BMC Medical Genomics

CASE REPORT

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Identification of novel compound heterozygote variants in the *PCCB* gene in a fetus with undetectable fetal phenotype

Xingyu Feng[1], Yao Hu[1], Huiming Yan[1], Lin Zhou[1], Na Ma[1], Chulong Xiong[1] and Hui Xi[1*]

Abstract

Propionic acidemia (PA) is a rare autosomal recessive metabolic disorder caused by functional deficiency of propionyl-CoA carboxylase, clinically characterized by life-threatening ketoacidosis, hyperammonemia, and multiorgan dysfunction. Due to its nonspecific clinical manifestations, PA is frequently misdiagnosed or only identified during severe metabolic crises. This study reports a Chinese family with a history of offspring affected by PA. Through whole-exome sequencing and Sanger validation of fetal amniotic fluid and parental peripheral blood samples, two novel compound heterozygous variants in the *PCCB* gene were identified in the fetus, initially classified as variants of uncertain significance (VUS) per ACMG guidelines. Subsequent functional studies and amniotic fluid metabolomic analyses were performed. The results demonstrated that the paternal *PCCB* c.366_372 + 7del variant caused exon 3 skipping(p.Phe102_Gln124del) or exon 2–3 skipping (p.Gly62_Gln124del), while the maternal c.183 + 6T > G variant resulted in intron 1 retention (p.Gly62Valfs*10), both leading to protein truncation and aberrant mRNA splicing. Metabolomic analysis demonstrated significantly elevated C3 levels and an increased C3/C2 ratio, consistent with PA diagnosis. These novel *PCCB* splicing variants expand the mutational spectrum of PA and demonstrate the clinical utility of integrated genomic-metabolomic analysis for prenatal diagnosis and genetic counseling in high-risk PA families.

Keywords Propionic Acidemia, *PCCB* Gene, Splicing Variant, Prenatal Diagnosis, Genetic Counseling

Introduction

Propionic acidemia (PA) (OMIM: 606054), is a rare autosomal recessive metabolic disorder, classified as organic acidemia, with an estimated incidence of 1:100,000 to 1:150,000 [1].The disease is primarily caused by variants in the *PCCA* and *PCCB* genes [2]. The *PCCA* gene, located at 13q32.3, and the *PCCB* gene, located at 3q22.3, encode theαand βsubunits of propionyl-CoA carboxylase (PCC), respectively [3]. Variants in these genes result in PCC deficiency, impairing the conversion of propionyl-CoA to (D)-methylmalonyl-CoA. This leads to accumulation of toxic metabolites, causing respiratory chain dysfunction, urea cycle impairment, and other metabolic disturbances, ultimately resulting in multiorgan damage, particularly affecting the brain, liver, and muscles [4, 5].

PA presents with severe clinical manifestations which may progress rapidly. Early-onset PA typically manifests in neonates as feeding difficulties, vomiting, and Kussmaul respiration. If untreated, the condition may rapidly progress to ketosis, metabolic acidosis, hyperammonemia, seizures, coma and even death [2, 6]. The diagnosis of PA primarily relies on genetic testing and metabolic

*Correspondence:
Hui Xi
54548660@qq.com
[1]Department of Medical Genetics, NHC Key Laboratory of Birth Defect for Research and Prevention (Hunan Provincial Maternal and Child Health Care Hospital), Changsha, China

profiling [3]. While numerous variants in the *PCCA* and *PCCB* genes have been reported, novel variants - particularly splicing variants - are continually being identified [4]. This study reports a case of PA caused by novel compound heterozygous variants in the *PCCB* gene. These findings further expand the variant spectrum of this gene.

Materials and methods

General data

A 32-year-old pregnant woman, who had previously given birth to a child suspected of having PA, was referred to the Prenatal Diagnosis Center of Hunan Provincial Maternal and Child Health Hospital in February 2024. The proband had passed away (family pedigree shown in Fig. 1C). The proband presented with constipation, progressive lethargy, and widespread petechiae at one month of age. Laboratory tests revealed anemia, thrombocytopenia, and elevated propionylcarnitine (C3) and C3/C2 ratios, suggesting PA or methylmalonic acidemia. Urine organic acid analysis showed elevated levels of propionylglycine, 3-hydroxypropionate, methylcitrate, and 3-methylcrotonylglycine, leading to a suspected diagnosis of PA. Despite supportive treatment, the child passed away six months later. No genetic testing was performed on the proband. Other family members were phenotypically normal with no similar medical history.

