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Pontocerebellar hypoplasia type 1 for the neuropediatrician: genotype-phenotype correlations and diagnostic guidelines based on new cases and overview of the literature

Author 1: I. Ivanov *

Department of Pediatrics, Medical University - Plovdiv, Bulgaria
ivanovist@gmail.com

Author 2: D. Atkinson*

VIB Center for Molecular Neurology, University of Antwerp, Belgium
derek.atkinson@molgen.vib-ua.be

*- equally contributing first authors

Author 3: I. Litvinenko

Department of Pediatrics, Medical University - Sofia, Bulgaria
ilitvinenko@excite.com

Author 4: L. Angelova

Department of Medical Genetics, University Hospital "St. Marina", Varna, Bulgaria
lyudmila_angelova@abv.bg

Author 5: S. Andonova

National Genetic Laboratory, Maichin Dom University Hospital, Sofia, Bulgaria
sandonova@netscape.net

Author 6: H. Mumdjiev

Department of Neonatology, Prof. Stoyan Kirkovich University Hospital, Stara Zagora, Bulgaria
hristom2000@yahoo.com

Author 7: I. Pacheva

Department of Pediatrics, Medical University - Plovdiv, Bulgaria
inapatcheva@hotmail.com

Author 8: M. Panova

Department of Pediatrics, Medical University - Plovdiv, Bulgaria
panova_marg@mail.bg

Author 9: R. Yordanova

Department of Pediatrics, Medical University - Plovdiv, Bulgaria
ralitsa_iordanova@yahoo.com

Author 10: V. Belovejdov

Department of Pathology, Medical University - Plovdiv, Bulgaria
vesbel@abv.bg

Author 11: A. Petrova.

Department of Radiology, Medical University - Plovdiv, Bulgaria
petrova_ar@abv.bg

Author 12: M. Bosheva
Department of Pediatrics, Medical University - Plovdiv, Bulgaria
bosheva@mail.bg

Author 13: T. Shmilev
Department of Pediatrics, Medical University - Plovdiv, Bulgaria
t.shmilev@gmail.bg

Author 14: A. Savov
National Genetic Laboratory, Maichin Dom University Hospital, Sofia, Bulgaria
alexey.savov@abv.bg

Author 15: A. Jordanova
VIB Center for Molecular Neurology, University of Antwerp, Belgium
Molecular Medicine Center, Department of Medical Chemistry and Biochemistry, Medical
University-Sofia, Sofia, Bulgaria
albena.jordanova@molgen.vib-ua.be

Corresponding authors:

Prof. Ivan Ivanov
Department of Pediatrics, Medical University - Plovdiv, Plovdiv, Bulgaria

Prof. Albena Jordanova
VIB Center for Molecular Neurology, University of Antwerp, Belgium

Abstract

Pontocerebellar hypoplasia type 1 (PCH1) is a major cause of non-5q spinal muscular atrophy (SMA). We screened 128 *SMN1*-negative SMA patients from Bulgaria for a frequent mutation -p.G31A in *EXOSC3*, and performed a literature review of all genetically verified PCH1 cases.

Homozygous p.G31A/*EXOSC3* mutation was identified in 14 Roma patients, representing three fourths of all our *SMN1*-negative Roma SMA cases. The phenotype of the p.G31A/*EXOSC3* homozygotes was compared to the clinical presentation of all reported to date genetically verified PCH1 cases. Signs of antenatal onset of disease present at birth were common in all PCH1 sub-types except in the homozygous p.D132A/*EXOSC3* patients. The PCH1 sub-types with early death (between ages 1 day and 17 months), seen in patients with p.G31A/*EXOSC3* or *SLC25A46* mutations have a SMA type 1-like clinical presentation but with global developmental delay, visual and hearing impairment, with or without microcephaly, nystagmus and optic atrophy. Mutations with milder presentation (homozygous p.D132A/*EXOSC3* or *VRK1*) may display additionally signs of upper motor neuron impairment, dystonia or ataxia and die at age between 5 and 18 years. Other *EXOSC3* mutations and *EXOSC8* cases are intermediate - SMA type 1-like presentation, spasticity (mostly in *EXOSC8*) and death between 3 months and 5 years. There is no correlation between neurological onset and duration of life. We add marble-like skin and congenital laryngeal stridor as features of PCH1. We show that imaging signs of cerebellar and pontine hypoplasia may be missing early in infancy. EMG signs of anterior horn neuronopathy may be missing in PCH1 patients with *SLC25A46* mutations. Thus, there is considerable phenotypic variability in PCH1, with some cases being more SMA-like, than PCH-like. Detailed clinical evaluation and ethnicity background may guide genetic testing and subsequent genetic counseling.

