Heliyon 10 (2024) e27438

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

PIGN c.776T>C (p.Phe259Ser) variant present in trans with a pathogenic variant for PIGN-congenital disorder of glycosylation: Bella-Noah syndrome

Lyvia Neves Rebello Alves [a,b], Lívia Valle dos Santos Silveira [c], Raquel Silva dos Reis Trabach [a], Débora Dummer Meira [a,b], Eldamária de Vargas Wolfgramm dos Santos [a,b], Iúri Drumond Louro [a,b,*]

[a] *Núcleo de Genética Humana e Molecular, Departamento de Ciências Biológicas, Universidade Federal do Espírito Santo (UFES), Vitória, 29075-910, ES, Brazil*
[b] *Programa de Pós-Graduação Em Biotecnologia, Universidade Federal do Espírito Santo, Vitória, 29047-105, ES, Brazil*
[c] *Centro de Ciências da Saúde, Curso de Medicina, Universidade Federal do Espírito Santo (UFES), Vitória, 29090-040, ES, Brazil*

ARTICLE INFO

Keywords:
Glycosylation
Congenital disorder of glycosylation
PIGN-CDG
Epileptic seizures
Hypotonia

ABSTRACT

Glycosylation is the most common protein and lipid post-translational modification in humans. Congenital disorders of glycosylation (CDG) are characterized by both genetic and clinical heterogeneity, presenting multisystemic manifestations, and in most cases are autosomal recessive in inheritance. The *PIGN* gene is responsible for the addition of phosphoethanolamine to the first mannose in the glycosylphosphatidylinositol (GPI)-anchor biosynthesis pathway, a highly conserved process that enables proteins to bind to the cell surface membrane. Here, we report a family with two siblings pediatric cases with the exact same compound heterozygous variants in *PIGN*. The (c.776T > C) variant of uncertain significance (VUS) together with a known pathogenic variant (c.932T > G), resulting in clinical features compatible with PIGN-related conditions, more specific the CDG. This is the first time that *PIGN* variant c.776T > C is reported in literature in individuals with PIGN-congenital disorder of glycosylation (PIGN-CDG), and the current submission in ClinVar by Invitae® is specifically of our case. Detailed clinical information and molecular analyses are presented. Here, we show for the first time two affected siblings with one pathogenic variant (c.932T > G) and the c.776T > C VUS in trans. In honor of the family, we propose the name Bella-Noah Syndrome for disorder.

1. Introduction

Glycosylation is the most common protein and lipid post-translational modification in humans. It is an essential process for the development, growth and functioning of organisms [1]. Patients with symptoms that affect many organs should be investigated for

* Corresponding author. Núcleo de Genética Humana e Molecular, Departamento de Ciências Biológicas, Universidade Federal do Espírito Santo (UFES), Vitória, 29075-910, ES, Brazil.

E-mail addresses: lyviarebello@gmail.com (L. Neves Rebello Alves), liviavssilveira@gmail.com (L. Valle dos Santos Silveira), raquel.sreis@outlook.com.br (R. Silva dos Reis Trabach), debora.dummer.meira@gmail.com (D. Dummer Meira), eldamariavw@yahoo.com.br (E. de Vargas Wolfgramm dos Santos), iurilouro@yahoo.com (I. Drumond Louro).

https://doi.org/10.1016/j.heliyon.2024.e27438
Received 16 November 2023; Received in revised form 12 December 2023; Accepted 28 February 2024
Available online 5 March 2024

defects in glycosylation, once incorrect protein glycosylation can trigger complex clinical manifestations and organ pathology [1,2].

Congenital disorders of glycosylation (CDG), first reported in 1980, are inborn errors of metabolism (IEM) characterized by both genetic and clinical heterogeneity [3–5]. CDG usually present multisystemic manifestations, but the most common symptoms are related to neurological abnormalities, including psychomotor retardation/cognitive disorders, epileptic seizures, hypotonia, ataxia, polyneuropathy and stroke-like events [1]. Furthermore, eye, skin, and cardiac disease, as well as facial dysmorphisms, may also occur [3]. CDG patients that have early-onset neurovisceral features have some signs and symptoms since birth [4].