Methods

Exome sequencing(ES)

Amniotic fluid (15 mL) was collected from the pregnant woman, and genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Germany). Peripheral blood samples (2 mL) were collected from the parents, and genomic DNA was extracted using the same kit.Exome Sequencing was performed on the fetus and parents using Roche Exome Sequencing capture probes. Sequencing reads were aligned to the human reference genome (hg19/GRCh37) using the Burrows-Wheeler Aligner (BWA). PCR duplicates were removed using Picard v1.57. Variant calling was performed using Berry Genomics' Verita Trekker® variant detection system and GATK. Variant annotation and interpretation

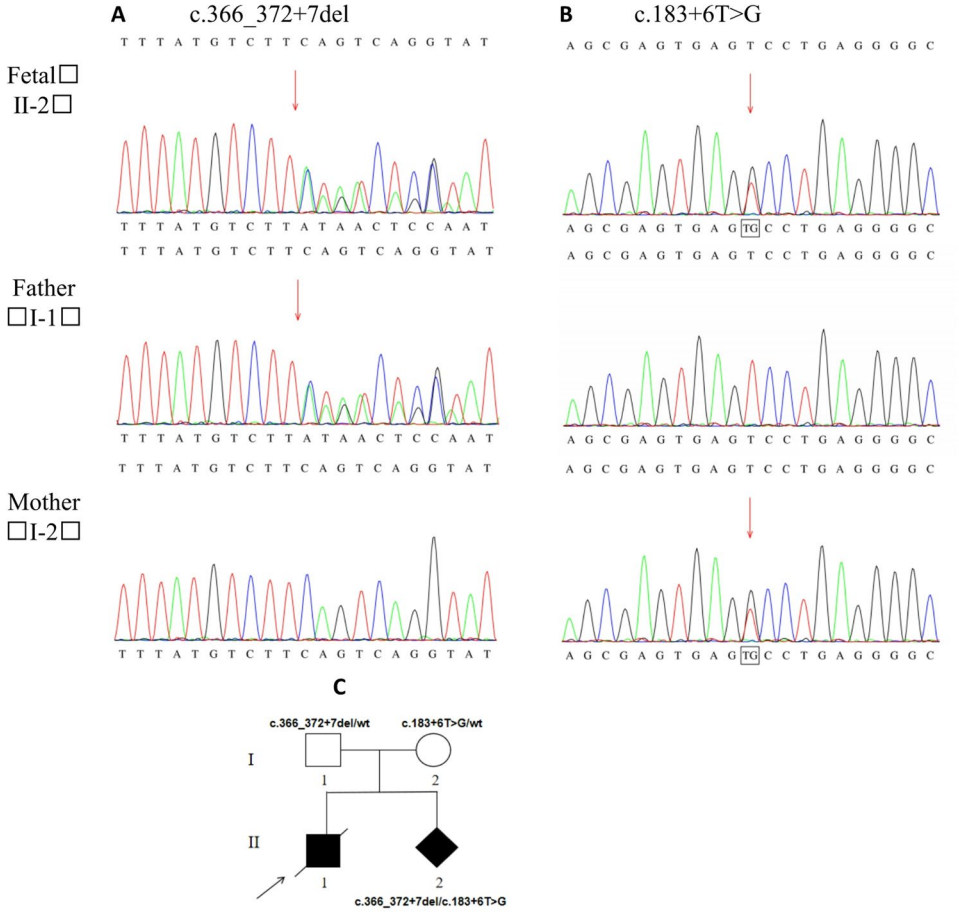

Fig. 1 Genetic testing results of *PCCB* fetuses and their parents. **A** Sanger sequencing results of the c.366_372 + 7del variant of the PCCB gene in the fetuses and their parents. **B** Sanger sequencing results of the c.183 + 6T > G variant of the PCCB gene in the fetus and its parents. **C** Family lineage map of the prior witness

were conducted using VEP and Berry Genomics' Enliven® system.

