DOI: 10.4149/BLL_2022_090

Bratisl Med J 2022; 123 (8)

568 – 572

CLINICAL STUDY

Molecularly confirmed pontocerebellar hypoplasia in a large family from Slovakia with four severely affected children

RADVANSKA Eva[1,2*], POS Zuzana[3,4,5*], ZATKOVA Andrea[3], HYBLOVA Michaela[6,7], BAUER Frantisek[1], SZEMES Tomas[4,5,8], KADASI Ludevit[3,8], RADVANSZKY Jan[3,5,8]

Institute of Clinical and Translational Research, Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, Slovakia. **andrea.zatkova@savba.sk**

ABSTRACT

BACKGROUND: Pontocerebellar hypoplasia type 1 (PCH1) is characterized by a central and peripheral motor dysfunction associated with anterior horn cell degeneration, similar to spinal muscular atrophy (SMA).
OBJECTIVES: We analysed three probands (later discovered to be siblings) suspected to have severe SMA, however, not confirmed by genetic test.
METHODS: Clinical-exome analysis (Illumina) was performed to identify causative variants, followed by Sanger sequencing confirmation in probands and other 10 family members.
RESULTS: Homozygous pathogenic variant c.92G>C (p.(Gly31Ala)) in the *Exosome Component 3 (EXOSC3)* gene was found in all 3 probands, thus confirming the diagnosis of a severe form of PCH1B. The parents and six siblings were carriers, while one sibling was homozygous for a reference allele. This variant is frequent in the Czech Roma population, where it is considered a founder mutation. Haplotype analysis in this largest reported PCH1B family showed that our patients inherited from their father (of Roma origin) a haplotype identical to that found in the Czech Roma population, thus indicating these alleles have a common origin.
CONCLUSION: This *EXOSC3* variant is rare among the general population but most likely frequent also among Roma people in Slovakia. PCH1B should be considered for a differential diagnosis in infants manifesting SMA-like phenotype, especially if of Roma origin *(Tab. 1, Fig. 1, Ref. 22)*. Text in PDF *www.elis.sk*
KEY WORDS: pontocerebellar hypoplasia, PCH1B, *EXOSC3*, SMA plus syndromes, pathogenic sequence variant.

Introduction

Pontocerebellar hypoplasia (PCH) is a rare and heterogenous group of neurodegenerative disorders with typically prenatal onset. The cerebral structures such as *pons* and *cerebellum* are usually affected the most severely and manifest a strong underdevelopment, but also other parts of the brain might show an impaired growth resulting in microcephaly (1). Currently, 13 subtypes of PCH are known (OMIM, #607596). They are, classified based on the clinical picture, spectrum of pathologic changes and molecular features. One of the major forms of PCH is type 1 (PCH1), characterized by a central and peripheral motor dysfunctions associated with anterior horn cell degeneration similar to spinal muscular atrophy (SMA). Due to the similarity with SMA, PCH1 has also been included in the group of *SMA plus syndromes*, or so-called infantile variants of SMAs with additional or atypical features (2). Distinctive features of this PCH subtype are motor neuron degeneration, muscle weakness, hypotonia, respiratory insufficiency and congenital contractures (1). The mode of inheritance is autosomal recessive.

The first symptoms of PCH1, such as reduced foetal movement and polyhydramnios, are usually seen during the prenatal period. During the new-born period, respiratory insufficiency, hypotonia and joint contractures can be observed, but also some other symptoms such as visual impairment, nystagmus, ataxia, muscle weakness, and feeding problems (3–6).

PCH1 is further subdivided into four groups: PCH1A, PCH1B, PCH1C, and PCH1D (1). PCH1A, PCH1C and PCH1D are caused by variants in *Vaccinia-Related Kinase 1 (VRK1)* (#607596) gene involved in neuronal migration (7–9), *Exosome Component 8 (EXOSC8)* (#616081) involved in mRNA degradation (10), and *Exosome Component 9 (EXOSC9* gene) (*606180), another component of exosome, respectively (summarised in 11).

[1]Department of Neonatology, Faculty Hospital Nove Zamky, Nove Zamky, Slovakia, [2]Szalamed Ltd., Nove Zamky, Slovakia, [3]Institute of Clinical and Translational Research, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia, [4]Geneton, Ltd., Bratislava, Slovakia, [5]Comenius University Science Park, Bratislava, Slovakia, [6]Trisomy Test Ltd., Bratislava, Slovakia, [7]Medirex Inc., Bratislava, Slovakia, and [8]Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

[*]These authors contributed equally to this work.