To date, more than 150 CDG subtypes have been reported and nomenclature is designated by the affected gene name (non-italicized) followed by -CDG [4]. It is important to highlight that most cases of CDG are autosomal recessive in inheritance, with one mutation inherited from each asymptomatic parent [3]. However, autosomal dominant and X-linked forms have also been described [4].

The *PIGN* (phosphatidylinositol glycan anchor biosynthesis class N) gene (OMIM* 606097) is located on chromosome 18q21.33 and is composed of 31 exons (29 coding) spanning 142.8 kb, and encoding a glycosylphosphatidylinositol ethanolamine phosphate transferase 1, a protein with 931 amino acids involved in the glycosylphosphatidylinositol (GPI)-anchor biosynthesis pathway, a deeply conserved process that enables proteins to bind to the cell surface membrane [6–10]. *PIGN* is responsible for the addition of phosphoethanolamine to the first mannose in the GPI [8].

Multiple congenital anomalies-hypotonia-seizures syndrome1 (MCAHS1) (OMIM# 614080) and Fryns syndrome (OMIM# 229850) are usually caused by mutations in the PIGN gene, resulting in a GPI anchor deficiency [10,11]. Nonetheless, some individuals who have PIGN mutations do not meet all the criteria for syndrome diagnosis [10].

In this study, we report a family with two pediatric siblings, in whom, Next Generation Sequencing (NGS) yielded compound heterozygous variants in the *PIGN* gene. One of them (c.776T > C) is a new variant that together with a known pathogenic variant (c.932T > G) resulted in clinical features compatible with PIGN-related conditions, more specifically the congenital disorder of glycosylation. Detailed clinical information and molecular analyses are presented.

This is the first time that the *PIGN* variant c.776T > C is reported in individuals with PIGN-congenital disorder of glycosylation and the ClinVar entry by Invitae® refers to this case (PIGN-CDG).

2. Materials and methods

Peripheral blood samples were obtained from both patients and sent out to Invitae®, CA for panel analysis. Family members signed a written informed consent. The study was approved by the Research Ethics Committee involving Human Beings of the Health Sciences Center of the Universidade Federal do Espírito Santo, protocol number 6.048.483.

2.1. Clinical information

Two siblings with symptoms of hypotonia and seizures, one male and one female, children of healthy non-consanguineous parents with no familial disease background, were evaluated by a geneticist. Pregnancies were reported as uncomplicated.

Owing to the proband history of hypotonia and epileptic seizures since birth and after a long investigation with many clinical, genetic and chromosomal tests without a clear diagnosis, parents sought assistance of the geneticist in April 2022. Documentation about the child clinical history and examinations was systematically reviewed from a clinical and genetic perspective.

During evaluation of the first child, a new pregnancy was presented and after birth, the second child began to show symptoms similar to those of her brother.

2.2. Patient 1

N.B.R., male, one year and eight months old, weighed 10 kg and measured 0.85 m, first child of healthy non-consanguineous parents. The patient had a medical history of neuromotor development delay associated with dysmorphic clinical signs, such as lip hypotonia, macrostomia, overlapping fingers, dorsiflexed hallux and two accessory nipples. Patient lacked motor coordination, had a bell-shaped chest and legs apart. Hypotonia was observed soon after birth and infantile spasms in the first month of life.

A neuropediatrician excluded the diagnosis of West syndrome due to electrocardiographic findings and the fact that the patient did not have seizures when sleeping.

At the current age, he is not able to speak or sit, only eats porridge and soft food, choking when eating semi-solid food, such as rice. At eight months-old, he started taking topiramate, which led to an improvement in the crises.

Before the case was brought to our attention, a series of tests and examinations were performed as described below.

