

Milder form of Vici syndrome due to novel missense variant in EPG5 gene affecting splicing: A case report

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Objective. Vici syndrome is a rare neurodevelopmental disorder with multisystem involvement, caused by mutations in the *EPG5* gene encoding a protein involved in autophagy. It includes dysgenesis of the corpus callosum, cataracts, hypopigmentation, cardiomyopathy, and immunodeficiency. Here we report a case of a 9-year-old boy of Roma ethnicity with a milder form of Vici syndrome and a novel variant in the *EPG5* gene.

Methods. DNA and RNA were extracted from the whole blood. Whole exome sequencing was performed and analysed with an in-house bioinformatics pipeline. A mini-gene assay was performed for *EPG5* exon 23 with or without the tested variant.

Results. The patient was born prematurely and presented with hypotonia, severe hypotrophy and growth retardation, developmental delay, congenital heart defects, mild brain atrophy, and a thin corpus callosum. Whole exome analysis identified a novel variant c.4205G>A, p.(Arg1402Lys) in the *EPG5* gene, suggesting the diagnosis of Vici syndrome. Further examination of symptoms commonly associated with Vici syndrome confirmed hypopigmented skin areas and immunodeficiency. No seizures, cataracts, or cardiomyopathy were observed. As the variant is located at the last base of exon 23, we sequenced the patient's *EPG5* mRNA and detected aberrant transcripts in addition to correctly spliced ones. The mini-gene assay confirmed decreased inclusion of the mutated exon compared with the wild-type (40% vs. 72%, respectively).

Conclusion. Novel variant *EPG5*:c.4205G>A, p.(Arg1402Lys) causes aberrant splicing only in a small proportion of transcripts; therefore, the milder presentation of Vici syndrome in our patient is probably due to the residual presence of *EPG5* protein.

Keywords: rare disease, Vici syndrome, *EPG5*, splicing, mini-gene assay, phenotypic spectrum

Vici syndrome (MIM#242840) is a rare autosomal recessive disorder belonging to the congenital disorders of autophagy. It is caused by mutations in the gene *EPG5* (MIM#615068) encoding ectopic P granules protein, a key tethering factor facilitating fusion of autophagosomes and lysosomes. Its deficiency results in failure of this fusion,

accumulation of autolysosomes and hybrid endosomal structures, and defect of cellular waste clearance (Cullup et al. 2013; Zhao et al. 2013; Yuan et al. 2025). Vici syndrome was initially described as a neurodevelopmental disorder with multisystem involvement, including global developmental delay, failure to thrive, postnatally acquired microcephaly,

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corpus callosum agenesis, cataracts, hypertrophic and/or dilated cardiomyopathy, pigmentary defects, and immunodeficiency (Vici *et al.* 1988; Cullup *et al.* 2013; Byrne *et al.* 2016). However, more than 200 cases have been reported in the literature to date summarized in Yang *et al.* (2025) and Dafsari *et al.* (2025), showing that the phenotypic spectrum can be wider, ranging from antenatally lethal presentations to milder isolated neurodevelopmental disorders such as adolescent-onset parkinsonism and dystonia with cognitive decline, and myoclonus (Dafsari *et al.* 2025). The majority of pathogenic variants are loss-of-function, but some missense or splicing variants that do not significantly reduce mRNA expression were found in patients with milder phenotypes. It has been hypothesized that the severity of the phenotype may be related to the residual functional EPG5 level (Kane *et al.* 2019). In agreement with this, increased risk of movement disorders was observed in heterozygous EPG pathogenic variant carriers, indicating a gene dosage effect (Byrne *et al.* 2016).

Here we report a case with a clinical picture consistent with Vici syndrome and a homozygous variant of unknown significance in the EPG gene with evidence of impact on splicing.