Highlights

- Pontocerebellar hypoplasia type 1
- *SMN1*-negative spinal muscular atrophy
- Clinical and genetic heterogeneity
- Roma population

Keywords

Pontocerebellar Hypoplasia, Spinal Muscular Atrophy, *EXOSC3*, Roma population

1.Introduction

Pontocerebellar hypoplasia type 1 (PCH1) is a major entity in the differential diagnosis of spinal muscular atrophy (SMA)⁽¹⁾. It combines progressive cerebellar and brainstem lesions with degeneration of motor neurons in the anterior spinal horn⁽²⁾. PCH1 patients present a broad phenotypic spectrum, ranging from neonatal death to survival into puberty and displaying various combinations of lower and upper motor neuron signs, extrapyramidal signs, ataxia, visual and hearing impairment, seizures, microcephaly, global developmental delay and contractures⁽¹⁻⁴⁾. Impairment of RNA processing and protein synthesis are at the basis of SMA and PCH1 in particular^(5, 6). Secondary mitochondrial dysfunction is proposed as an additional etiological factor in few cases⁽⁷⁾.

More than half of the PCH1 cases are caused by mutations of the *EXOSC3* gene⁽³⁾ and are marked as PCH1B. The gene encodes subunit 3 of the human exosome complex - a major RNA processing machinery in cells⁽⁶⁾. There are at least 11 mutations of the *EXOSC3* gene reported so far⁽⁸⁾. The null mutations (frameshift and others) have a severe presentation in heterozygous state and are supposed to be lethal in homozygous state⁽⁹⁾. The missense mutations are usually hypomorphic and their presentation is variable⁽⁹⁾.

According to S. Rudnik-Schöneborn *et al.* (2013) who analyzed 10 families with bi-allelic *EXOSC3* mutations, p.D132A (c.395A>C) is the most common mutation, accounting for 55% of all pathogenic alleles. Patients homozygous for this missense mutation present with milder phenotype that includes also upper motor neuron and extrapyramidal signs⁽⁴⁾. Compound heterozygosity of this with another missense mutation p.V80F (c.238G>T) in one family was found to cause an even milder than PCH1 phenotype that is compatible with hereditary spastic paraparesis⁽¹⁰⁾. Similarly, homozygosity for the *EXOSC3* mutation p.G191C (c.571G>T), was discovered in another family with hereditary spastic paraparesis⁽¹¹⁾.

The p.G31A (c.92G>C) missense mutation in *EXOSC3* was first described by J. Wan *et al.*, (2012) and only 12 homozygous patients are reported in the available literature so far^(3, 4, 12-14). The phenotype of the homozygous and heterozygous cases is severe with rapid progression and early death⁽⁴⁾. J. Schwabova *et al.* (2013) revealed that this is a founder mutation among the Roma population in Czechoslovakia. Homozygosity for other missense mutations (excluding the cited above with mild phenotypes) or compound heterozygosity with the p.D132A mutation are found also to cause severe phenotype^(3, 4).

Other causes of PCH1 are mutations in the *EXOSC8* gene (classified as PCH1C), found in 22 patients, belonging to two Roma and one Palestinian families, as well as in *VRK1* gene (PCH1A) in 7 patients, belonging to one Jewish and one Iranian families^(15, 16). Recently, J. Wan *et al.* (2016), T. van Dijk *et al.* (2017) and M. C. Braunisch *et al.* (2017) reported that mutations in the *SLC25A46* gene may cause very severe congenital PCH1 with death soon after birth⁽¹⁷⁻¹⁹⁾. Although several genetic studies on the cause of PCH1 have been published so far, there is no head-to-head comparison of the phenotypes of all PCH1 cases that might help everyday work of the neuropaediatrician.

Cerebellar hypoplasia with variable affection of the pons is mandatory for diagnosis of PCH^(2, 4). All of the above cited investigations searched for mutations in pools of patients with clinical and radiological or pathological diagnosis of PCH1. We could not find studies for PCH1 among *SMN1*-negative SMA patients.

Aiming to test the frequency and clinical presentation of the common p.G31A/*EXOSC3* mutation, we screened a group of 128 *SMN1*-negative SMA cases. We identified 14 p.G31A/*EXOSC3* homozygous individuals, all of Roma origin, who represented the world-largest cohort of patients with this mutation. Additionally, we present a comparative comprehensive and up-to-date review of the clinical characteristics of all reported genetically verified PCH1 patients that might guide the clinical and genetic workup.