Magnetic Resonance Imaging of the skull, held in February 2021, revealed hypersignal on T2, restriction of bilateral and symmetric diffusion affecting the thalamus, globus pallidus, cerebral peduncles, periaqueductal white matter, central tegmental tracts and dentate nucleus. The patient had been using vigabatrin for 40 days before the exam, so the main diagnostic hypothesis was related to toxicity due to vigabatrin and neurometabolic disease. After that, the patient received cofactors replacement, such as biotin, pyridoxine and folic acid, but there were no signs of improvement.

A G and C band karyotype test was requested, which showed a normal result: 46, XY, the reference value of male karyotype. Whole Exome Sequencing (WES) was performed in April 2021 on the patient and his healthy parents. Alterations that justify the clinical condition were not detected. No pathogenic copy number variations (CNV) were identified by the next-generation sequencing (NGS) method. However, a variant p.Asn903Ser (ENST00000335727) was identified in the *TNRC6B* gene. This variant is also present in the

Table 1
Genetic variants found in patients 1 and 2 and their parents.

GENE	VARIANT	VARIANT CLASSIFICATION	RESULTS							
			Patient 1		Patient 2		Mother		Father	
			Zigozity	Result	Zigozity	Result	Zigozity	Result	Zigozity	Result
PIGN	c.932T > G (p.Leu311Trp)	Pathogenic	Heterozygous	Detected	Heterozygous	Detected	Heterozygous	Detected	N/A	Not detected
	c.776T > C (p.Phe259Ser)	Uncertain significance	Heterozygous	Detected	Heterozygous	Detected	N/A	Not detected	Heterozygous	Detected
CLN6	c.461_463del (p.Ile154del)	Pathogenic	Heterozygous	Detected	Heterozygous	Detected	Heterozygous	Detected	N/A	Not detected
FA2H	c.131C > A (p.Pro44Gln)	Pathogenic	Heterozygous	Detected	N/A	Not detected	Heterozygous	Detected	N/A	Not detected
GAA	c.1064T > C (p.Leu355Pro)	Pathogenic	Heterozygous	Detected	N/A	Not detected	N/A	Not detected	Heterozygous	Detected
ALG9	c.1173 + 18G > A (Intronic)	Uncertain significance	Heterozygous	Detected	Heterozygous	Detected	N/A	Not detected	Heterozygous	Detected
CNTNAP1	c.2703G > A (p.Met901Ile)	Uncertain significance	Heterozygous	Detected	Heterozygous	Detected	N/A	Not detected	Heterozygous	Detected
NAGA	c.1208T > C (p.Ile403Thr)	Uncertain significance	Heterozygous	Detected	N/A	Not detected	N/A	Not detected	Heterozygous	Detected
PRF1	c.310C > T (p.Arg104Cys)	Uncertain significance	Heterozygous	Detected	Heterozygous	Detected	Heterozygous	Detected	N/A	Not detected
PRKDC	c.7649T > C (p.Ile2550Thr)	Uncertain significance	Heterozygous	Detected	N/A	Not detected	Heterozygous	Detected	N/A	Not detected
RAB3GAP1	c.1187G > C (p.Gly396Ala)	Uncertain significance	Heterozygous	Detected	N/A	Not detected	N/A	Not detected	Heterozygous	Detected
SYNE1	c.4843G > A (p.Ala1615Thr)	Uncertain significance	Heterozygous	Detected	Heterozygous	Detected	Heterozygous	Detected	N/A	Not detected
PYGM	c.855+4C > T (Intronic)	Uncertain significance	Heterozygous	Detected	NT	NT	NT	NT	NT	NT
SPEG	c.7153G > T (p.Val2385Leu)	Uncertain significance	Heterozygous	Detected	NT	NT	NT	NT	NT	NT

N/A: not applicable; NT: Not Tested.

healthy patient's mother, and therefore, it was considered unlikely to be responsible for the clinical condition.

In July 2021, a qualitative analysis of urinary organic acids was made. The main organic acids analyzed were aromatic amino acids, branched chain amino acids, fatty acids, Krebs cycle and respiratory chain, lactic acid and ketone bodies, glycine, serine and lysine. The analysis showed a normal profile.