Sanger sequencing

To confirm the candidate variants detected by Trio-exome sequencing, Sanger sequencing validation was conducted using peripheral blood samples collected from the fetal parents. Primers were designed by Primer 5 software. Primer P1 (P1F: TGTAAAACGACGGCCAGTCT CCCTACAGCCAGCCGATG, P1R: CAGGAAACAGCT ATGACCTACCCGCCTCCACGAGAAAC) was used to amplify c.366_372 + 7del, the product was 485 bp in size. Primer P2 (P2F: TGTAAAACGACGGCCAGTTTCAT TGAGGCATAGTGG, P2R: CAGGAAACAGCTATGA CCCAAGCAAGTTTTCATTCC) was used to amplify c.183 + 6T > G, the product wa-s 280 bp in size. Target DNA fragments of the family members were amplified by PCR: initial denaturation at 95 °C for 5 min, followed by 34 cycles at 95 °C for 20 s, 60 °C for 20 s and 72 °C for 20 s and a final hold at 72 °C for 4 min. Purified PCR product-s were sequenced (ABI 3730xl, BigDye™ kit) and analyzed against GenBank NM_000532.5 using Chromas.

Bioinformatics analysis

The UniProt database was used to identify the functional domains of the *PCCB* protein. The HGMD and ClinVar databases were searched for previously reported *PCCB* gene variants up to December 25, 2024. The Ensembl genome database was used to compare the variants with homologous sequences in other species. Splice AI software was used to predict the impact of the variants on protein function. The Rare Diseases Data Center (RDDC) was used to predict the effects of the variants on splicing patterns.

Reverse transcription polymerase chain reaction (RT-PCR)

Since the fetus carried compound heterozygous *PCCB* gene variants inherited from both parents, RNA was extracted from parental peripheral blood samples using the Rapid Total RNA Extraction Kit (Cat. No. RP4001) from Biotech Company. cDNA was synthesized following standard protocols. PCR amplification was performed using the primers listed in Table 1. The PCR conditions were as follows: 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min. PCR products were separated by 2% agarose gel electrophoresis, and bands were excised and sequenced.

Table 1 RT-PCR amplification primers were designed according to the variant sites and gene sequences of the *PCCB* gene

Primer name	Primer sequence 5′-3′
-183–1 F	GACGCGCCGGCACAGCAAAA
-183–2 F	CAAGGCTCAGCGTTCTGG
-183-2R	CATTGATTCGGCCTCGTCCA
-183-1R	AGATCTTTTGGGCATGTGCT
-366–1 F	CAAGGCTCAGCGTTCTGG
-366–2 F	CTGTTAACGAACGCATCGAA
-366-2R	GCATAGCCAGCCAAAGACTC
-366-1R	TGTTAGGGCTGGGGAGTAGA

Protein model

Use AF-Q5H8C1-F1-model_v4 as template, models are computed by the SWISS-MODEL serverhomology modelling pipeline which relies on ProMod3, an in-house comparative modelling engine based on OpenStructure, Mutations evaluated using the Swiss-PdbViewer [7, 8].

Amniotic fluid metabolite analysis

Amniotic fluid supernatant (3 μL) was analyzed using tandem mass spectrometry (MS/MS) on an Applied Biosystems API 2000 to measure C3, C2, and the C3/C2 ratio. The concentrations were calculated using ChemoView v1.2 software. Organic acid levels were quantified using gas chromatography-mass spectrometry (GC-MS) on a Shimadzu QP2010.

Results

Exome sequencing and sanger sequencing results

Exome Sequencing and Sanger sequencing revealed two heterozygous variants in the *PCCB* gene (NM_000532.5) in the fetus: c.366_372 + 7del and c.183 + 6T > G, inherited from the father and mother, respectively (Fig. 1A and B).

Bioinformatics analysis

The c.366_372 + 7del variant in the *PCCB* gene (NM_000532.5) is not predicted to undergo nonsense-mediated mRNA decay (NMD) according to the DECIPHER database. SpliceAI prediction (score:0.99) suggests this variant is likely to cause aberrant splicing. Located at a non-canonical splice position (+ 7), this variant occurs in an exon where loss-of-function (LoF) variants are rarely observed in the general population, collectively indicating its potential to disrupt mRNA splicing (PVS1_Moderate). This variant was not found in the ExAC, 1000 Genomes, or gnomAD databases (PM2_Supporting). VarSEAK software predicted a high likelihood of splicing disruption (Fig. 2). RDDC predicted two splicing patterns: a 220 bp insertion leading to premature termination or a 62 bp deletion causing exon skipping (Fig. 3A). According to ACMG guidelines, this variant was classified as a variant of uncertain significance (PVS1_Moderate + PM2_Supporting).