Address for correspondence: Andrea ZATKOVA, PhD, Institute of Clinical and Translational Research, Biomedical Research Center of the Slovak Academy of Sciences, Dúbravská cesta 9, SK-845 05 Bratislava, Slovakia. Phone: +421911466599

Acknowledgements: This work was supported by several grants: Slovak Research and Development Agency: APVV-18-0319; Grant Agency of The Ministry of Education, Science, Research and Sport of the Slovak Republic and Slovak Academy of Sciences: VEGA_2/0167/20; VEGA_02/0040/20, and the OP Integrated Infrastructure for the project: ITMS: 313011V578 (PreveLYNCH), co-financed by the European Regional Development Fund.

Tab. 1. Details on clinical history of 3 siblings suffering from a severe form of PCH1B.

	Patient 1	Patient 2	Patient 3
Gender	male	male	female
Pregnancy (number)	4th	7th	8th
Pregnancy (findings)	chronic foetal distress	chronic foetal distress	chronic foetal distress
Year of birth	2009	2012	2013
Gestational age at birth (Petrussa)	37^{th} – 38^{th}	40^{th}	38^{th}
Weight	2300 g	2500 g	2600 g
Length	46 cm	46 cm	46 cm
Ofc	n.a.	33 cm	n.a.
Apgar score	6/8	3/8	8/9
Neonatal icterus	yes	yes	yes
Respiratory distress syndrome	yes	yes	yes
Feeding difficulties	yes	yes	yes
Muscular hypotonia	yes	yes	yes
Stridor	yes	yes	wheezing, stridorous crying
Other findings	Patient 1	Patient 2	Patient 3
At birth	hypoglycemia, prenatal hypotrophy due to chronic foetal distress with somatofacial stigmatisation: micrognathia, microphthalmia, gothic palate, dysphonic voice, dorsal flexion of the toes	hypotonia (floppy baby), prenatal hypotrophy due to chronic foetal distress, mild asphyxia	hypotonia, lack of reflexes, horizontal nystagmus, defect of atrial septum - ASD II and pulmonary insufficiency of the 1st grade; EMG: suspected myasthenic syndrome; MRI of the brain: possible atrophy of the cerebellum; follow-up EMG: excluded myasthenia gravis and pointed to polyneuropathy; the neurologist concluded central hypotonic syndrome and suspected SMA type 1.
At the age of one month	MRI: terminal hypoplasia of cerebellum, a frame of mega cisterna magna without direct communication with 4th chamber	hospitalisation: cardiopulmonary compensated, without signs of acute infection; neurological finding: central hypotonic syndrome, hyporeflexia to areflexia, fasciculation of the tongue, kinesiologically at the level of the first trimenon; EMG: axonal-demyelinating neuropathy; USG: preventricular hypoxic-ischemic changes of the 1st level	n.a.
At the age of 2 month	hospitalisation: cardiopulmonary compensated, with stridor, purulent conjunctivitis o. sin. was present. Marked axial hypotonia with lower limb areflexia, upper limb hyporeflexia, reduced active movement of lower limb, suspected lesion of nerves (?) IX-XII, and suspected hearing impairment; EMG: axonal-demyelinating polyneuropathy and a neurogenic lesion; USG: slightly more spacious occipital corners of the lateral ventricles and mega cisterna magna	n.a.	tracheostomy due to progressive respiratory insufficiency; hypotonic syndrome with hyporeflexia to areflexia, bulbar syndrome and hypomyelinating neuropathy or congenital myopathy was suspected.
At the age of 3 month	n.a.	n.a.	somatic stigmatisation with severe hypotonia, hyporeflexia and sign of microcephaly; feeding through nasogastric tube
At the age of 5 month	hospitalisation because of worsened clinical status: axial hypotonia and areflexia, visual and hearing impairment, pseudobulbar syndrome, hypoproteinemia with hypoalbuminemia due to the feeding difficulties, infections of urinary and respiratory tract; USG of the brain: marked atrophy of cerebral cortex and hypoxic changes in white matter	n.a.	n.a.
Age at death	6 months	2 months	4 months
SMN1 deletions of exons 7 and 8	not present	not present	not present
EXOSC3 mutations	c.92G>C (p.(Gly31Ala)) c.92G>C (p.(Gly31Ala))	c.92G>C (p.(Gly31Ala)) c.92G>C (p.(Gly31Ala))	c.92G>C (p.(Gly31Ala)) c.92G>C (p.(Gly31Ala))

