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Dyslipidemia and hypercalciuria in a patient with pantothenate kinase 2 deficiency: A novel variant and case report

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Abstract

Pantothenate kinase-associated neurodegeneration (PKAN, OMIM: 234200) results from biallelic pathogenic variants in *PANK2* which encodes pantothenate kinase 2, a crucial mitochondrial enzyme involved in coenzyme A biosynthesis. Pantothenate kinase-associated neurodegeneration patients typically exhibit the distinctive "eye of the tiger" sign on brain magnetic resonance imaging in the globus pallidus, along with psychiatric symptoms, extrapyramidal movements such as parkinsonism and dystonia, eventual speech and gait impairments, and the presence of dysphagia. An 11-year-old girl, with fifth-degree consanguinity, demonstrated typical psychomotor development and growth until the age of 5, when she began experiencing psychiatric symptoms. At the age of 9, she developed hand tremors, progressing to generalized muscular dystonia. By age 10, she exhibited gait and speech impairment. Physical examination revealed extensive generalized dystonia, hand tremors, speech impairment, dysphagia, inability to walk, and heightened osteotendinous reflexes. Metabolic analysis identified dyslipidemia with partial response to statin treatment and normocalcemic hypercalciuria. Exome sequencing revealed a novel likely pathogenic variant in *PANK2* (NM_001386393.1:c.526C > G) in a homozygotic state. Pantothenate kinase-associated neurodegeneration typically manifests with generalized dystonia and psychiatric symptoms. Here, we present a Pantothenate kinase-associated neurodegeneration patient with dyslipidemia and hypercalciuria as potentially previously undescribed metabolic phenotype.

Keywords

PKAN, PANK2, pantothenate kinase 2, dystonia

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Introduction

Pantothenate kinase-associated neurodegeneration (PKAN, OMIM: 234200) falls under the category of Neurodegeneration with Brain Iron Accumulation (NBIA), a diverse group of progressive disorders characterized by the abnormal accumulation of iron in the brain.[1] The distinctive radiological feature among all NBIA disorders is the presence of elevated levels of brain iron, particularly within the basal ganglia. NBIA exhibits genetic heterogeneity, with 16 identified genes thus far: *FTL, CP, PLA2G6, C19orf12, WDR45, FA2H, ATP13A2, DCAF17, COASY, GTPBP2, SCP2, REPS1, CRAT, AP4M1, FBXO7*, and *PANK2*.[2] Pantothenate kinase 2 (PANK2) is the only member of the PANK family that is present in mitochondria as well as in the nucleus. PANK serves as a crucial regulatory enzyme in the biosynthesis of coenzyme A (CoA), catalyzing the cytosolic phosphorylation of pantothenate

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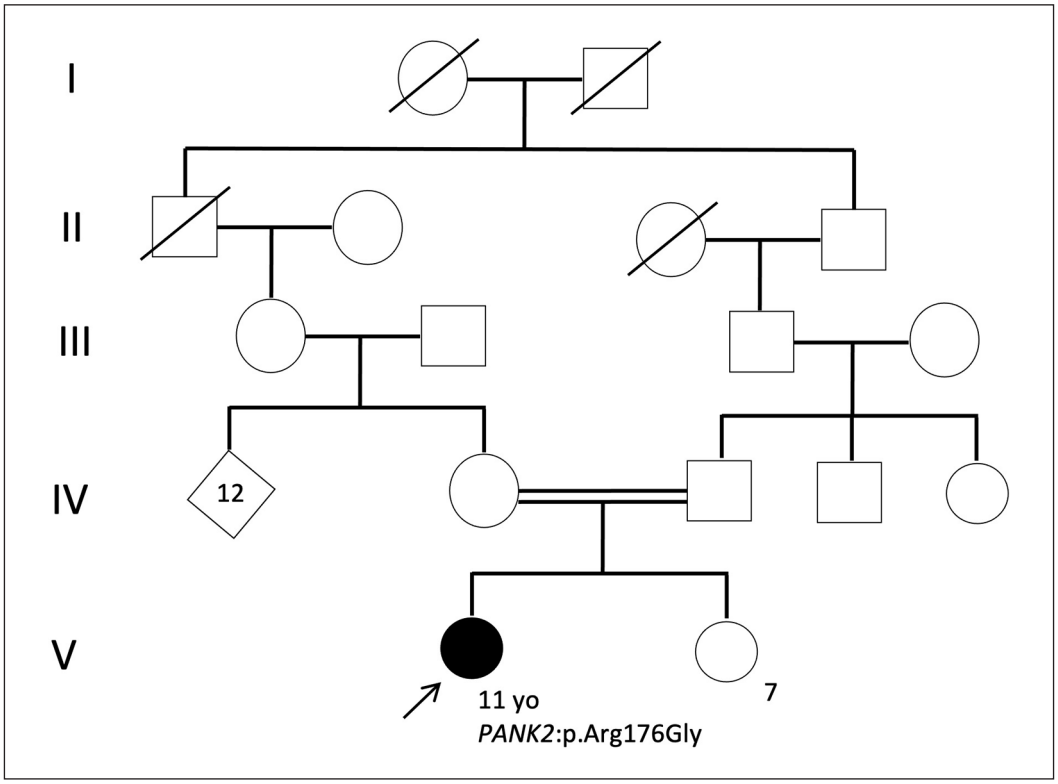