2. Materials and methods

2.1 Cohort description

Among the patients referred to the National Genetics Laboratory of Bulgaria at the Medical University-Sofia a cohort of *SMN1*-negative SMA patients was selected using the following inclusion criteria: chronic progressive disorder; muscle hypotonia, weakness and atrophy, starting in infancy or childhood; absent or diminished tendon reflexes; neurogenic EMG with normal conduction velocities; neurogenic atrophy on muscle biopsy; no *SMN1* gene deletion⁽²⁰⁾. The patients that satisfied these criteria were 128. All of them were Bulgarian citizens but with variable ethnic origin. Nineteen of them were of Roma ethnicity. In 126 (98.5%) individuals, the clinical picture was dominated by proximal muscle weakness, whereas in 2 (1.5%) persons, distal

muscles were affected mostly. The subjects with proximal SMA were further classified as having type 1 (34%), type 2 (13%), type 3 (50%), or type 4 (3%).

2.2 Clinical assessment

Retrospective analysis of the data from medical records of the p.G31A mutation positive patients was done. A literature review of all available published cases of PCH1 with an identified genetic cause was also performed after search in Pubmed, OMIM and Google scholar.

All subjects gave written consent for genetic analysis. The study was approved by scientific ethics committee of the institutions involved.

2.3p.G31A mutation analysis

Mutation analysis was performed by bi-directional Sanger sequencing. Primers were designed to cover the first exon of *EXOSC3* (F-primer: 5'-TCCAACGGACCGCTACTG-3 and R-primer: 5'-GGCTACCACTCTCACTGCC-3'). The PCR products were purified with ExoSAP-IT (USB, Cleveland, OH). Sequencing was achieved with a Big Dye Terminator v.3.1 kit (Applied Biosystems, Foster City, CA) and electrophoretically separated on an ABI3730xl DNA Analyzer (Applied Biosystems).

3.Results

3.1. Phenotype description of the newly identified patients carrying homozygous p.G31A mutation

From the investigated 128 *SMN1*-negative SMA patients, we identified 14 homozygous individuals from 12 families, representing 11% of the cases. Remarkably, all mutation carriers were of Roma origin, making prevalence of 73% (14/19) of this mutation among the *SMN1*-negative SMA patients of Roma ethnic origin.

All 14 patients were born between 1995 and 2016. The families originated from southern and eastern regions of Bulgaria - from the districts of Pazardjik, Plovdiv, Haskovo, Stara Zagora, Burgas, Varna and Shumen. Consanguinity was reported for the parents of one patient (fourth cousins marriage). There were two pairs of sibs among these 14 patients. Close relatives with neuromuscular problems or malformations were reported for seven out of 12 infants; data were missing for two patients.

Basic features of the phenotype of our 14 patients are listed in Supplementary Table1. Seven infants were admitted to hospital at age 1 to 3 months for diagnostic evaluation of generalized hypotonia and weakness ("floppy baby s-me"). Four other had respiratory failure that urged their admittance to hospital in the neonatal period or prevented them from dehospitalization after birth. Detailed clinical data were missing for three patients.

All patients displayed severe muscle hypotonia, severe reduction of spontaneous mobility (Supplementary Video 1 and 2) and tendon hypo-/areflexia. In two patients there were no active movements except facial and oculomotor. Besides tongue fasciculations or fibrillations observed in four infants, facial fasciculations were also noted in one. Congenital laryngeal stridor was observed in three neonates; laryngoscopy revealed no malformations. Skin abnormalities were described in four patients: marble-like in three (without hypercarbia), loose skin in two and edematous in one (Fig. 1). All the six infants with available data that survived the neonatal period didn't have head control by the age of 3 months. Delay in emotional-social and vocal development with absent smile and cooing was also observed.

EMG at ages 1 to 4 months revealed anterior horn neuronopathy in five infants. Two of these five patients had normal EMGs at 1 month but abnormal at 2 or 4 months. EMG of a sixth patient was inconclusive showing neurogenic and myopathic findings. Pontine and cerebellar hypoplasia were found in 2 patients at ages 2 and 3 months by ultrasonography (US) and MRI, being accompanied by hypoplastic corpus callosum in one of them. Two other patients had only cerebellar hypoplasia after examination only by US at 1 and 3 months. Six patients had no cerebellar or pontine abnormalities in the first 3 months, five of them being examined only by US, while the sixth one had 2 CT investigations in the neonatal period (Fig.3).

Creatine kinase was grossly elevated in one patient (1785IU/l; upper limit of the reference range was 175IU/l), mildly elevated in two other infants (346 - 361 IU/l) and normal in other four infants. Muscle biopsy revealed grouped atrophy in one patient and unspecific muscle atrophy in another.