All carried a homozygous variant c.92G>C (p.(Gly31Ala)) of the *EXOCS3* gene. OFC – occipital frontal circumference, EMG – electromyography, ASD- atrial septal defect, MRI – magnetic resonance imaging, SMA – spinal muscular atrophy, USG – head ultrasound, n.a. – not available

PCH1B (OMIM 614678) is associated with *EXOSC3* (*Exosome Component 3*) (#614678) pathogenic variants that account for up to 50 % of all PCH1 cases (1, 12–15). For now, there are 24 *EXOSC3* variants in the HGMD professional database 2021.4, the majority of which are missense/nonsense. *EXOSC3* variants have not been found in patients with other PCH subtypes (14).

EXOSC3 (also known as hRrp40) is one of the three subunits (EXOSC1/2/3) of the RNA binding cap of the exosome complex (in total composed of 9 EXOSC subunits), which is responsible for the processing of mRNA, rRNA and presumably tRNA of RNA precursor, transcript and unspliced degradation, and regulation of RNA processing in cerebellar and spinal neurons (13). EXOSC3 contains 3 domains: N-terminal domain, putative RNA-binding S1 domain, and putative RNA-binding KH domain (16). To date, several variants have been identified in this gene, including large deletions, nonsense and missense variants that affect interactions of EXOSC3 with other exosome subunits and result in impaired function of the RNA exosome (16). Genetic heterogeneity is reflected in the broad phenotypic spectrum of PCH1B, ranging from a relatively mild to severe, while genotype-phenotype correlations have been observed (1, 12, 14–17).

Material and methods

Subjects

During the years 2009–2013, DNA samples of three phenotypically similar new-borns (patients P1-3) were sent from social institutions to our laboratory for genetic analysis (clinical history summarized in Table 1). All of them manifested severe hypotonia and hypoplasia of cerebellum (ranging from possibly atrophy of cerebellum to hypoplasia of cerebellum) and were suspected to have SMA. However, no deletions of exon 7 and exon 8 of the *SMN1* gene were found. Later, their familiarity was noted, and another older sister (born in 2006) was identified, who also died early (a few months after birth), most probably due to similar symptoms. Her medical records were not available though. Both parents were apparently healthy, and no consanguinity was reported. The father is of Roma origin.

An ethical statement

The reported core analyses were not performed due to research since they were carried out as a result of a routine process of molecular diagnostics. At the time of blood sampling, each analysed individual, in the case of children, parents or legal guardians, signed written informed consent forms consistent with the Helsinki declaration. The genetic data for the population of the Slovak Republic were derived from the following studies approved by the Ethical Committee of the Bratislava Self-Governing Region (Sabinovska ul.16, 820 05 Bratislava): PreveLynch (approval on 30 November 2020 under the decision ID 09834/2020/HF) and Reuse of NIPT data (approval on 30 April 2015 under the decision ID 03899_2015).

Methods

Exon 7 and 8 deletions of *SMN1* gene were tested during routine DNA diagnostic procedure. Post-mortem DNA samples of patients 1–3 (from archived DNA) and of individual 4 (healthy sister) were analysed by massively parallel sequencing using a TruSight