Materials and Methods

Whole exome sequencing (WES). Genomic DNA was isolated from peripheral blood using standard procedures. WES was done by a service provider (BGI, Hong Kong). DNA library was prepared using BGI 59M Human Exome kit and was sequenced on Complete Genomics Black Bird platform (BGI, Shenzhen, China). Sequencing data was processed by BGI's standard bioinformatics pipeline, which encompassed base calling, alignment of generated reads to the GRCh37 reference genome without the unplaced or alternate loci, and variant calling of SNPs, short insertions and deletions up to approximately 50 base pairs. Aligned reads and called variants were obtained in standard bioinformatics formats and subjected to the following bioinformatics pipeline. Variants were decomposed and normalized using vt (Tan *et al.* 2015). The Variant Effect Predictor (McLaren *et al.* 2016) was used to annotate variants with respect to their potential effects on genes and transcripts. The Gemini framework (Paila *et al.* 2013) was used to insert variants into a SQLite database and to add additional data from genome annotation databases like dbSNP (Sherry *et al.* 2001), or gnomAD (Chen *et al.* 2024). Variants were filtered using 0.011 minor allele frequency (max_aaf_all

<0.01 parameter in Gemini) and prioritized using VEP annotations.

In silico analysis. Functional consequences of the candidate EPG5 missense variant were evaluated using PolyPhen-2, SIFT, PROVEAN and AlphaMissense prediction algorithms (Kumar *et al.* 2009; Adzhubei *et al.* 2010; Choi and Chan 2015; Cheng *et al.* 2023). The consequences involving splicing were assessed using MaxEntScan, Human Splicing Finder, NNSplice, and SpliceAI (Reese *et al.* 1997; Yeo and Burge 2004; Desmet *et al.* 2009; Jaganathan *et al.* 2019). Pathogenicity assessed by combining multiple *in silico* tools was predicted by CADD and REVEL metapredictors (Kircher *et al.* 2014; Ioannidis *et al.* 2016).

Sanger sequencing. Sanger sequencing was performed to confirm the presence of the candidate variant in the genome and to analyse EPG5 mRNA after reverse transcription into cDNA. Primers for amplification of exon 23 of the EPG5 gene and the region from the exon 20 – exon 21 junction to the exon 25 exon 26 junction of EPG5 mRNA by polymerase chain reaction (PCR) were designed using Primer BLAST software (NM_020964.3 was used as a reference; primer sequences available on request). Unincorporated primers and nucleotides were removed from PCR products using *Exo I* and FastAPTM enzymes. Sequencing reactions of purified PCR products were carried out using BigDye Terminator v3.1 chemistry and separated on ABI 3500 genetic analyser (Applied Biosystems, Foster City, USA) according to the manufacturer's instructions. The sequencing data was analysed using SeqScape software.

Minigene assay. The minigene assay was performed as described in Kishore *et al.* (2008). The pSpliceExpress vector was a gift from Stefan Stamm (Addgene plasmid # 32485). Briefly, EPG5 exon 23 was amplified from patient peripheral blood DNA using primers with attB1 and attB2 tails and cloned into pSpliceExpress using Clonase BP (Invitrogen). Competent cells (SIG10 5α, Sigma) were transformed, and positive clones were selected on ampicillin (100 mg/ml). The sequence of selected clones was verified using colony PCR and sequencing. Purified plasmids from two clones, one wild type and one carrying the tested mutation, were used for lipofection of HeLa (Sigma) cells. Cells were lysed after 24 hours, and RNA was extracted with a DNase I step included and reverse transcribed. The minigene cDNA was amplified, and the presence/absence of EPG5 exon 23 was tested on agarose gel electrophoresis and verified by sequencing. Quantification of band densities was carried out with ImageJ (Schneider *et al.* 2012), and the counted

ratio was corrected for the molecular weight of the compared fragments.

Biochemical analyses. All metabolic and biochemical parameters were measured in the serum, cerebrospinal fluid, and urine in the central laboratory of the Children Faculty Hospital in Bratislava by standardized methods.

Ethics. Legal guardians of the proband provided written informed consent for this study, which was approved by the ethics committees of the National Institute of Children's Diseases in Bratislava, Slovakia, and adhered to the tenets outlined in the Declaration of Helsinki.