Two patients died in the neonatal period (at ages 4 and 25 days), four in the second month after birth, another four between 4 and 8 months of age. Five patients died from respiratory failure and its complications, one from hemolytic-uremic syndrome. There are no certain data on the causes of death of the other four patients. Four patients were lost for follow-up with last visit being at 3, 4, 4 and 8 months.

3.2. Literature review of PCH type 1

Data of all PCH1 cases with identified mutations available in the literature is displayed in Table 1, Fig. 4 and Supplementary Table 1. The only data that has not been included in this review are the reports of a single family with *TSEN54* mutation⁽²¹⁾ and of a single patient with *RARS2* mutation⁽²²⁾. Although published already 7 years ago, these genes are not included in the OMIM classification of PCH1 (OMIM # 607596, 614678, 606489, 616081, 606019). PCH1 cases with unknown genetic defects are also excluded^(4, 23).

4. Discussion

4.1. Phenotype of the p.G31A mutation

The present study describes the **largest sample** of cases with the p.G31A/*EXOSC3* mutation. It outnumbers the sum of all reported patients with this mutation in the available literature and provides detailed information on the phenotypic presentation. Our findings support the conclusions of J. Schwabova *et al.* (2014) that p.G31A is a founder mutation in the Roma population. The prevalence of PCH1 among this minority might be even higher knowing that other PCH1 mutations (p.S272T/*EXOSC8*⁽²⁴⁾, p.R538* /*VRK1*^(15, 25)) were also found in the Roma population. Thus, PCH1 should be considered as a major entity in *SMN1*-negative SMA among Romas because just one mutation (p.G31A/*EXOSC3*) accounts for almost **three fourths of *SMN1*-negative Roma SMA cases**.

Although the clinical presentation of our patients is in agreement with previous reports of p.G31A/*EXOSC3*, there are some phenotypic features that have not been described yet - congenital stridor, skin abnormalities, hypoplastic corpus callosum, and most importantly - the possible absence of cerebellar and pontine hypoplasia in the first months of life. The presentation of the p.G31A/*EXOSC3* mutation could be summarized as "more SMA-like than PCH-like" as pontocerebellar hypoplasia may not be evident in the first months after birth and markers of cerebral involvement like upper motor neuron signs or non-motor developmental delay are absent or not easily recognized.

4.2. Presentation of PCH1 according to the mutation type

The present study offers a **comprehensive review** of all genetically solved PCH1 cases published so far, including the newly reported *SLC25A46* cases^(18, 19).

After adding the present 14 patients to the 12 patients reported by previous authors, the pool of p.G31A cases becomes the **largest among PCH1 patients** (Supplementary Table 1), outnumbering the p.D132A/*EXOSC3* mutation that has been accepted as the most prevalent in all ethnic groups so far⁽⁴⁾. The **duration of life** in PCH1 patients is a major indicator of disease severity (Fig. 4). p.G31A/*EXOSC3* homozygotes live considerably shorter than all other PCH1 patients except those with *SLC25A46* mutations who die in the first weeks after birth^(17, 18).

Neurological signs *per se* are evident in the neonatal period in the majority of patients in all PCH1 subtypes, with the exception of the infants with *EXOSC8* mutations whose presentation is mainly in the next 6 months after birth. There is no correlation between neurological onset and duration of life suggesting that the disease course is more complex than the simple paradigm "early onset-early death" and that the rate and course of disease progression is different in each mutation group.

Motor signs in PCH1 can be divided into **two models**: **(i)** pure lower motor neuron impairment, observed in the most severe phenotypes, like PCH1B due to p.G31A/*EXOSC3* or other mutations, different from homozygous p.D132A/*EXOSC3*, and PCH1 due to mutation in *SLC25A46*; **(ii)** combination of upper and lower motor neuron signs, found in patients with severe phenotypes, like PCH1C, as well as in milder presentations like the p.D132A/*EXOSC3* mutation and the mutations in *VRK1*. Dystonia and dyskinesia, that are characteristic for PCH2⁽²⁾ might be an additional motor finding, specific for the p.D132A/*EXOSC3* mutation.

Ataxia is specific for *VRK1* mutations being the major impairment in two of the three patients with available data in this PCH1 subtype⁽¹⁵⁾. The suggestion that only patients with longer life may develop ataxia seems not to be true as ataxia was absent in the numerous patients with the p.D132A/*EXOSC3* mutation having the longest survival.