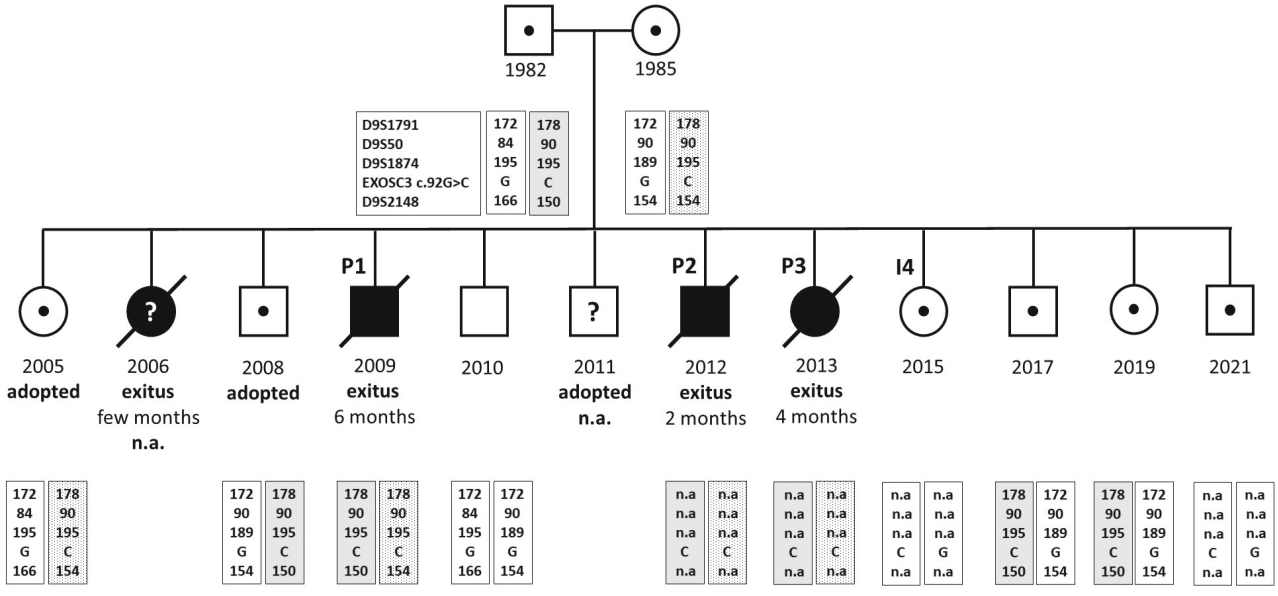

Fig. 1. Family with 4 siblings suffering from a severe form of PCH1B caused by homozygous variant c.92G>C (p.(Gly31Ala)) within the *EXOSC3* gene. Year of birth is indicated. Haplotypes based on four STR markers (D9S1791, D9S50, D9S1874, D9S2148) flanking *EXOSC3* gene are shown. Haplotype associated with variant c.92G>C in the father (shaded in grey) is the same as in patients of Roma origin reported by Schwabova et al (17), while the mother's haplotype differs only in marker D9S2148 (allele 154 instead of 150); n.a. = DNA samples not available for analyses or markers not analysed, P1 - P3 = Patients 1-3, I4 = Individual 4.

One Sequencing Panel kit (Illumina, San Diego, CA, USA) in order to identify the disease causing the DNA change. After sequencing on MiSeq (Illumina) sequence, reads were aligned/mapped against the GRCh37/UCSC hg19 reference genome by BWA (Burrows-Wheeler Aligner) and variant calling was performed by GATK variant caller. Variant filtering and annotation from the generated VCF files were performed using the Ingenuity Variant Analysis software (Qiagen, Hilden, Germany). Genomic DNA of the remaining family members, from whom blood samples were obtainable, was isolated from peripheral blood leukocytes (Puregene™ DNA Purification Kit, Qiagen). The respective genomic region harbouring the identified pathogenic variant was PCR amplified (primers: forward 5´-GAGGGGGTTTGTCACTTGG-3´, reverse 5´-AACACAGAGGCAGAGGGAGA- 3´) and sequenced (BigDye Terminator v3.1 Cycle Sequencing kit - Life Technologies, ABI Prism Genetic Analyzer 3130xl - Applied Biosystems, 3130 Data Collection and Sequencing Analysis v5.4 software - Applied Biosystems, ChromasPro v1.6 - Technelysium Pty Ltd). STR markers D9S1791, D9S50, D9S1874 and D9S2148 flanking *EXOSC3* gene were analysed for haplotype analysis as described in (17).

Data availability statement

The data that support the findings of this study are available on reasonable request from the corresponding author. Full whole-exome sequencing data are, however, not publicly available due to privacy or ethical restrictions.

Results and discussion

DNA *post mortem* samples of patients P1–3 (from archived DNA) and of individual 4 (I4, healthy sister) were analysed by massively parallel sequencing. A variant that was previously reported to cause PCH type 1B, namely NM_016042.4**:**c.92G>C, NP_057126.2:p.(Gly31Ala) (dbSNP: rs387907196, ClinVar ID: 31691, HGMD: CM125759) in exon 1 of the *EXOSC3 gene* (chromosome 9) was found (13, 17). All three patients were found to be homozygous, while their healthy sister (I4) was heterozygous (Fig. 1). As further shown by Sanger sequencing, the unaffected parents and six siblings were heterozygous carriers, while one healthy sibling carries a reference genotype (Fig. 1).