Figure 1. The family pedigree of propositus (V,1) shows a fifth-degree consanguinity, both parents are second-degree cousins.

(vitamin B5), N-pantothenoyl-cysteine, and pantetheine. CoA plays a pivotal role as the primary acyl carrier in intermediary metabolism (fatty acid metabolism) and protein modification. Pathogenic variants in *PANK2* are anticipated to cause CoA depletion, disruptions in energy generation, heightened oxidative stress, and impaired membrane synthesis, resulting in symptoms primarily affecting tissues with high membrane turnover rates, such as the central nervous system and retina.[3,4]

In normal brains, nonheme iron accumulates in specific regions like the medial globus pallidus and the substantia nigra pars reticulata, which are affected by PKAN. In PKAN, there is a deficiency of phosphopantothenate, leading to the accumulation of cysteine, which rapidly autoxidizes in the presence of iron, thereby generating free radicals. The cytotoxicity of cysteine, along with lipid peroxidation and impaired membrane biosynthesis, is proposed as the underlying mechanism for neurodegeneration in PKAN.[5] All this results in psychiatric symptoms appearing in the early stages of the disease,[6] which later progress to extrapyramidal movements, such as parkinsonism and dystonia, eventually affecting speech, and gait, leading to the presence of dysphagia. Brain magnetic resonance imaging (MRI) of PKAN patients typically reveals the distinctive "eye of the tiger" sign in the globus pallidus, indicative of iron accumulation and gliosis. Understanding the various mechanisms underlying the pathophysiology of PKAN is essential for the development of effective therapeutic approaches. These may include strategies aimed at mitigating iron accumulation, replenishing phosphopantothenate levels in PKAN cells, activating CoA biosynthesis, and exploring gene therapy options.[7] PKAN is classified into classic and atypical forms, with distinctions based on age of onset and rate of progression. Classic PKAN typically manifests in early childhood (within the first decade of life) and progresses rapidly, often resulting in loss of gait by the second decade of life. By contrast, atypical PKAN presents in the second decade of life and progresses more gradually over time.[8]

Case report/case presentation

We present an 11-year-old female patient with a fifth-degree consanguineous family background (shown in Figure 1), born after an uncomplicated pregnancy. Her parents and a 7-year-old sister are healthy. The proband exhibited typical psychomotor development and growth until the age of 5 when she started experiencing psychiatric symptoms, including visual and auditory hallucinations as well as irrational fears. At the age of 9, she began to develop hand tremors, which later evolved into generalized muscular dystonia. There was a loss of gait and speech by the age of 10. Physical examination reveals extensive generalized dystonia, hand tremors, speech impairment, dysphagia, inability to walk, and heightened osteotendinous reflexes.