In August 2021, a multiplex ligation-dependent probe amplification (MLPA) test of genes *SMN1* and *SMN2* was requested to check the possibility of spinal muscular atrophy. However, MLPA did not detect the presence of large deletions in the regions included in the analysis.

In November 2021, a high-density SNP-Array test did not detect gains or losses of chromosomal segments considered pathogenic, probably pathogenic or of uncertain clinical significance, which excluded syndromes known due to microdeletion or microduplication in the genome.

2.3. Patient 2

B.B.R, female, 10 days of birth, second child of healthy non-consanguineous parents, younger proband's sister. Immediately after hospital discharge, she was tested for the variants found in her brother and the exact same *PIGN* pathogenic variant and the variant of uncertain significance (VUS) were found in her. At this point, it was hard to determine if she had signs of disease, but her father felt she was hypotonic like her brother. Two months after birth, epileptic seizures and hypotonia became clear symptoms.

2.4. Biological samples

DNA extracted from blood samples were tested by next-generation sequencing with simultaneous detection of both sequence and exon-level copy number variants as previously described [12–14]. Briefly, each gene was targeted with oligonucleotide baits (Agilent Technologies, Santa Clara, CA; Roche, Pleasanton, CA; IDT, Coralville, IA) that were designed to capture exons and 10 bases of flanking intronic sequences. Genes were sequenced to an average of 350X high-depth coverage (50X minimum). Reads were aligned, and single nucleotide variants, small insertions or deletions, large indels, structural variants, and exon-level copy number variants were identified by standard and custom algorithms [12,13].

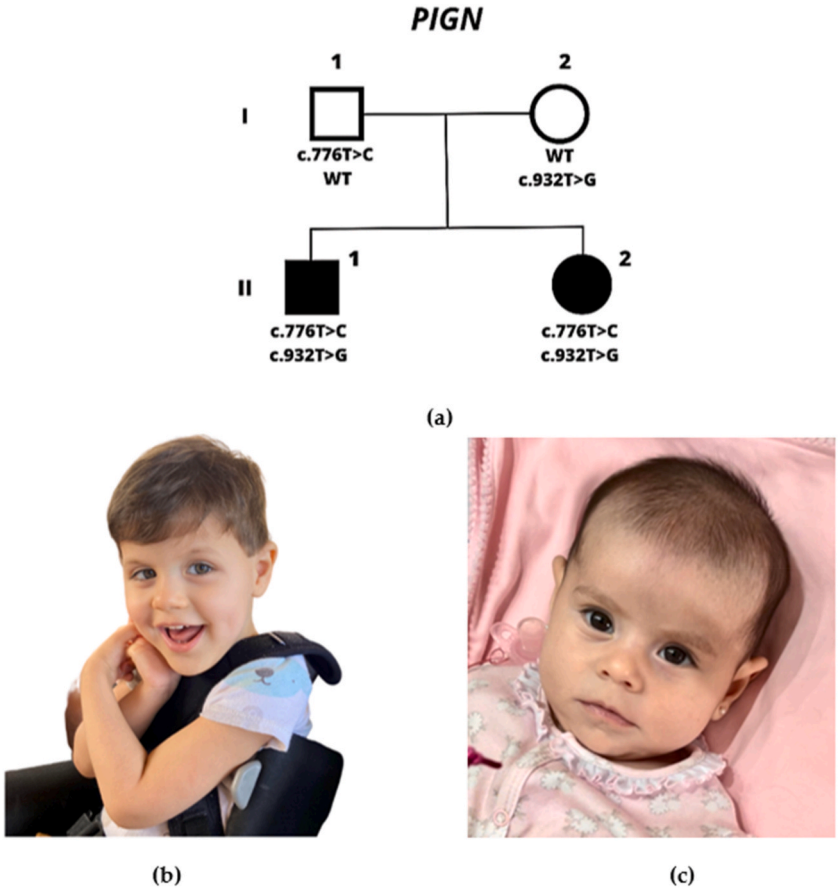

Fig. 1. (a) Family pedigree and PIGN gene variants; photographs of (b) Patient 1 and (c) Patient 2.