Results

Patient description. The patient is currently a 10-year-old boy of Roma ethnicity. He was born prematurely at 28 weeks of gestation. The pregnancy was terminated due to premature rupture of membranes after a complete dexamethasone treatment. Birth weight of 940 g, length of 36 cm, and head circumference of 24 cm were within the lower normal range according to the Fenton growth charts for preterm infants (Fenton et al. 2025) (Table 1). His Apgar score was 2/7/8. After delivery, the child was resuscitated for apnea and bradycardia and placed on N-CPAP ventilation support with FiO₂ 0.30. A 5-day antibiotic treatment was administered for perinatal infection. Due to a heart murmur, a cardiology examination was performed, revealing a persistent ductus arteriosus (PDA). At 9 weeks

of life, desaturations were observed due to bronchopulmonary dysplasia, and the child was placed on long-term oxygen therapy, followed by a slight improvement in feeding and thriving. At 7 months of age, the child was acutely hospitalized for apneic pauses. Right-sided pneumonia was diagnosed, and despite treatment, on the 4th day of hospitalization, circulatory arrest occurred, requiring repeated resuscitation. A follow-up cardiology evaluation revealed dilatation of the left cardiac ventricle and angio CT and high-resolution CT of the lungs showed a combined congenital anomaly of the great vessels (right-sided aortic arch, an isolated branch of the left subclavia from the original persistent left-sided arterial ductus arteriosus – which was found to be occluded at the time of examination – and hypoplastic left branch of the pulmonary artery). In the following days, the child's condition deteriorated critically as a result of a catheter-related bloodstream infection. Due to impaired oxygenation, it was necessary to address the stenosis of the pulmonary artery branch – stents were introduced via catheterization. After this procedure, no further significant breathing disturbances were observed. During the first years of life, the child presented with hypotonia, developmental delay, severe hypotrophy, and growth retardation (Figure 1A). Anthropometric parameters were influenced by prematurity and congenital heart defects, but he failed to catch up in growth after the resolution of his heart condition. At 10 months, he had dolichocephaly and a head circumference below the 1st percentile, but proportional to his small height.

Table 1
Anthropometric parameters of the patient showing postnatally delayed growth and postnatal progression of microcephaly

	Length/Height			Weight			Head circumference			Growth charts
	[cm]	[SD]	[centile]	[kg]	[SD]	[centile]	[cm]	[SD]	[centile]	
Birth (28 gw)	36	-0.25	40%	0.94	-1.25	11%	24.0	-1.17	12%	Fenton (2025)*
11 weeks (39 gw)	44	-2.65	0%	2.42	-2.39	1%	33.8	-0.77	22%	
8 months	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	40.0	-2.27	1%	
10 months	58.5	-4.80	0%	n.a.	n.a.	n.a.	40.3	-3.14	0%	CDC growth charts valid from 0 to 36 months with correction for gestational age at birth*
18 months	68.5	-3.78	0%	6.17	-5.69	0%	42.0	-4.17	0%	
24 months	79	-1.76	4%	7.45	-4.80	0%	n.a.	n.a.	n.a.	
36 months	n.a.	n.a.	n.a.	7.68	-6.28	0%	43.0	-3.71	0%	
3.5 years	88	-2.71	1%	8.77	-4.36	0%	n.a.	n.a.	n.a.	CDC growth charts and Nellhaus (1968) [#]
6.5 years	103	-2.89	0%	12.00	-4.03	0%	45.0	-4.90	0%	
7.5 years	105	-3.51	0%	13.00	-4.03	0%	n.a.	n.a.	n.a.	
9 years	116	-2.80	0%	14.50	-4.03	0%	46.0	-5.10	0%	

*Counted using PediTools (Chou et al. 2020); #Counted using Head Circumference Calculator at www.simulconsult.com; n.a. – not available