Laryngeal stridor has been described already in *SMN1*-negative SMA⁽¹⁾ and in early reports on PCH1 with unknown genetic cause⁽²⁶⁾ (Table 1). Because of its appearance in the neonatal period, it is named "congenital" although no malformations are disclosed in PCH1 and it might be considered as an early sign of muscle weakness due to bulbar loss of vagal nerve neurons, before other signs of bulbar palsy become evident. Thus, although the list of differential diagnoses of this major pediatric entity "congenital stridor" is long ⁽²⁷⁾, we support the inclusion of severe PCH1 in it.

Skin abnormalities have not been reported in PCH1 yet. Marble-like skin is a striking finding not seen in *SMN1*-positive SMA type 1⁽²⁸⁾ and is probably not related to hypokinesia unlike skin edema and loose skin. In our cases, marble-like skin is not related to hypercarbia, too. We suggest that this finding is a sign of an autonomous nervous system dysfunction and needs further proof and elucidation.

Nystagmus is an important specific sign of cerebellar dysfunction that differentiates PCH1 from *SMN1*-positive SMA. Up to now, it is reported only in PCH1B^(3, 4). J. Schwabova et al. (2013) described these abnormal eye movements as "bursts of nystagmus", while J. Wan et al. (2012) - as "oculomotor apraxia". Similar movements were observed in one of our patients (Supplementary material).

Optic atrophy was described initially in PCH3⁽²⁾ and in *SLC25A46* mutations without PCH⁽²⁹⁾, but was later seen in some of the PCH1 cases with severe presentation - mutations in *SLC25A46* or in *EXOSC3* (excluding homozygous p.D132A). It may be one of the presentations of neuropathy, characteristic of *SLC25A46* mutations⁽¹⁹⁾.

Delay in motor and non-motor development is universal in PCH1, but with variable severity. Motor delay is not correlated directly with the duration of life, as, i.e., some patients reported by J. Wan et al. (2012) never reached any milestone but survived until 18 years. Probably, the duration of life is correlated more robustly with the rate of progression of the respiratory muscles weakness than with the grade of motor delay.

Although **EMG** signs of spinal motor neurons impairment is a major feature of SMA, including PCH1⁽²⁸⁾, signs of axonal and/or demyelinating neuropathy might also be found in PCH1. Even more, they may be the only EMG finding in patients with *SLC25A46*⁽¹⁷⁾ or *VRK1* mutations⁽¹⁵⁾.

Cerebellar hypoplasia or atrophy is considered mandatory for PCH1⁽²⁾ but the present study and review reveals some contradictions. Cerebellar hypoplasia was missing on MRI performed in the age period 2-19 months in 4 PCH1C cases of V. Boczonadi et al. (2014); their inclusion in the group of PCH1 was based on clinical signs and on the imaging data for vermian hypoplasia in other (non-PCH1) patients of the same kindred⁽²⁴⁾. In addition, the absence of cerebellar hypoplasia on CT twice in the neonatal period in one of our 14 patients, and on brain ultrasound in 5 other our patients whose diagnosis of PCH1 was verified by EMG and genetics, supports the observation that cerebellar hypoplasia may be missing in some cases, at least in early infancy,

even with severe clinical presentation. Thus, in clinical grounds, the absence of cerebellar hypoplasia cannot rule out PCH1, at least in the first months (or even years) after birth.

5. Conclusion

At present PCH1 is associated with four genes - *EXOSC3*, *EXOSC8*, *VRK1* and *SLC25A46*. Mutations in *EXOSC3* are the most common cause and explain more than 60% of all PCH1 cases.

The p.G31A mutation in *EXOSC3* is frequent in the Roma population and is responsible for about three fourths of the *SMN1*-negative SMA cases in this ethnic group in Bulgaria. Therefore, *SMN*-negative Roma patients with severe SMA-like presentation should be first tested for the p.G31A/*EXOSC3* mutation.

There is variability and specificity in the clinical presentation of the different mutation groups. Those with severe presentation and early death (e.g. *SLC25A46*, as well as *EXOSC3* mutations different from homozygous p.D132A, and *EXOSC8* mutations) have SMA-like clinical presentation plus global developmental delay, visual and hearing impairment, with or without microcephaly, nystagmus and optic atrophy. EMG signs of neuropathy in severe cases should imply mutation in the *SLC25A46* gene, while spasticity - in the *EXOSC8* gene. Imaging signs of cerebellar and/or pontine hypoplasia may be missing in the first months in patients with severe presentation. On the other hand, there are mutations with milder presentation (e.g. homozygous p.D132A/*EXOSC3* or *VRK1* mutations). They may display additionally signs of upper motor neuron impairment, dystonia or ataxia. The present study may guide the neuropsychiatrician in the diagnosis of PCH1 and the planning of genetic investigations.