The c.183 + 6T > G variant in the *PCCB* gene (NM_000532.5) was also not found in the Ex-AC(http://exac.broadinstitute.org/),1000 Genomes(http://browser.1000genomes.org), or gnomA-D databases (https://gnomad.broadinstitute.org/) (PM2_Supporting).Splice AI(

Fig. 2 Results predicted by VarSEAK software The higher the rating, the higher the likelihood of affecting splicing. c.366_372 + 7del variant and c.183 + 6T > G variant ratings of the *PCCB* gene were both scored 5

Fig. 3 RDDC predicts two modes affecting shearing. **A** PCCB gene c.366_372 + 7del variant predicts two modes affecting shearing, one is inserting 220 bp, altering the shear donor, leading to premature termination of transcription, and the other is making a deletion of 62 bp, leading to exon jumping. **B** PCCB gene c.183 + 6T > G variant predicts two modes affecting shear, one inserting 20 bp, altering the shear donor and causing a frameshift variant, leading to premature termination of transcription, and the other making a 219 bp deletion, leading to exon skipping

Table 2 *PCCB* gene variation splice AI score

Gene	nucleotide change	Acceptor Loss	Donor Loss	Acceptor Gain	Donor Gain
	c.366_372+7del	0.00	0.99	0.00	0.00
	c.183+6T>G	0.00	0.59	0.00	0.39

https://spliceailookup.broadinstitute.org/) (Table 2) and VarSEAK software predicted that this variant could lead to abnormal splicing (Fig. 2), affecting protein function (PP3). RDDC predicted tw-o splicing patterns: a 20 bp insertion causing a frameshift or a 219 bp deletion causing e-xon skipping (Fig. 3B). According to ACMG guidelines, this variant was classified as a variant of uncertain significance (PM2_Supporting+PP3).

Splicing analysis of *PCCB* RT-PCR

RT-PCR analysis showed that the c.366_372+7del variant resulted in three splicing patterns: exon 3 skipping or exon 2 and 3 skipping at the same time, and normal splicing (Fig. 4B). Agarose gel electropherogram of RT-PCR products (Fig. 4A), electrophoretic detection showed that the wild-type PCR product had three bands, named band a, band b, and band c, in descending order; c.366_372+7del The PCR product had three bands, named band a, band b, and band c, in descending order. The electrophoretic pattern of splice bands a, b, and c, along with their corresponding sequencing chromatograms for the c.366_372+7del variant, are presented in Fig. 4C. The skipping of Exon 3 is represented at the cDNA and protein levels as: c.304_372del, p.Phe102_Gln124del. The skipping of Exon 2 and Exon 3 is denoted at the cDNA and protein levels as: c.184_372del, p.Gly62_Gln124del.The c.183+6T>G variant caused abnormal splicing with a 20 bp retention in intron 1 (Fig. 5B). Agarose gel electrophoresis of RT-PCR products, electrophoretic detection showed that: the wild-type PCR product was a single band, which corresponded to the expected size (312 bp) and was named as band a; c. 183+6T>G PCR product had two bands, of which the large band was named as band b and the small band was named as band a (Fig. 5A). Electrophoretic analysis of splice bands a and b with their corresponding sequencing traces for the c.183+6T>G variant is presented in Fig. 5C. The retention of 20 bp from the left flanking region of Intron 1 is represented at the cDNA and protein levels as: c.183_184insGTGAGGCCTGAGGGGCCTAA, p.Gly62ValfsTer10.

Protein model of *PCCB* variants

To predict the effects of the c.366_372+7del (p.Phe102_Gln124del and p.Gly62_Gln124del) and c.183+6T>G (p.Gly62ValfsTer10) variants on the three-dimensional structure of *PCCB*, we performed computational modeling