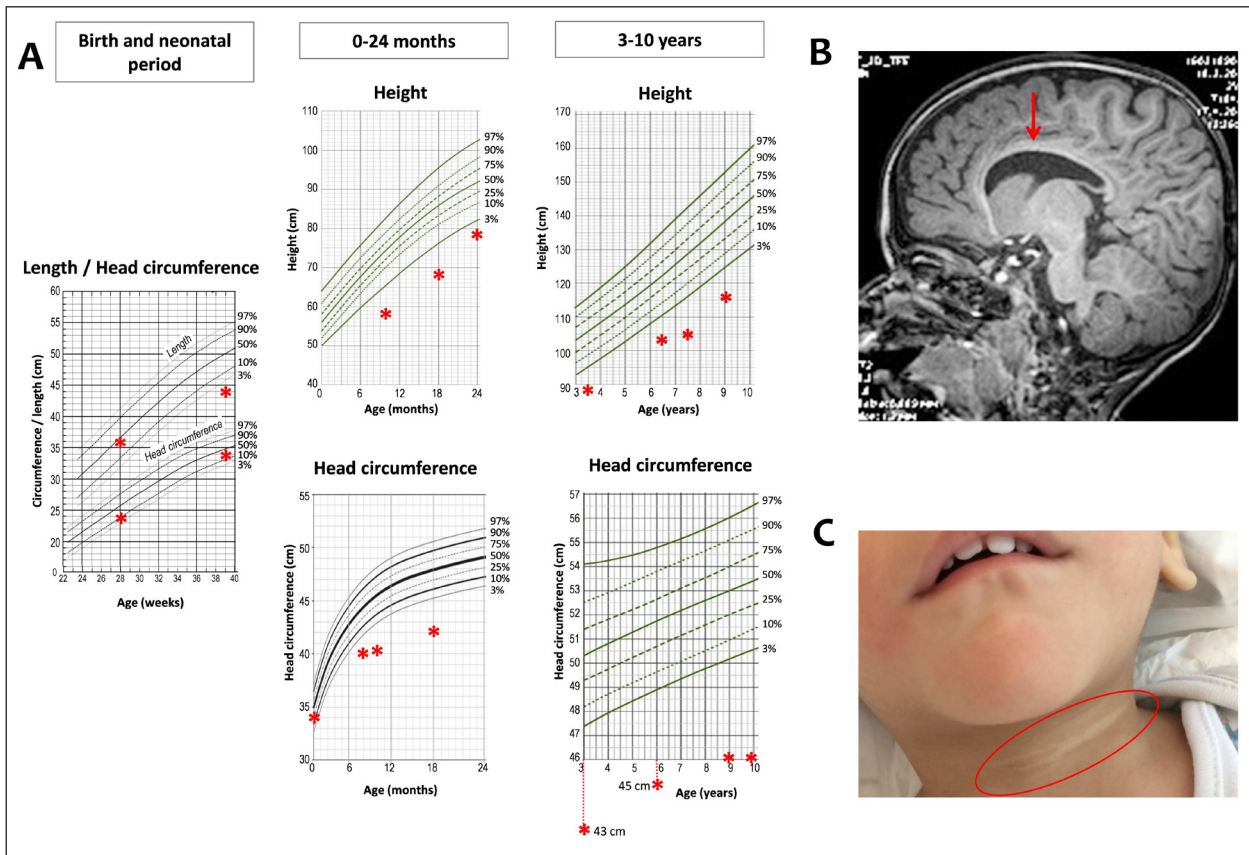


Figure 1. (A) Length/height and head circumference percentile charts with values of the patient marked red documenting growth delay and microcephaly. (B) Brain MRI at age 25 months showed mild brain atrophy and thin corpus callosum (red arrow). (C) Hypopigmented area at the neck of the patient (red ellipse).

However, he developed microcephaly (Figure 1A, Table 1) and turricephaly later in his life. The current height of 116 cm is below the 1st percentile, and the head circumference is profoundly below the 1st percentile, with SD -5.3 (Table 1).