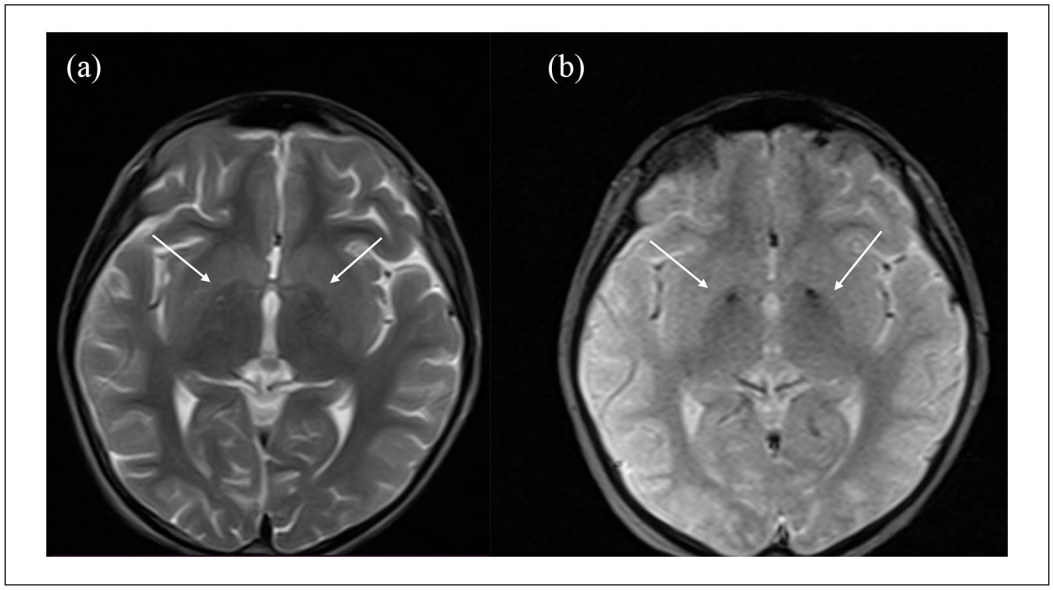

Figure 2. T2-weighted brain (a) and T2-weighted GRE magnetic resonance imaging (b) revealed hypointensity with central hyperintensity in both globi pallidi, indicative of the "eye-of-the-tiger" sign.

T2-weighted MRI images revealed evidence of iron accumulation, indicated by the "eye of the tiger" sign, displaying a ventral hyperintensity in the globus pallidus (shown in Figure 2). No additional abnormalities were detected in the MRI image pattern.

Biochemical analysis revealed normocalcemic hypercalciuria (urine calcium range: 13.29–42.5 mg/dL (mean: 21.64)), dyslipidemia (total cholesterol range: 157–240 mg/dL (mean: 192 mg/dL, total low-density lipoprotein range (LDL): 102–148 mg/dL (mean: 125 mg/dL)), triglycerides range: 142–237 mg/dL (mean: 177 mg/dL), which had a partial response to statins. Atorvastatin 20 mg daily was initiated when LDL cholesterol was at 148 mg/dL, resulting in actual control levels of LDL cholesterol at 102 mg/dL, and normal levels of ceruloplasmin (23 mg/dL).

The peripheral blood of the patient was collected and stored as a dry blood spot (DBS) sample for exome analysis. Genomic DNA was extracted from the DBS specimen using standard protocol. Exome capture was performed using xGen Exome Research Panel v2 (Integrated DNA Technologies, Coralville, Iowa, USA). Sequencing was performed using NovaSeq 6000 (Illumina, San Diego, CA, USA). In total, 8,171,963,246 bases of sequence were generated and uniquely aligned to the Genome Reference Consortium Human Build 38 (GRCh38) within Galaxy Project using the BWA-MEM2 protocol.[9] Approximately 99% of the targeted bases were covered to a depth of $>20\times$. Alignments were analyzed using IGV software (San Diego, CA, USA. Ver. 2.15.4). Variant calling was performed using the DeepVariant protocol[10] and analyzed within the Franklin platform by Genoox.[11]

One likely pathogenic variant was located in chr20:3,908,153C > G (shown in Figure 3) (c.526C > G:p.Arg176Gly) in homozygosity. This results in a transversion from cytosine to guanine at position 526 of the exon 2 of the *PANK2* gene (NM_001386393.1), leading to a change from arginine to glycine in the position 176 of the protein. According to the American College of Medical Genetics (ACMG) guidance, this variant is classified as likely pathogenic, with the following characteristics: Extremely low frequency in gnomAD population databases (PM2), a different amino acid change as a known pathogenic variant (PM5), a non-synonymous variant is located in a mutational hot spot and critical and well-established functional domain (PM1), missense variant in a gene with a low rate of benign missense mutations and for which missense mutations is a common mechanism of disease (PP2), computational prediction tools unanimously support a deleterious effect on the gene (PP3).