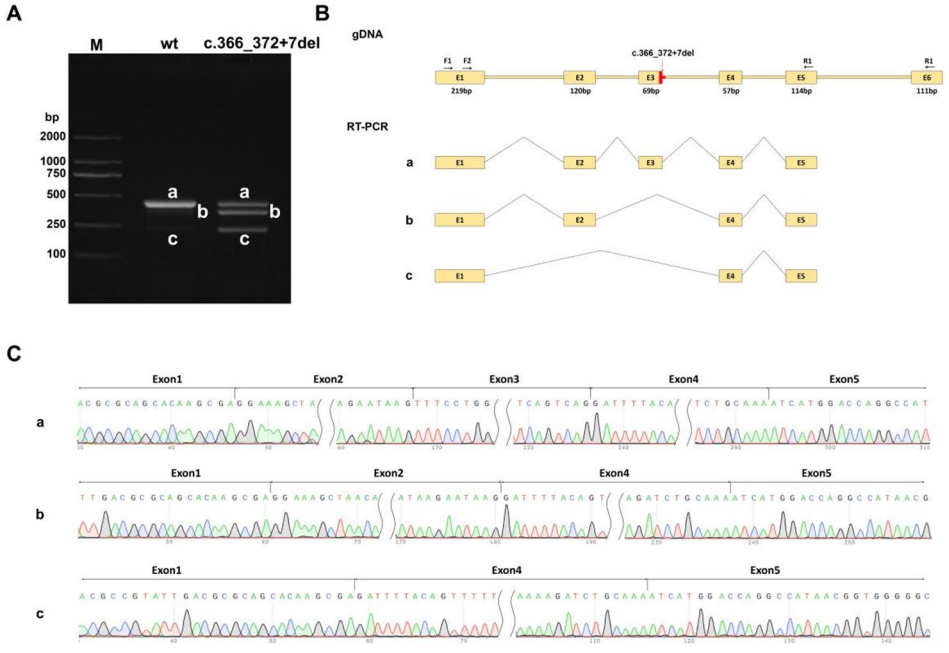

Fig. 4 Effect of c.366_372+7del variant on mRNA shearing. **A** Agarose gel electropherogram of RT-PCR products, electrophoretic detection showed that the wild-type PCR product had three bands, named band a, band b, and band c, in descending order; c.366_372+7del The PCR product had three bands, named band a, band b, and band c, in descending order. **B** Schematic of primer design and shear schematic, where red arrows point to variant sites. **C** Plot of sequencing results corresponding to shear bands a, b, and c. c.366_372+7del variant: band a is a normal shear band with Exon1 (183 bp)-Exon2 (120 bp)-Exon3 (69 bp)-Exon4 (57 bp)-Exon5 (114 bp); Band b is an anomalous shear band, Exon3 jumping, which is sheared in Exon1 (183 bp)-Exon2 (120 bp)-Exon4 (57 bp)-Exon5 (114 bp); Band c is an anomalous shear band, Exon2 and Exon3 jumping, which is sheared in Exon1 (183 bp)-Exon4 (57 bp)-Exon5 (114 bp)