2.5. Genetic panels for suspected disorders

Genomic DNA was enriched for targeted regions using a hybridization-based protocol (described above) and sequenced using Illumina technology.

In May 2022, three genetic panels by Invitae® Corporation were performed on the proband: a Leukodystrophy and Leukoencephalopathy Panel, which analyzes 697 genes; a Neuromuscular Disorder Panel of 230 genes; and a Lysosomal Disease Panel of 266 genes.

After that, parental studies were performed for significant genetic variants found in the proband to investigate if the variants were in trans allelic pattern of inheritance. Finally, patient 2 underwent a genetic test after birth.

3. Results

Results of the three genetic panels identified pathogenic variants in *PIGN*, *CLN6*, *FA2H* and *GAA* genes and variants of uncertain significance in *PIGN*, *ALG9*, *CNTNAP1*, *NAGA*, *PRF1*, *PRKDC*, *RAB3GAP1*, *SYNE1*, *PYGM* and *SPEG* genes (Table 1).

The proband was a *PIGN* gene compound heterozygous for two variants: c.932T > G (p.Leu311Trp), a pathogenic variant in exon 11, and c.776T > C (p.Phe259Ser), a variant of uncertain significance in exon 9. Together these variants were considered the most likely causes of the clinical condition.

The *PIGN* gene is associated with an autosomal recessive PIGN-Congenital Disorder of Glycosylation (PIGN-CDG), and one pathogenic variant is normally inherited from each parent. Because the proband's clinical manifestations could be attributed to this disorder, a genetic test was performed on his parents to confirm inheritance.

A *PIGN* pathogenic variant (c.932T > G) was found in the proband's mother and the *PIGN* VUS (c.776T > C) in his father. Alone, these variants are insufficient to cause the disorder, conferring only reproductive risk, but together, considering that this VUS is potentially a pathogenic variant, they could result in the PIGN-CDG diagnosis.

Genetic tests of Patient 2 showed the same *PIGN* variants as Patient 1: the pathogenic variant c.932T > G (p.Leu311Trp) and the VUS c.776T > C (p.Phe259Ser).

Other variants were also identified, but are insufficient to cause specific diseases. The genetic tests summary is shown in Table 1. Family pedigree, mutations and patient photographs are shown in Fig. 1 (a – c).

4. Discussion

Defects of human glycosylphosphatidylinositol (GPI) anchor biosynthetic pathway are associated with a broad range of clinical phenotypes and can cause congenital disorders of glycosylation [11,15]. *PIGN* is one of 26 genes involved in GPI pathway and biallelic variants are associated with a spectrum of phenotypes from Fryns syndrome, MCAHS, to those mainly neurological manifestations without visceral congenital anomalies [11,16]. The reason for this phenotype diversity remains unknown, but the different clinical conditions depend on the nature and location of the genetic variant [17].

Siavriene et al. [17] reviewed 76 individuals previously reported with *PIGN* variants, 33 males and 43 females from 66 unrelated families and with a wide range of clinical manifestations. Phenotype–genotype correlation analysis showed that there is a predominance of missense *PIGN* variants, but there are also many splicing and nonsense variants. Furthermore, approximately 70% of reported DNA sequence variants were found in compound heterozygotes of the *PIGN* gene [17].

Loong et al. [16] described 21 patients that together with other 40 patients presented in the literature account for 61 patients with biallelic *PIGN* variants [16]. The symptoms of biallelic *PIGN* variants usually includes psychomotor development delay, hypotonia, seizures and dysmorphic features [10]. The most commonly reported *PIGN* disease related variant is the c.932T > G. All reported individuals with this missense variant were compound heterozygous with a different second variant [17].