The variant c.92G>C has a low population frequency (MAF: G = 0.,000010 in ExAC and G = 0.000020 in gnomAD) and fulfills the following criteria of pathogenicity of the American College of Medical Genetics and Genomics (18): The classification of PP5, PM2, PP2, PP3 was performed using manual curation of the automatically generated results of the Varsome annotation tool (19). The variant locates in the EXOSC3 N-terminal domain that is important for intersubunit interactions: residue Gly31 has been shown tightly packed against the surface of EXOSC5, indicating its importance for EXOSC3-EXOSC5 interactions (16, 20).

The variant c.92G>C has not been found in the general Slovak population (21). However, it was reported to be frequent in the Czech Roma population, where 4 of 90 (4.4 %) unrelated Roma control individuals tested heterozygous and the haplotype analysis with four STR markers (D9S1791, D9S50, D9S1874 and D9S2148) flanking *EXOSC3* gene suggested a founder effect (17). The analysis in our family showed that the haplotype in the father is identical to haplotypes found in the Czech Roma patients described by Schwabova et al (17). The mother's haplotype associated with the variant c.92G>C differs from that of the father only in the marker D9S2148 (allele 154 instead of 150), thus indicating a simple crossing over event or a repeat number change possibly due to a replication error in the respective microsatellite marker (Fig. 1). Based on the close connection of the populations of Slovakia and Czech Republic, we can hypothesise that alleles have a common origin. Interestingly, also in the study of Eggens et al (14) all patients who carried the p.(Gly31Ala) mutations were of Roma/Gypsy descent, although living in different countries (Sweden and Hungary), which also suggests a common founder (12, 17).

Taken together, this *EXOSC3* variant can be added to the list of variants causing autosomal recessive disorders that are rare in the general Slovak population but frequent among the Roma populations. Thus, it is important in Slovakia to consider *EXOSC3*-associated PCH1B for a differential diagnosis in infants with SMA-like symptoms, especially if of Roma origin.

In their genotype-phenotype correlation study, Eggens et al (14) showed that some variants such as the homozygous p.(Asp132Ala) variant, lead to a more chronic form of PCH with survival into childhood and preservation of the pons. Instead, the compound heterozygosity for the p.(Asp132Ala) mutation and nonsense or p.(Tyr109Asn) allele, homozygous p.(Gly31Ala) mutation or p.(Gly135Glu) mutation causes a severe disease course including death during infancy and hypoplasia of the pons (14). Roma patients from the study of Schwabova et al (17), as well as our patients, manifested a severe form of the disorder, with death in the first year of life.

Recently, Duc et al (22) reported on extensive clinical heterogeneity in 2 siblings, a 21-year-old young man with more severe manifestations with rapid progression, as compared to his 8-year-old mildly affected sister with non-progressive manifestations. Both probands are compound heterozygous for c.395A>C (p.(Asp132Ala)) and novel variant c.572G>A (p.(Gly191Asp)) in *EXOSC3* while no other variants were identified in them by clinical exome sequencing (22). As authors discussed, this unexpected intrafamilial variability can depend on the varying level of EXOSC3 expression leading to variable neuroradiological findings. It can be speculated that the male sex is a risk factor, but further studies are required to verify the effect of gender hormones.

There were two patients reported to be compound heterozygous for the c.92G>C (p.(Gly31Ala)) variant and c.712T>C (p.(Trp238Arg)). However, the variant p.(Trp238Arg) is a severe one, affecting the interaction of EXOSC3 with EXOSC9 subunit (16, 20), thus, both patients showed a severe phenotype (12, 13) while no heterogeneity was observed.

Patients in our large PCH1B family are homozygous for the p.(Gly31Ala) variant and both females and males showed the same severe disease course and died within a few months. It indicates that the clinical phenotype in cases with *EXOSC3* compound heterozygosity might be different than in cases homozygous for

the same mutations, depending on the combination of the effect of individual mutations on the structure and function of the exosome, as well as on the level of the EXOSC3 expression from individual mutated alleles. In the future, it may be interesting to identify and analyse patients who carry p.(Gly31Ala) and some other less severe variant.

References

1. van Dijk T, Baas F, Barth PG, Bwee Tien Poll-The. What's new in pontocerebellar hypoplasia? An update on genes and subtypes. Orphanet J Rare Dis 2018; 13. DOI: 10.1186/s13023-018-0826-2.

2. Kizilates SU, Talim B, Sel K, Köse G, Caglar M. Severe lethal spinal muscular atrophy variant with arthrogryposis. Pediatr Neurol 2005; 32: 201–204.