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Table 1. Major features of pontocerebellar hypoplasia type 1 according to the cause.

PCH1 subtype	PCH1B	PCH1B	PCH1B	PCH1A	PCH1C	PCH1D?
Gene	<i>EXOSC3</i>	<i>EXOSC3</i>	<i>EXOSC3</i>	<i>VRK1</i>	<i>EXOSC8</i>	<i>SLC25A46</i>
Mutation	p.G31A hz	p.D132A hz	other <i>EXOSC3</i>	R358X hz	2 hz mut.	various
References:	This study and 3, 4; 12, 13, 14	3, 4, 7, 12, 29	3, 4, 12	15, 16, 28	27	17, 18, 19
Frequency§	frequent	frequent	frequent	rare	frequent	rare
Specific ethnicities	Roma	no	no	Ashkenazi Jewish, Iranian	Roma, Palestinian	no
Mean age of death (min-max)	5,52m (4d-17m)	9.27 y (5-18y)	1,20y (3m-5y)	9,33y (8-11y)	1,61y (8-19m)	25d (1day-3mo)
Malformations observed at birth‡	+++	-	++	+++	+	++
Microcephaly	++	++	+	+++	?	++
Neonatal neurol. presentation#	+++	++	+++	+	±	+++
Neurol. present. at age2-6m. #	±	+	-	?	++	-
Lower motor neuron signs†	+++	++	+++	++	++	+++
Tongue fibrilat./fascicul.	+	-	+	++	±	+
Upper motor neuron signs‡	-	++	-	++	++	-
Dystonia/dyskinesia	-	+	-	-	-	-
Ataxia	-	-	?	++	-	ed
Cong. laryngeal stridor	3/11	0/5	no data	0/3	0/11	0/11
Abnormal skin	4/11	0/5	no data	0/3	0/11	0/11
Feeding difficulties	+++	++	++	++	++	+++
Onset of respirat. failure‡	early	late	early	late	early	early
Nystagmus	+	++	+	-	?	-
Optic atrophy	+	-	++	-	?	++
Visual/ hearing impairment	+++	-	+++	-	+++	ed
Motor delay	severe	variable	severe	mild	no data on severity	ed
Non-motor delay	severe	severe	severe	mild	no data on severity	ed
EMG ant. horn neuronopathy	+++	+++	+++	++	++	++
EMG demyel./axon. neuropat.	-	±	±	+	-	++
Cerebellar hypoplasia	++*	+++	+++	++	++	+++
Pontine hypoplasia	+*	+	++	+	-	++

Hypoplast. corpus callosum	±*	-	±	-	+	+
Mild brain atrophy	+*	±	±	+	+	+

Legend: **hz** - homozygous; §- frequent - >15% group frequency rate; rare - <15% group frequency rate; ĺ - at least 1 contracture or dysmorphism or microcephaly or malformation; (+++) - very frequent (in 80-100%); (++) - frequent (in 50-79%); (+)- possible (20-49%); (±) - rare (0-19%); (-)-absent; ?- no data; ed - early death; # - excluding cong. malform., microcephaly, fetal hypokinesia, contractures; †- muscle hypotonia, weakness, atrophy, tendon hypo-/areflexia; ‡ - spasticity, tendon hyperreflexia, Babinski sign; † early - in the first 2 years after birth; late - after the 2-nd year; * - seven patients had only ultrasonographic examination done in the first 4 months after birth.

Fig. 1. Facial dysmorphism, hypotonic posture and marble-like skin in two patients with in p.G31A/*EXOSC3* mutation.

Fig. 2. Marked hypoplasia of cerebellar hemispheres with "dragonfly-like" pattern and suspicious cerebellar cysts, hypoplastic vermis and pons and mild ventricular dilatation at age 2 months of a patient with p.G31A/*EXOSC3* mutation and death at 4 months (MRI - coronal T2 FLAIR and sagittal 3D FSPGR T1).

Fig. 3. Normal cerebellum on CT at age 1 day of a neonate with p.G31A/*EXOSC3* mutation, performed because of muscle hypotonia after perinatal asphyxia. A second CT at age 15 days had the same results.

Fig.4. **Survival of patients with pontocerebellar hypoplasia type 1 according to the mutation type** (for mutation description - see Supplemental Table 1)

Supplementary table 1. Detailed presentation of pontocerebellar hypoplasia type 1 according to the cause.

