptiform	Focal neonatal epilepsy; 3 y sporadic focal anomalies; at 7 y: normal
Brain MRI (age)															
Foliar distortion of the left cerebellar hemisphere, MCM ^a (5 y)	IVH with prominent foramen and cisterna magna, severe foliar distortion of cerebellar hemispheres with centrifugal orientation, fusion anomaly (5 y)	Increased sub-rachnoid spaces	Mild IVH with a patulous foramen Magendie and mega cisterna magna, mild distortion of the cerebellar folia (3 wk)	Retrocerebellar arachnoidal cyst, IVH with prominent foramen Magendie, MCM, severe foliar cerebellum distortion with centrifugal orientation, fusion anomaly (7 d)	Normal ^a (10 d); normal ^a (4 mo)	IVH, left retrocerebellar cyst, causing distortion of the smaller left cerebellar hemisphere and thinning of the overlying bones ^a (2 mo)	Normal (birth)	Mega cisterna magna and patulous foramen Magendie, subtle cerebellar foliar distortion hypothalamic fusion anomaly (1 wk)	Mega cisterna magna, patulous foramen Magendie (1 mo)	Thick corpus callosum, inferior vermis hypoplasia ^a (12.5 y)	MCM, severe foliar distortion with centrifugal orientation (3 mo)	Moderate cerebellar foliar distortion (23 mo)	Mild scattered subarachnoid haemorrhage (2 mo); Prominent MCM and patulous foramen Magendie with subtle foliar distortion (31 mo)	Abnormal cerebellar foliation orientation of the right cerebellum MCM and hemisphere, Iso-liquoral cyst on the right lateral ventricle	

TABLE 1 (Continued)

Olson et al ¹¹															Our patient	
Clinical features	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14		
Additional clinical features																
							1mc café au lait spots, poor feeding, CHI	None	Dysphasia, accessory nipples	None	Hypertrophic pyloric stenosis, velopharyngeal hypotonia	Brachycephaly, inverted nipples	Sacral pit			

Abbreviations: ASD, atrial septal defect; CHI, conductive hearing impairment; CPP, central precocious puberty; DC, dextrocardia; EEG, electroencephalogram; F, female; IVH, inferior vermillion hypoplasia; m, median; M, male; MCM, mega cisterna magna; MF, multifocal; NA, not available; OCD, obsessive compulsive disorder; SB, strabismus; STPC, single transverse palmar crease; VSD, ventricular septal defect.

^a Immediate recurrence after withdrawal of valproate.

appear generally within normal ranges prenatally, as well as postnatally, notwithstanding the limited availability of data. By contrast, the present patient presented with postnatal growth delay that became evident at age 5, when GH treatment was started. WES analysis did not reveal any putative functionally relevant variant linked to processes controlling growth, suggesting that postnatal growth failure may represent part of the phenotypic spectrum of this disorder.

Six of 15 individuals, including the present case, were reported to have epilepsy. While details on the nature of epilepsy in this cohort are limited, it appears to have generally a neonatal/early-infantile onset, with 12/14 of cases having seizures during the first week of life, and often challenging to control in infancy. Consistently, our patient epilepsy manifested at 3 days of life and it was difficult to control in the first 30 days, which however was followed by a seizure-free period that still persists until now, even after the discontinuation of treatment. CNS malformations appear to be a recurrent feature of subjects with PACS2 mutations, described in 12/14 individuals, 10/14 of which presenting with cerebellar involvement.¹¹ In the present case, abnormal cerebellar foliation orientation along the basal and posterior portions of the right cerebellum hemisphere was found, together with a quantitative reduction of the white matter of the parietal region and lateral ventricular asymmetry.

Other defects are less common, including congenital heart disease (3/15 patients), ocular abnormalities with myopia and strabismus, and mild distal limb anomalies, including fifth finger brachyclinodactyly.

In conclusion, the present patient confirms that de novo PACS2 mutations underlie a complex disorder characterized by developmental and epileptic encephalopathy with mild to moderate ID, variable CNS malformations with cerebellar dysgenesis, and facial dysmorphism. Reduced postnatal growth with GH deficiency could be an additional feature. Further studies are required for delineating the natural history of this newly recognized syndrome.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

Ethics approval

The study follows the principles outlined in the Helsinki Declaration and the patient's family gave written informed consent for molecular study and publication.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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