Here, we describe two siblings who are *PIGN* compound heterozygotes with clinical symptoms since birth of neuromotor development delay, dysmorphic clinical signs, lack of motor coordination, hypotonia and spasms. The known pathogenic variant c.932T > G (p.Leu311Trp) was inherited from the mother and implies the substitution of leucine to tryptophan at *PIGN* codon 311. The variant c.776T > C (p.Phe259Ser) of uncertain significance was inherited from the father and leads to the replacement of phenylalanine for a serine at codon 259.

This is the first time that *PIGN* variant c.776T > C is reported in individuals with PIGN congenital disorder of glycosylation. Currently, this variant of uncertain significance has 2.5 pathogenic points according to Invitae® VUS score and needs to reach a score of 4 to be considered likely pathogenic. Initially, the variant was awarded 1 pathogenic point due to population models giving the variant a moderate to highly predictive pathogenic score; 0.5 pathogenic point was due to *in silico* models predicting the variant to be deleterious; 1 additional point was due to the variant being found in trans with a pathogenic variant in the proband presented here. Now, we report a second individual (Patient 2) with *PIGN* variant c.776T > C in trans pattern of inheritance with the pathogenic variant c.932T > G. This is new evidence, showing for the first time two affected siblings with one pathogenic variant and the c.776T > C VUS in trans, which may increase the pathogenic score over the current 2.5 points.

5. Conclusions

Owing to the evidences presented here, we suggest that the variant c.776T > C (p.Phe259Ser) identified on *PIGN* gene exon 9 is potentially a pathogenic variant, since its combination with a pathogenic variant in trans caused the PIGN-congenital disorder of

glycosylation in two siblings. As per the family request and in honor of the patients that allowed for this conclusion, we propose the name Bella-Noah Syndrome for this PIGN-congenital disorder of glycosylation.

Data availability statement

Data included in article/supp. material/referenced in article.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Lyvia Neves Rebello Alves: Writing – review & editing, Writing – original draft, Supervision, Methodology, Data curation, Conceptualization. **Lívia Valle dos Santos Silveira:** Writing – original draft, Data curation. **Raquel Silva dos Reis Trabach:** Writing – review & editing, Data curation. **Débora Dummer Meira:** Writing – review & editing, Data curation. **Eldamária de Vargas Wolfgramm dos Santos:** Writing – review & editing, Data curation. **Iúri Drumond Louro:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are grateful to the family for participating in this study.