PCH1 subtype	<i>PCH1B</i>	PCH1B	PCH1B	PCH1B	PCH1A	PCH1C	PCH1D?
Gene	EXOSC3	EXOSC3	EXOSC3	EXOSC3	VRK1	EXOSC8	SLC25A46
Mutation	<i>p.G31A homozygous</i>	p.G31A homozygous	p. D132A homozygous	other <i>EXOSC3</i> mutations	R358X homozygous	p. S272Thomozyg.; p.A2Vhomozyg.	p.L341P homozyg.; p.R231*/exon3 ₋ del; p.A246X homozyg.; p.T14X/c.462+1G>A
Reports:	<i>Present study only (14 cases)</i>	Present 14 cases; Wan et al., 2012; Rudnik-S et al., 2013; Schwabova, 2013; Eggens et al., 2014; Giovambattista et al., 2016	Wan et al., 2012; Rudnik-S et al., 2013; Eggens et al., 2014; Biancheriatl., 2013; Schottmann G et al. 2017	Wan et al., 2012; Rudnik-S et al., 2013; Eggens et al., 2014	Renbaum P. et al, 2009; Najmabad i 2011 (also in OMIM 607596#4); Vinograd-Byk H et al., 2015	Bozconadi V et al., 2014	Wan J et al., 2016; van Dijk et al., 2017; Braunschetal., 2017
Number of pts	14	26	18	17	8	22	11
Males:females	9:5	13:13	11:4	5:7	2:2	6:5	5:6
Specific ethnicities	<i>Roma</i>	Roma	no	no	Ashkenazi Jewish, Iranian	Roma, Palestinian	no
Alive (min- max age of last observation)	4/14 (at 3 - 8mo)	4/26 (at 3 - 8 mo)	8/17 (7- 20y)	0/17	1/4 -4yrs	2/11 (9m; 5y)	0/11
Dead	10/14	22/26	10/17	17/17	3/4	9/11	11/11
Mean age of death (min-max)	3.28m (4d- 8m)	5,52m (4d- 17m)	9.27 y (5- 18y)	1,20y (3m-5y)	9,33y (8- 11y)	1,61y (8- 19m)	25d (1day-3mo)
Congenital malformations:							
Cong. contractures (+arthrogrip.mult.)	6/12 ^a	12/23	0/12	4/15	0/3	0/11	3/11
Fetalhypokinesia	0/11	0/23	0/12	2/15	0/3	0/11	0/8
Polyhydramnios	0/11	0/23	0/9	1/10	0/3	1/11	7/11
Facial dysmorphism	6/11 ^b	12/22	0/12	2/15	0/3	3/11	0/11
Other dysmorphic features/malformations	5/11 ^c	5/11	1/8	2/5	0/3	3/11	0/11
Antenatal microcephaly	2/4	3/7	0/11	0/3	3/3	no data	2/6
Antenatal onset (at least 1 contracture/dysmorphism/microceph./malform)	9/11	15/18	0/12	10/15	3/3	4/11	8/11
Pregnancy and birth							
Other pregnancy abnormalities	1/11	1/11	0/8	0/15	0/3	1/1	2/9

Low birthweight for age	9/11	11/16	0/4	?	0/3	0/11	1/6
Preterm birth	5/11	5/11	1/10	0/15	0/3	0/11	1/6
Perinatal asphyxia	6/11	6/11	0/4	?	0/3	0/11	3/6
Neurological findings:							
Microcephaly (progressive)	4/7 (1/2)	11/19 (4/5)	12/16 (7/7)	3/10 (no data)	3/3 (3/3)	no data	2/4 (no data)
Age of neurol. presentation (excluding cong. malform. dysmorphism, or microcephaly)							
in the 1st. month	10/14	21/26	7/12	15/15	1/3	1/11	11/11
in the 2nd month	2/14	3/26				3/11	
in the 3rd month	1/14	1/26				4/11	
at age 3-6months	1/14	1/26	5/12			3/11	
no data					2/3		
Hypotonia	12/12	24/24	12/13	10/10	2/3	2/11	11/11
DTR hypo-, areflexia	8/8	20/20	2/8	5/5	0/3	no data	4/4
Muscle weakness	11/11	23/23	7/13	no data	2/3	11/11	11/11
Muscle atrophy	11/11	11/11	5/9	4/4	3/3	8/11	no data
Tongue fibrilat./fascicul.	4/11	7/23	0/8	1/5	2/3	1/11	1/4
Spasticity	0/11	0/23	5/9	0/10	1/3	8/11	0/11
Dystonia/dyskinesia	0/11	0/23	4/12	0/13	0/3	0/11	0/11
DTR hyperreflexia	0/11	0/23	9/12	0/15	3/3	1/11	0/7
Babinski sign	0/11	0/23	4/5	no data	0/2	no data	0/7
Ataxia	0/11	0/11	0/5	no data	2/3	0/11	early death
Tremor	0/11	0/11	0/5	no data	1/3	5/11	early death
Seizures	0/11	0/23	2/12	3/15	0/3	0/11	3/11
Cong. laryngeal stridor	3/11	3/11	0/5	no data	0/3	0/11	0/11
Abnormal skin	4/11	4/11	0/5	no data	0/3	0/11	0/11
Feeding difficulties (age tube-fed)	8/11 (1st month)	14/17 (birth to 2wks)	7/12 (3-7y)	9/15 (birth-8m)	2/3	5/11	11/11
Respiratory failure (age)	11/11 (neon. - 4/11; 3-6mo - 7/11) ^d	25/25 (birth -6m)	4/9 (4-13y)	8/10 (birth-2,16y)	2/3 (9y)	11/11 (first year)	11/11 (at birth)
Nystagmus	4/11	7/19	10/16	3/13	0/2	no data	0/7
Strabismus	2/11	2/12	7/9	0/9	0/2	no data	0/7
Optic atrophy	1/4	3/9	0/6	2/4	0/2	no data	4/7
Impaired vision and/or hearing	2/3	10/11	0/7	3/3	0/3	9/11	early death
Developmental delay:							
Motor delay (grade)	11/11 (severe)	10/10 (severe)	12/12 (variable)	10/10 (severe)	3/3 -mild	11/11 (no data)	early death