After the patient's diagnosis, treatment consisted of benzodiazepines and muscle relaxants, resulting in a mild improvement in dystonia but a favorable response in pain management. Due to chronic dysphagia, the patient developed malnutrition and low body weight. As a result, a pediatric gastroenterologist and pediatric surgeon performed a gastrostomy placement. The family received comprehensive education on how to utilize the gastrostomy, and as a result, the patient has gained some body weight.

Discussion

We present a patient exhibiting typical PKAN syndrome characterized by dystonia, psychiatric symptoms, and

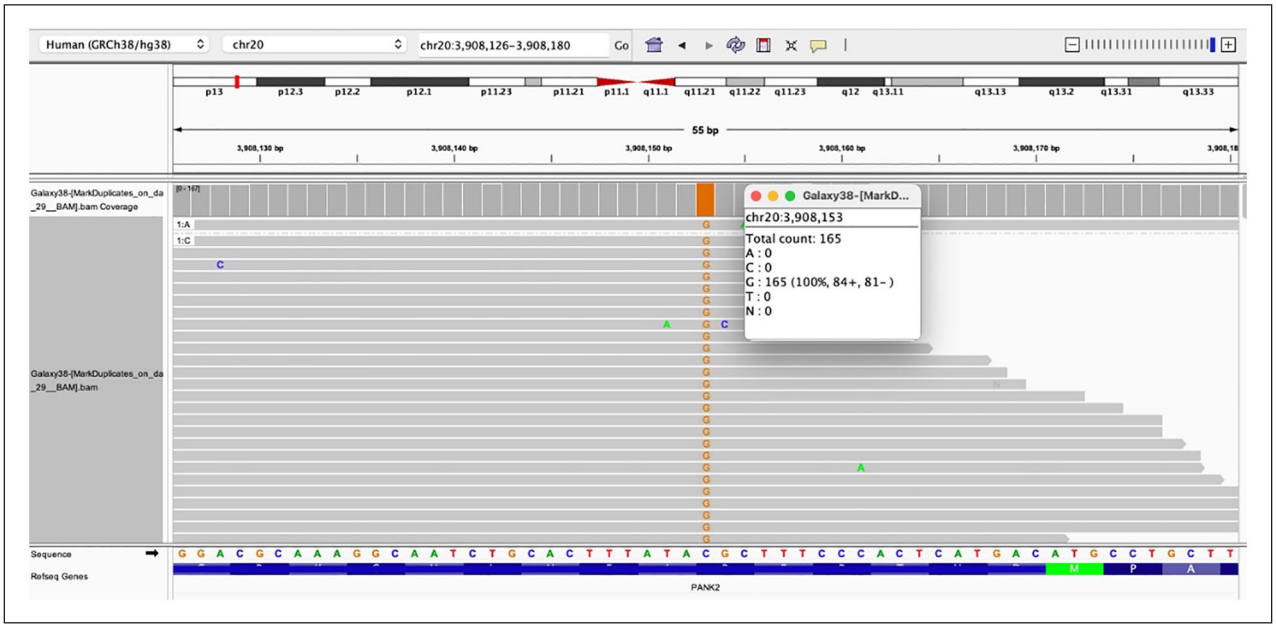

Figure 3. Screenshot of IGV software showing BAM file of exome alignment, position chr20:3,908,153, indicating a cytosine to guanine transversion in 100% of the analyzed sequences (165× depth).

metabolic disorders such as dyslipidemia and hypercalciuria, along with a novel likely pathogenic variant in *PANK2*. Psychiatric symptoms manifested as early as 5 years of age, followed by the onset of hand tremors, speech impairment, dysphagia, and inability to walk at age 9. These symptoms progressed to generalized dystonia and inability to walk, consistent with typical PKAN cases.[12] Notably, we emphasize the presence of dyslipidemia and hypercalciuria, which have not been previously reported in the literature.