MRI at the age of 2 years showed mild brain atrophy and a thin corpus callosum (Figure 1B). Selective metabolic screening was performed due to hypotonia and developmental delay. The organic acid profile in urine repeatedly showed elevated 3-methylglutaconate (78.7 and 38 $\mu\text{mol}/\text{mmol}$ of creatinine; N: <20 $\mu\text{mol}/\text{mmol}$ of creatinine), suggesting mitochondrial disease. TMEM70 deficiency with 3-methylglutaconate aciduria, frequent in Roma paediatric patients in Slovakia, was excluded by genetic testing. After the WES analysis suggested Vici syndrome in the patient, targeted reverse phenotyping confirmed hypopigmented skin areas (Figure 1C) and mild, non-specific cellular immunodeficiency. He had no seizures, cataracts, or cardiomyopathy. He has dysmorphic features – mild micro- and retrognathia,

prominent upper lip, low-set and posteriorly rotated ears, high forehead with protruding glabella, and bilateral epicanthus. In his 10 years, he is nonverbal with autistic features, concomitant strabismus, has axial hypotonus and spastic paraparesis, has stereotypic arm movements, and walks on his toes. He had frequent respiratory infections and nonspecific secondary cellular immunodeficiency combined with progressive IgA hyper-gammaglobulinemia (serum IgA: 3.15, 3.94, 4.17, 5.05 g/l; N: 0.70–2.30) and activation of the alternative complement pathway. The hypopigmented skin patches disappeared by the time he was 9 years old. Clinical features are summarized and compared with published cases in Table 2. The patient's parents were unavailable for genetic testing as he had been raised in a foster home.

Genetics. Analysis of the patient's exome data showed the presence of a homozygous variant c.4205G>A, p.(Arg1402Lys) in the *EPG5* gene. This variant has not been reported in patients with Vici syndrome to date in the literature, but has been

Table 2

Frequency of selected features in Vici syndrome (Dafsari et al. 2022) and in all reported cases with EPG-related disorder, also including cases with milder presentation, adapted from (Dafsari et al. 2025), compared to the case described in this study

Feature		% of Persons with Feature		This case
		(Dafsari et al. 2022) N=90 patients	(Dafsari et al. 2025) N=211 patients (147 families)	
Most common findings				
Brain malformations	Corpus callosum agenesis/thinning	100%	89%	+, thin
	Thalamic abnormalities	20%	8%	–
	Neuronal migration abnormalities or polymicrogyria	20%	n.a.	–
	Pontocerebellar hypoplasia	n.a.	27%	–
	Schizencephaly	n.a.	2%	–
	Heterotopias	n.a.	2%	–
Neurologic	Developmental delay/ Intellectual disability	100%	94%	+
	Neonatal muscular hypotonia	70%	n.a.	+
	Seizures	70%	55%	–
	Movement disorders	65%	38%	+
	Neuromuscular phenotypes	n.a.	66%	+
	Spasticity	n.a.	26%	+
	Parkinsonism with dystonia	n.a.	8%	–
	Myoclonus	n.a.	9%	–
Microcephaly		90%	50%	+
Immunodeficiency		95%	n.a.	+
Failure to thrive		90%	55%	+
(Oculo-)cutaneous hypopigmentation		90%	51%	+
Cardiac	Cardiomyopathy	60%–68%	n.a.	–
	Congenital heart defects	8%–15%	n.a.	+
Ophthalmologic	Cataract	70%	37%	–
	Retinal abnormalities	35%	n.a.	–
	Optic nerve atrophy	30%	27%	–
	Sensorineural hearing loss	65%	18%	–
Anaemia, leucopenia, thrombocytopenia		50%	n.a.	–
Dysmorphic features		n.a.	37%	+
Less common findings				
Thyroid agenesis or dysplasia		10%	n.a.	–
Thymic aplasia		10%	n.a.	–
Pulmonary hypoplasia		10%	n.a.	–
Renal tubular dysfunction		10%	n.a.	transient

n.a. – not available

submitted to ClinVar in two cases, with a VUS classification. *In silico* tools estimating the impact of missense variants provided inconsistent predictions

(Table 3), as did tools estimating impact on splicing and metapredictors (Table 4). However, this G to A is located at the last base of exon 23 (Figure 2A), where G

at this position is highly conserved in typical GT-AG splice sites (Sibley et al. 2016) (Figure 2B). Given the patient's clinical picture consistent with features of Vici syndrome, we decided to test the possibility of splicing impairment experimentally.