Non-motor delay (grade)	3/4 (severe)	7/8 (severe)	16/16 (severe)	5/5 (severe)	3/3 (mild)	11/11 (no data)	early death
EMG:							
Ant. horn neuronopathy	5/6 ^e	12/13	11/12	10/10	2/3	1/2	2/4
Axonal neuropathy	0/6	0/16	1/11	0/10	0/3	0/2	2/4
Demyelinating neuropathy	0/6	0/10	0/11	1/10	1/3	0/2	1/4
Morphology:							
Cerebellar hypoplasia/atrophy	4/10 ^f	16/22	14/14	15/15	7/7	5/9	10/10
Pontine hypoplasia	2/10 ^f	10/21	3/14	10/15	1/3	0/9	5/8
Retrocerebellar cyst	0/10 ^f	0/22	0/14	3/15	0/3	0/9	0/8
Cerebellar cysts	1/10 ^f	1/22	0/14	4/15	0/3	0/9	0/8
Hypoplastic corpus callosum	1/10 ^f	1/13	0/14	1/15	0/3	4/9	2/8
Cortical atrophy (mild)	2/10 ^f	6/21	0/14	1/15	1/3	3/9	2/8
Atrophic ventricular dilat. (mild)	3/10 ^f	4/21	1/14	0/15	1/3	0/9	0/8
Muscle biopsy-neurogen. atrophy	1/2	5/6	5/6	9/9	2/2	1/2	0/1

^a - Contractures affected the hip joints in 2 pts, knee-joints in 1, rocker-bottom feet in 1, pedes planovalga in 1, clinodactily in 2, hammer-toes in 1, and unspecified deformities of the extremities in 2 pts.

^b - Mild to moderate facial dysmorphism: upslanting palpebral fissures, deeply set eye globes, blue sclerae, microretrognathia or low set ears.

^c - Multiple renal cysts, thin umbilical cord, umbilical hernia, cryptorchidism and partial sagittal synostosis at birth.

^d - Additional features were weak cry in 4, failure to thrive in 4, obtundation or somnolence in 3 patients.

^e - Spontaneous activity in 3 pts, reinnervation potentials in 1, "giant action potentials" in 1pt; no detailed report for 1 pt.

^f - Seven patients had only ultrasonographic examination done in the first 4 months after birth.

Supplementary video material:

1. Atonic quadriparetic neonate in NICU on artificial ventilation, grimacing to pain.
2. Nystagmoid eye movements, probably gaze-evoked, in a 3-months-old infant with paretic posturing, atonia, amimia, severely reduced active movements, right-sided convergent strabismus and normal vestibulo-ocular reflex. The patient had no visual fixation and pursuit by the age of the last examination at 8 months.

AUTHOR DECLARATION FORM

We wish to confirm, on behalf of all co-authors of our manuscript "*Pontocerebellar hypoplasia type 1 for the neuropediatrician: genotype-phenotype correlations and diagnostic guidelines based on new cases and overview of the literature*" that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

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Signed on behalf of all co-authors:

Prof. Ivan Ivanov, MD, PhD

Prof. Albena Jordanova, PhD,

10/11/2017

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Signed on behalf of all co-authors:

Prof. Ivan Ivanov, MD, PhD

Prof. Albena Jordanova, PhD,

10/11/2017