We performed whole-exome sequencing on the proband, prompted by clinical findings and a family pedigree exhibiting autosomal recessive characteristics. Following bioinformatic filtration of numerous variants, a likely pathogenic variant was identified in *PANK2* (c.526C > G:p.Arg176Gly). Whole Exome Sequencing (WES) is a potent diagnostic tool for identifying genetic variants across the entire coding region of a patient's genome. In patients with a high suspicion of a genetic disease, diagnostic rates with WES range from 25% to over 50%. Furthermore, utilizing various bioinformatic tools, both short-read or medium-read WES can potentially identify large copy number variants (CNVs), enhancing its diagnostic utility. Even with emerging sequencing technologies, such as large-read WES, it remains possible to identify CNVs with high sensitivity.[13]

Pathogenic and likely pathogenic variants have been documented in all seven exons of *PANK2*. The amino acid Arg176 is situated within the intermediate/regulatory region of PANK2 (residues 46–211),[14] and is a position commonly regarded as pathogenic. Previous reports[5] described a transition (c.556C > T) from cytosine to thymine at position 556 of the coding sequence, leading to a change from arginine to cysteine in position 176 of the protein sequence (p.Arg176Cys) in a patient with classic PKAN. However, our report highlights a transversion (c.556C > G) from cytosine to guanine at the same point in position 556 of the coding sequence, resulting in a change from arginine to glycine in position 176 of the protein sequence (p.Arg176Gly). Arginine is a positively charged polar amino acid, while glycine and cysteine are both small hydrophobic amino acids, hence leading to a similar outcome in the mutated protein, explaining the classic phenotype in the patient.

PANK2 is a mitochondrial enzyme involved in the CoA synthesis and lipid homeostasis. CoA plays a crucial role in lipid synthesis, and studies in animal models such as *Drosophila melanogaster* have shown a reduction in triglycerides serving as stored fatty acids, indicating impaired lipid homeostasis in these models.[3] Alterations in lipid metabolism have been described in humans with pathogenic variants in *PANK2*, presenting as "HARP syndrome" (hypoprebetalipoproteinemia, acanthocytosis, retinitis pigmentosa, and pallidal degeneration),[15] which is an allelic disease to PKAN. Although the proper terminology for lipid metabolism in PKAN and its allelic diseases has been under discussion, it is evident that further analysis is needed.[16]

However, we present a patient with dyslipidemia (elevated LDL, total cholesterol, and triglycerides) who exhibited a partial response to statins, contrary to the expected phenotype[17,18] which entails lower levels of cholesterol due to impairment of lipid metabolism. It is important to note that we ruled out other causes of LDL dyslipidemia such as obesity (normal body mass index), thyroid disease, familial dyslipidemia, or nutritional factors due to the patient's

Table 1. Genetic and phenotypic characteristics of 14 patients with Pantothenate kinase-associated neurodegeneration in Mexico.

Variable	Proband	Gonzalez-Huerta LM, 2021[6]	Perez-Gonzalez EA, 2013[18]	Morales-Briseño H, 2014[19]
Genotype *PANK2* (NM_001386393.1)	c.556C > G (p.Arg176Gly) (exon 2, homozygotic)	1 Patient c.949G > A(p.Gly317Arg) (exon 2) c.1688T > C(p.Leu563Pro) (exon 7)	1 patient c.1561G > A (p.Gly521Arg) (exon 6) c.1663G > A: p.Gly555Ser (exon 7)	11 patients c.656G > T(p.Gly219Val) (exon 2) c.1211A > T(p.Asn404Ile) (exon 3) c.1405G > C(p.Ala469Pro) (exon 4)
Phenotype				
Sex	Female	Female	Male	6 Males 5 Females
Age at examination (years old)	11	16	26	21 (mean age)
Age of onset of symptoms (years old)	5	12	4	12 (mean age)
Consanguinity	Yes	No	No	No
Extrapyramidal symptoms				
Dystonia	Yes	Yes	Yes	Yes (8 patients) No (3 patients)
Parkinsonism	No	Yes	Not reported	Yes (6 patients) No (5 patients)
Tremor	Yes	Yes	Not reported	Not reported
Ataxia	No	Not reported	Not reported	Not reported
Chorea	No	Not reported	Yes	Not reported
Other neurological findings				
Extraocular movements	No	Not reported	Not reported	Yes (5 patients) No (6 patients)
Speech difficulty	Yes	Yes	Yes	Yes (11 patients)
Dysphagia	Yes	Not reported	Yes	Yes (1 patient)
Hyperreflexia	Yes	Yes		
Ocular findings	None	Not reported	Retinitis pigmentosa	Retinitis pigmentosa (1 patient)
Gait				
Dystonic	Not applicable	Yes	Yes	
Non-ambulant	Yes	No	No	Yes (3 patients)
Psychiatric symptoms				
Visual and auditory hallucinations	Yes	Yes	Not reported	Not reported
Obsessive-compulsive disorder	No	Not reported	Not reported	Yes (5 patients)
Self-injury	Yes	Yes	Not reported	Not reported
Aggressive behavior	Yes	Yes	Not reported	Yes (5 patients)
Metabolic findings				
Dyslipidemia	Yes	Not reported	Not reported	Not reported
Hypercalciuria	Yes	Not reported	Not reported	Not reported