Functional study. First, we extracted RNA from the patient's blood, reverse transcribed, and amplified and sequenced the region between exons 20 and 26 of the *EPG5* transcript (location of forward and reverse primers depicted in Figure 2A). RNA sequencing using the Sanger method confirmed the presence of the correctly spliced transcript (Figure 2C), but also

revealed low levels of aberrantly spliced transcripts in the background, suggesting exon-skipping events (Figure 2D). As misplaced transcripts are typically degraded in the process of nonsense-mediated decay, they may escape detection by sequencing. Therefore, to further assess the effect of the variant on splicing, we performed a mini-gene assay using the pSpliceExpress vector carrying *EPG5* exon 23 with or without the tested variant. The results have shown decreased inclusion of exon 23 compared to wild-type (40% vs. 72%, respectively, $p < 0.001$) (Figure 3). The inclusion was confirmed by Sanger sequencing (data not shown).

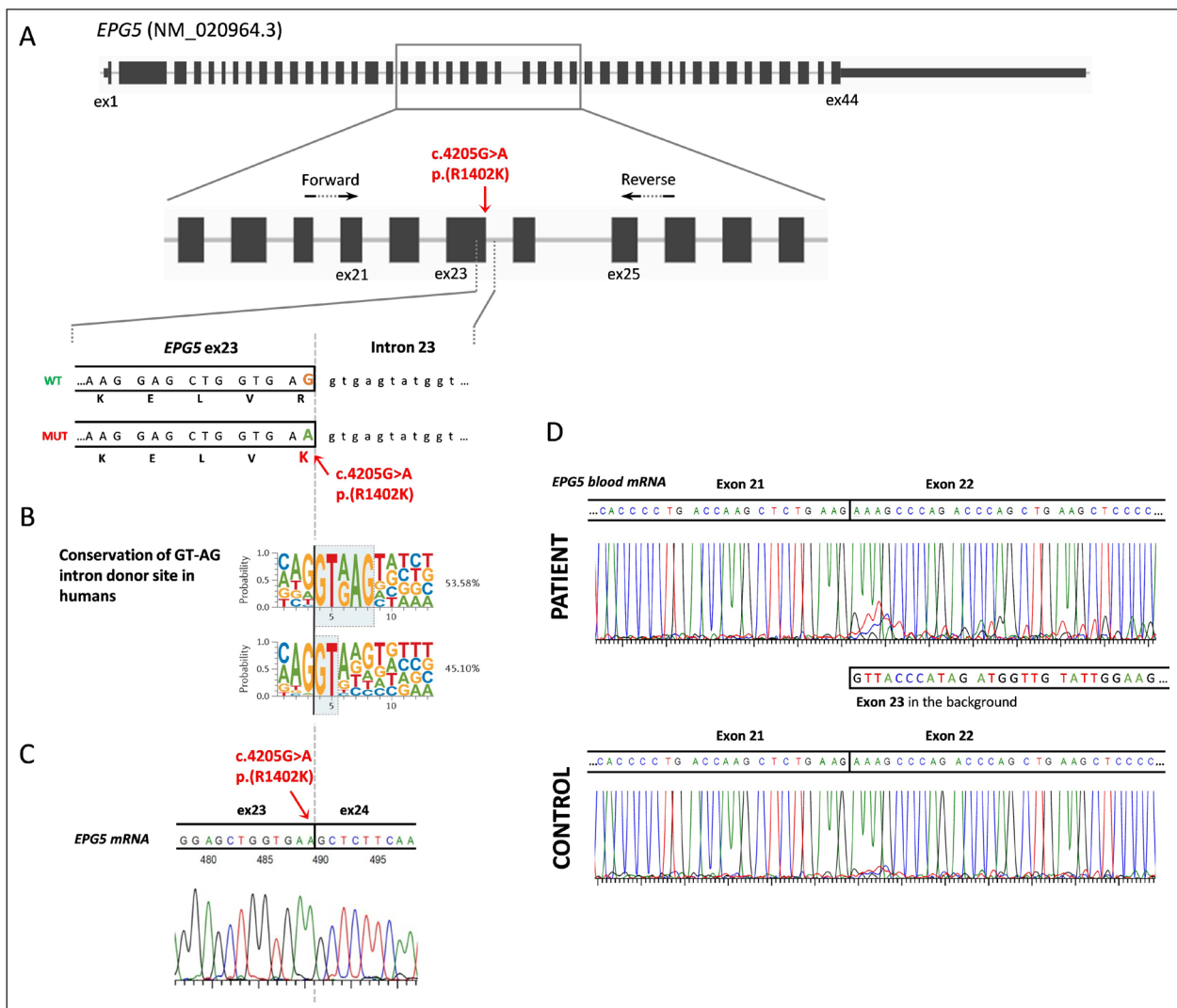


Figure 2. (A) *EPG5* (NM_020964.3) transcript scheme (intron lengths are not proportional). Position of the variant c.4205G>A, p.(Arg1402Lys) found in the patient in this study is shown with zoom on the exon-intron junction region. Primers used for mRNA sequencing are depicted. (B) Conservation of the two most frequent types of exon and intron sequences in the donor splice sites in human