References

[1] J. Paprocka, A. Jezela-Stanek, A. Tylki-Szymanska, S. Grunewald, Congenital disorders of glycosylation from a neurological perspective, Brain Sci. 11 (2021) 88, https://doi.org/10.3390/brainsci11010088.
[2] H.H. Freeze, E.A. Eklund, B.G. Ng, M.C. Patterson, Neurology of inherited glycosylation disorders, Lancet Neurol. 11 (2012) 453–466, https://doi.org/10.1016/S1474-4422(12)70040-6.
[3] I.J. Chang, M. He, C.T. Lam, Congenital disorders of glycosylation, Ann. Transl. Med. 6 (2018) 477, https://doi.org/10.21037/atm.2018.10.45.
[4] P. Lipinski, A. Tylki-Szymanska, Congenital disorders of glycosylation: what clinicians need to know? Front Pediatr 9 (2021) 715151 https://doi.org/10.3389/fped.2021.715151.
[5] N. Ondruskova, A. Cechova, H. Hansikova, T. Honzik, J. Jaeken, Congenital disorders of glycosylation: still "hot" in 2020, Biochim. Biophys. Acta Gen. Subj. 1865 (2021) 129751, https://doi.org/10.1016/j.bbagen.2020.129751.
[6] G. Maydan, I. Noyman, A. Har-Zahav, Z.B. Neriah, M. Pasmanik-Chor, A. Yeheskel, et al., Multiple congenital anoma-lies-hypotonia-seizures syndrome is caused by a mutation in PIGN, J. Med. Genet. 48 (2011) 383–389, https://doi.org/10.1136/jmg.2010.087114.
[7] A. Jezela-Stanek, E. Ciara, D. Piekutowska-Abramczuk, J. Trubicka, E. Jurkiewicz, D. Rokicki, et al., Congenital disorder of glycosylphosphatidylinositol (GPI)-anchor biosynthesis–The phenotype of two patients with novel mutations in the PIGN and PGAP2 genes, Eur. J. Paediatr. Neurol. 20 (2016) 462–473, https://doi.org/10.1016/j.ejpn.2016.01.007.
[8] X. Jiao, J. Xue, P. Gong, X. Bao, Y. Wu, Y. Zhang, et al., Analyzing clinical and genetic characteristics of a cohort with multiple congenital anomalies-hypotonia-seizures syndrome (MCAHS), Orphanet J. Rare Dis. 15 (2020) 78, https://doi.org/10.1186/s13023-020-01365-0.
[9] S.Q. Xiao, M.H. Li, Y.L. Meng, C. Li, H.L. Huang, C.X. Liu, et al., Case report: compound heterozygous phos-phatidylinositol-glycan biosynthesis class N (PIGN) mutations in a Chinese fetus with hypotonia-seizures syndrome 1, Front. Genet. 11 (2020) 594078, https://doi.org/10.3389/fgene.2020.594078.
[10] M. Tian, J. Chen, J. Li, H. Pan, W. Lei, X. Shu, Damaging novel mutations in PIGN cause developmental epileptic-dyskinetic encephalopathy: a case report, BMC Pediatr. 22 (2022) 222, https://doi.org/10.1186/s12887-022-03246-w.
[11] I. Thiffault, B. Zuccarelli, H. Welsh, X. Yuan, E. Farrow, L. Zellmer, et al., Hypotonia and intellectual disability without dysmorphic features in a patient with PIGN-related disease, BMC Med. Genet. 18 (2017) 124, https://doi.org/10.1186/s12881-017-0481-9.
[12] S.E. Lincoln, Y. Kobayashi, M.J. Anderson, S. Yang, A.J. Desmond, M.A. Mills, et al., A systematic comparison of traditional and multigene panel testing for hereditary breast and ovarian cancer genes in more than 1000 patients, J. Mol. Diagn. 17 (2015) 533–544, https://doi.org/10.1016/j.jmoldx.2015.04.009.
[13] R. Truty, J. Paul, M. Kennemer, S.E. Lincoln, E. Olivares, R.L. Nussbaum, et al., Prevalence and properties of in-tragenic copy-number variation in Mendelian disease genes, Genet. Med. 21 (2019) 114–123, https://doi.org/10.1038/s41436-018-0033-5.
[14] S.E. Lincoln, R. Truty, C.F. Lin, J.M. Zook, J. Paul, V.H. Ramey, et al., A rigorous interlaboratory examination of the need to confirm next-generation sequencing-detected variants with an or-thogonal method in clinical genetic testing, J. Mol. Diagn. 21 (2019) 318–329, https://doi.org/10.1016/j.jmoldx.2018.10.009.
[15] C. Ohba, N. Okamoto, Y. Murakami, Y. Suzuki, Y. Tsurusaki, M. Nakashima, et al., PIGN mutations cause congenital anomalies, developmental delay, hypotonia, epilepsy, and pro-gressive cerebellar atrophy, Neurogenetics 15 (2014) 85–92, https://doi.org/10.1007/s10048-013-0384-7.
[16] L. Loong, A. Tardivo, A. Knaus, M. Hashim, A.T. Pagnamenta, K. Alt, et al., Biallelic variants in PIGN cause Fryns syndrome, multiple congenital anomalies-hypotonia-seizures syndrome, and neurologic phenotypes: a genotype-phenotype correlation study, Genet. Med. 25 (2023) 37–48, https://doi.org/10.1016/j.gim.2022.09.007.
[17] E. Siavrienė, Maldžienė Ž, V. Mikštienė, G. Petraitytė, T. Rančelis, J. Dapkūnas, et al., PIGN-related disease in two Lithuanian families: a report of two novel pathogenic variants, molecular and clinical characterisation, Medicina 58 (2022) 1526, https://doi.org/10.3390/medicina5811152.