feeding difficulties (dysphagia and dystonia). We propose that dyslipidemia may be part of the phenotype that is not yet fully understood in humans.

The proband exhibits normocalcemic hypercalciuria, a manifestation not previously reported as part of the PKAN phenotype. Normocalcemic hypercalciuria arises from the kidney failure to adequately reabsorb calcium from the proximal convoluted tubule and thick ascending limb of the loop of Henle. Mitochondria play a pivotal role in calcium reabsorption from the tubules.[19] Although renal disturbances are not typically described in PKAN, the hypercalciuria observed in this patient may be attributed to mitochondria dysfunction at the renal level. It is important to consider other causes of hypercalciuria that have been ruled out in the patient, such as dehydration, excessive calcium intake, and pathogenic variants in genes related to calcium metabolism, such as vitamin D receptor and calcium-sensing receptor.

In this study, we compared propositus with 3 previous reports that included 13 patients from 7 families with the same diagnosis from Mexico[6,20,21] (as shown in Table 1). Our case is the first Mexican patient reported with a consanguineous family background and a more severe phenotype: the propositus has very strong dystonia that leads to the loss of gait. In addition, we report new clinical findings such as dyslipidemia and hypercalciuria. Common findings include psychiatric symptoms, such as visual and auditory hallucinations, self-injury, and aggressive behavior. However, there are signs not present in our patient, like abnormal ocular movements and retinitis pigmentosa. We consider that these clinical differences cannot be solely explained by genotype. Evidence shows that one factor predicting progression is an early age of onset ($<$10 years old) and the presence of two null variants in *PANK2*.[22] However, our patient only exhibits a very early age of onset (5 years old). There are no genotype–phenotype reports that demonstrate a correlation between pathogenic variants in the regulatory region of *PANK2* and phenotype severity.

Conclusion

We have presented the clinical profile of a typical patient with PKAN and compared it with existing literature. We also identified a novel likely pathogenic variant in *PANK2* in homozygosis. Furthermore, we described the metabolic findings in this patient that might be associated with PANK2 deficiency: dyslipidemia and hypercalciuria. Our study contributes to the expansion of the clinical and genetic spectrum of patients with PKAN.

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Author contributions

H.M.R.P. Medical management of the case, literature review, writing the initial draft of the manuscript; O.B.R.F. Medical management of the case, literature review, writing the initial draft of the manuscript, description of neurological images, and revising the manuscript critically.

Y.Q.P. Medical management of the case, conceptualization, description, and revision of the case, and revising the manuscript critically; Y.A.C.N. Medical management of the case, conceptualization, description, and revision of the case, revising the manuscript critically; C.G.V. Medical management of the case, conceptualization, description, and revision of the case, revising the manuscript critically; F.J.C.G. Medical management of the case, literature review, writing the initial draft of the manuscript, conducting genetic and bioinformatic studies, revising, and approving the final draft, and revising the manuscript critically.

Data availability statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethics approval

This case report was reviewed and approved by the Research and Ethics committee of The General Hospital "Dr. Agustin O'Horan," approval number CI-012-1-24.

Informed consent

Written informed consent was obtained from a legally authorized representative for anonymized patient information to be published in this article.

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