GT-AG introns with indicated percentage of junctions containing each motif - adapted from Sibley et al. (2016). (C) *EPG5* mRNA sequence at the exon 23–24 junction. (D) Mixed sequence detected at the exon 21–22 junction in the patient, suggesting the presence of multiple splice products.

Table 3
Prediction of the impact of the c.4205G>A, p.(Arg1402Lys) variant of the EPG5 protein function

	Missense predictors			
	SIFT	PolyPhen-2	PROVEAN	AlphaMissense
EPG5(NM_020964.3): c.4205G>A p.Arg1402Lys	0.359 (tolerated)	0.87 (possibly damaging)	-0.37 (neutral)	0.202 (benign supporting)

Table 4
Prediction of the impact of the c.4205G>A, p.(Arg1402Lys) variant on splicing of the EPG5 pre-mRNA and metapredictors

	Splicing predictors				Metapredictors	
	HSF	MaxEnt	SpliceAI	dbSNV Ada	CADD	REVEL
EPG5(NM_020964.3): c.4205G>A p.Arg1402Lys	WT site broken -10.9% (deleterious)	-20.5% (benign)	0.01 (benign)	1 (deleterious)	25.6	0.1809 (benign)

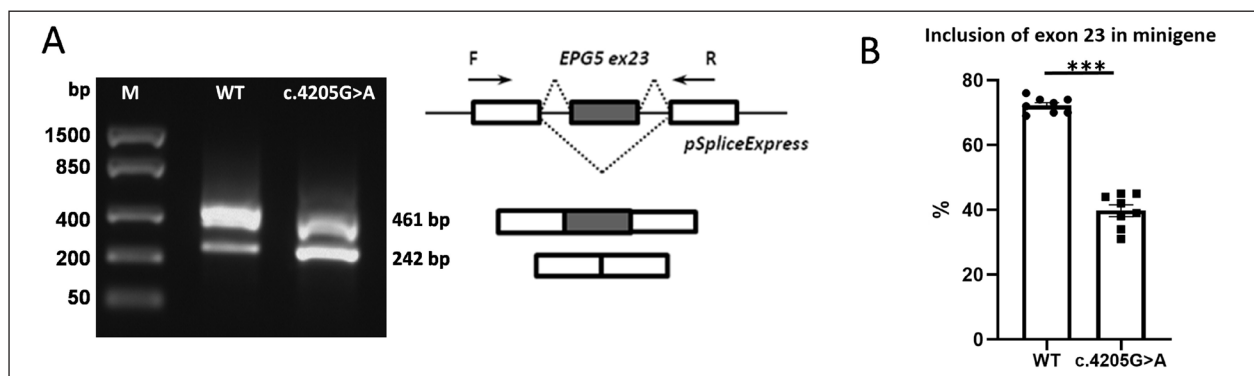


Figure 3. (A) Electrophoresis of PCR products using F and R primers amplifying the region included in the mature transcript expressed from pSpliceExpress vector. Two different amplicons were detected – without ex23 (242 bp) and with correctly included ex23 (461 bp). (B) Exon carrying the tested c.4205G>A variant had reduced inclusion into the minigene mature transcript compared to wild-type (WT) (40% vs. 72%, respectively, t-test, *** $p < 0.001$).