3. Chou SM, Gilbert EF, Chun RW et al. Infantile olivopontocerebellar atrophy with spinal muscular atrophy (infantile OPCA + SMA). Clin Neuropathol 1990; 9: 21–32.

4. Barth PG. Pontocerebellar hypoplasias. An overview of a group of inherited neurodegenerative disorders with fetal onset. Brain Dev 1993; 15: 411–422.

5. Szabó N, Szabó H, Hortobágyi T, Túri S, Sztriha L. Pontocerebellar hypoplasia type 1. Pediatr Neurol 2008; 39: 286–288.

6. Namavar Y, Barth PG, Poll-The BT, Baas F. Classification, diagnosis and potential mechanisms in pontocerebellar hypoplasia. Orphanet J Rare Dis 2011; 6: 50.

7. Renbaum P, Kellerman E, Jaron R et al. Spinal muscular atrophy with pontocerebellar hypoplasia is caused by a mutation in the VRK1 gene. Am J Hum Genet 2009; 85: 281–289.

8. Gonzaga-Jauregui C, Lotze T, Jamal L et al. Mutations in VRK1 associated with complex motor and sensory axonal neuropathy plus microcephaly. JAMA Neurol 2013; 70: 1491–1498.

9. Stoll M, Teoh H, Lee J et al. Novel motor phenotypes in patients with VRK1 mutations without pontocerebellar hypoplasia. Neurology 2016; 87: 65–70.

10. Boczonadi V, Müller JS, Pyle A et al. EXOSC8 mutations alter mRNA metabolism and cause hypomyelination with spinal muscular atrophy and cerebellar hypoplasia. Nat Commun 2014; 5: 4287.

11. Burns DT, Donkervoort S, Müller JS et al. Variants in EXOSC9 Disrupt the RNA Exosome and Result in Cerebellar Atrophy with Spinal Motor Neuronopathy. Am J Hum Genet 2018; 102: 858–873.

12. Rudnik-Schöneborn S, Senderek J, Jen JC et al. Pontocerebellar hypoplasia type 1: clinical spectrum and relevance of EXOSC3 mutations. Neurology 2013; 80: 438–446.

13. Wan J, Yourshaw M, Mamsa H et al. Mutations in the RNA exosome component gene EXOSC3 cause pontocerebellar hypoplasia and spinal motor neuron degeneration. Nat Genet 2012; 44: 704–708.

14. Eggens VR, Barth PG, Niermeijer J-MF et al. EXOSC3 mutations in pontocerebellar hypoplasia type 1: novel mutations and genotype-phenotype correlations. Orphanet J Rare Dis 2014; 9: 23.

15. Ivanov I, Atkinson D, Litvinenko I et al. Pontocerebellar hypoplasia type 1 for the neuropediatrician: Genotype-phenotype correlations and diagnostic guidelines based on new cases and overview of the literature. Eur J Paediatr Neurol 2018; 22: 674–681.

16. Morton DJ, Kuiper EG, Jones SK, Leung SW, Corbett AH, Fasken MB. The RNA exosome and RNA exosome-linked disease. RNA 2018; 24: 127–142.

17. Schwabova J, Brozkova DS, Petrak B et al. HomozygousEXOSC-3Mutation c.92G→C, p.G31A is a Founder Mutation Causing Severe Pontocerebellar Hypoplasia Type 1 Among the Czech Roma. Journal of Neurogenetics. 2013; 27: 163–169.

18. Richards S, Aziz N, Bale S et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17: 405–424.

19. Kopanos C, Tsiolkas V, Kouris A et al. VarSome: the human genomic variant search engine. Bioinformatics 2019; 35: 1978–1980.

20. Fasken MB, Losh JS, Leung SW et al. Insight into the RNA Exosome Complex Through Modeling Pontocerebellar Hypoplasia Type 1b Disease Mutations in Yeast. Genetics 2017; 205: 221–237.

21. Budis J, Gazdarica J, Radvanszky J et al. Non-invasive prenatal testing as a valuable source of population specific allelic frequencies. J Biotechnol 2019; 299: 72–78.

22. Duc DL, Le Duc D, Horn S et al. Novel EXOSC3 pathogenic variant results in a mild course of neurologic disease with cerebellum involvement. Eur J Med Genet 2020; 63: 103649.

Received February 24, 2022.
Accepted March 14, 2022.