Variant classification. We performed classification of the variant according to ACMG guidelines (Richards et al. 2015), with modifications to PS3 for splice variants (Walker et al. 2023) and to PP2 and PM3 (ClinGen Variant Classification Guidance 2025). The variant met the criteria PM2_supporting (low MAF 0.00002877), PP4 (specific phenotype), PM3_supporting (homozygous occurrence), and PS3_supporting (experimentally demonstrated effect on splicing but leaky), allowing us to classify it only as a variant of unknown significance. This might change in case more patients with this variant are identified.

Discussion

We present a patient with features overlapping with Vici syndrome. The diagnosis is supported

by the identification of a homozygous missense variant with experimentally demonstrated impact on splicing. However, the variant is still classified as of unknown significance using current classification recommendations.

The variant is rare, and homozygous individuals were not present in gnomAD databases in previous gnomAD versions (Chen et al. 2024). However, the recently released gnomAD v4.0 data include an individual with the same homozygous variant, which would argue against the variant's pathogenicity. In the gnomAD v4 database, as in the previous versions, severe paediatric cases are excluded. Nevertheless, the authors of gnomAD v4 note that many samples in this version are derived from large biobanks and may include individuals with disease (<https://gnomad.broadinstitute.org/help/dataset-selection>).

Recent clinical evaluation of a large cohort of patients with variants in the *EPG* gene has shown that the syndrome presents a phenotypic continuum (Dafsari *et al.* 2022, 2025). The most severe is classic Vici syndrome, defined as a neurodevelopmental disorder with multisystem involvement characterized by the combination of agenesis of the corpus callosum, cataracts, hypopigmentation, cardiomyopathy, combined immunodeficiency, microcephaly, and failure to thrive. Milder forms of Vici syndrome may manifest with various combinations of symptoms, and the patients live longer than those with the classical Vici syndrome. Some reported individuals had only mild intellectual disability, some were able to attend regular primary school with additional support, and one was reported to have normal learning abilities (Dafsari *et al.* 2025).

It was shown that disease severity depends on the mutation type. Median life expectancy was significantly lower in cases with bi-allelic truncating variants than in those with bi-allelic missense variants (28 months vs. 16 years) (Dafsari *et al.* 2025). The variant in our case appears to affect splicing, though not completely. Such “leaky” splice variants reduce but do not abolish normal splicing, and the proportion of aberrant transcripts may have a significant impact on the severity of an individual’s phenotype (variable expressivity) or be associated with reduced penetrance (Walker *et al.* 2023). This would be in line with the less severe phenotype in our patient, as well as with the current view of Vici syndrome as a spectrum. Variable expressivity and penetrance may also explain the presence of the homozygous case in gnomAD v4 database. Moreover, extreme prematurity in our patient could have emphasized the symptoms such

as hypotonia and spasticity, and his clinical picture might have been even milder if he had been born at term. In addition, even patients with the same homozygous variants showed variable presentations of hallmark signs, including callosal dysgenesis, cataracts, cardiac involvement, optic nerve atrophy, or hypopigmentation (Byrne *et al.* 2016; Vojcek *et al.* 2020; Chorin *et al.* 2022; Dafsari *et al.* 2025). The role of other genetic factors cannot thus be excluded. Our patient is now 10 years old and presents with most of the hallmark features (Table 2). The typical sign - pale skin and hair present in Caucasian patients was also not present, but he had hypopigmented skin areas (Figure 1C). These have disappeared over time, suggesting that the frequency of this feature may be underestimated in patients phenotyped later in life.

Conclusion

Novel variant *EPG5*:c.4205G>A, p.(Arg1402Lys) causes aberrant splicing in a proportion of transcripts; therefore, the milder presentation of Vici syndrome in our patient is probably due to the residual presence of the *EPG5* protein. The described case contributes to the view of the Vici syndrome as a spectrum with variable severity and symptom combinations dependent, at least in part, on the variant type.

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Conflict of interest: The authors declare no conflict of interest.

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