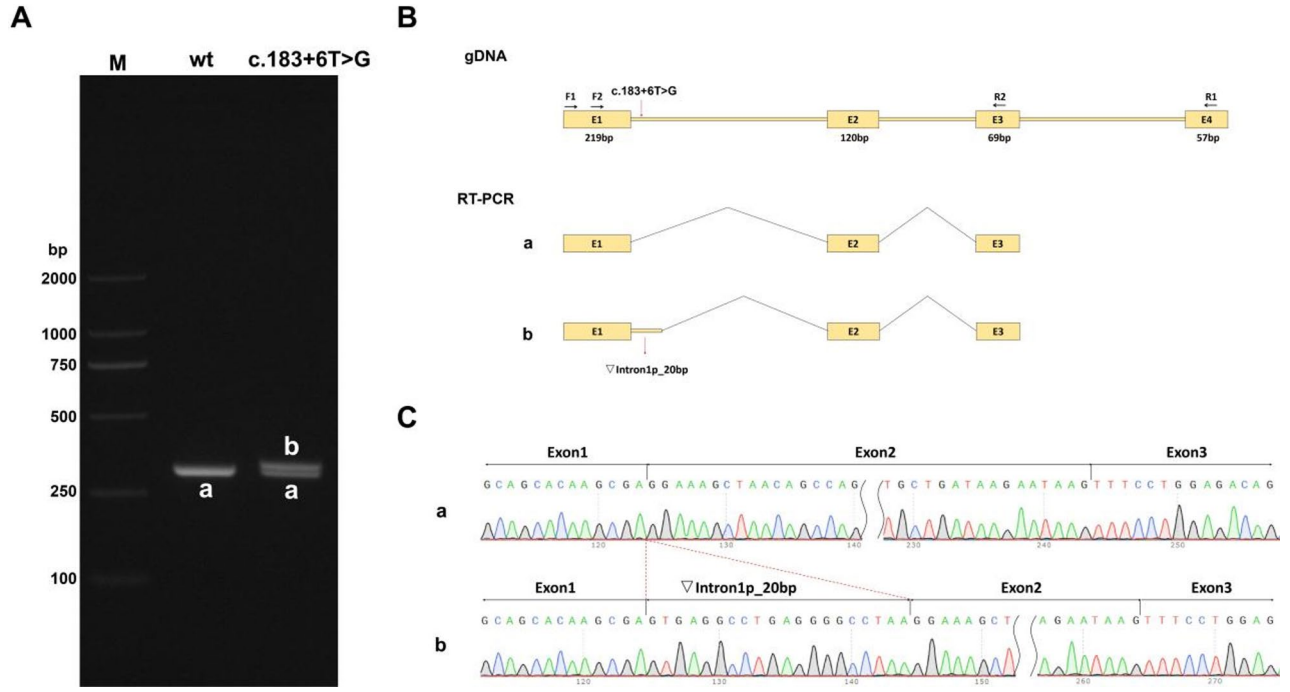

Fig. 5 Effect of the c.183 + 6T > G variant on mRNA shearing. **A** Agarose gel electrophoresis of RT-PCR products, electrophoretic detection showed that: the wild-type PCR product was a single band, which corresponded to the expected size (312 bp) and was named as band a; c. 183 + 6T > G PCR product had two bands, of which the large band was named as band b and the small band was named as band (a) (**B**) Schematic of primer design and shear schematic, where red arrows point to variant sites. **C** Plot of sequencing results corresponding to shear bands a and (b) c. 183 + 6T > G variant: band a is a normal shear band with Exon1 (183 bp)-Exon2 (120 bp)-Exon3 (69 bp); band b is an abnormal shear band with a 20 bp lagging on the left side of Intron1, and its shear mode is Exon1 (183 bp)-▽Intron1(20 bp)-Exon2(120 bp)-Exon3(69 bp)

analyses. The variants p.Phe102_Gln124del and p.Gly62_Gln124del occur in the N-terminal domain of CoA carboxyltransferase (Fig. 6A). These variants result in the deletion of 23 or 63 amino acids, generating truncated proteins that compromise the function of the N-terminal domain Fig. 6B and C). The c.183 + 6T > G (p.Gly62ValfsTer10) variant led to a premature stop codon, resulting in complete loss of the N-terminal domain (Fig. 6D).

Amniotic fluid metabolite analysis

MS/MS analysis of the amniotic fluid revealed significantly elevated levels of propionylcarnitine (C3) and an increased C3/C2 ratio, consistent with a diagnosis of PA.

Discussion

PA typically presents in the neonatal period, with toxic metabolite accumulation leading to metabolic acidosis, neurological symptoms, multi-organ dysfunction, and even death [9]. Most cases do not present with clinical symptoms during the fetal period, with only one reported case of antenatal nephromegaly [10]. The diagnosis of PA relies on genetic testing of the *PCCA* or *PCCB* genes or measurement of PCC enzyme activity [5].

To date, the HGMD database has reported 170 *PCCA* gene variants and 146 *PCCB* gene variants, with missense variants being the most common (58.9% for *PCCB*), while splicing variants account for only 12.3% (https://www.hgmd.cf.ac.uk/ac/index.php, accessed December 15, 2024). The variants identified in this study, c.366_372 + 7del and c.183 + 6T > G, have not been previously reported in the HGMD or ClinVar databases. Zhang et al. summarized the genetic variants in 61 Chinese PA patients, with 36 (59.0%) carrying *PCCB* variants, predominantly missense variants (51.4%, 37/72), located in exons 1, 3, 12, and 13 [5]. The novel compound heterozygote variants identified in this study, c.366_372 + 7del and c.183 + 6T > G, were predicted by bioinformatics tools to affect *PCCB* mRNA splicing. Bychkov et al. functionally analyzed 13 PA-related variants and found that most caused exon skipping, leading to loss of function at the protein level [11].

RT-PCR analysis demonstrated that the presence of minor variant bands in wild-type samples may represent conserved alternative splicing or somatic mosaicism. And the c.366_372 + 7del variant induces exon 3 skipping and increases the proportion of exon 2–3 double skipping events (Fig. 4A), resulting in a truncated protein of 516 amino acids (c.304_372del, p.Phe102_Gln124del). Based on the SpliceAI score (Donor Loss = 0.99) and RDDC-predicted aberrant splicing patterns, we propose that the c.366_372 + 7del variant alters the donor splice site and generates a novel donor site, leading to in-frame exon skipping. SWISS-MODEL was further employed to visualize the potential impact of this

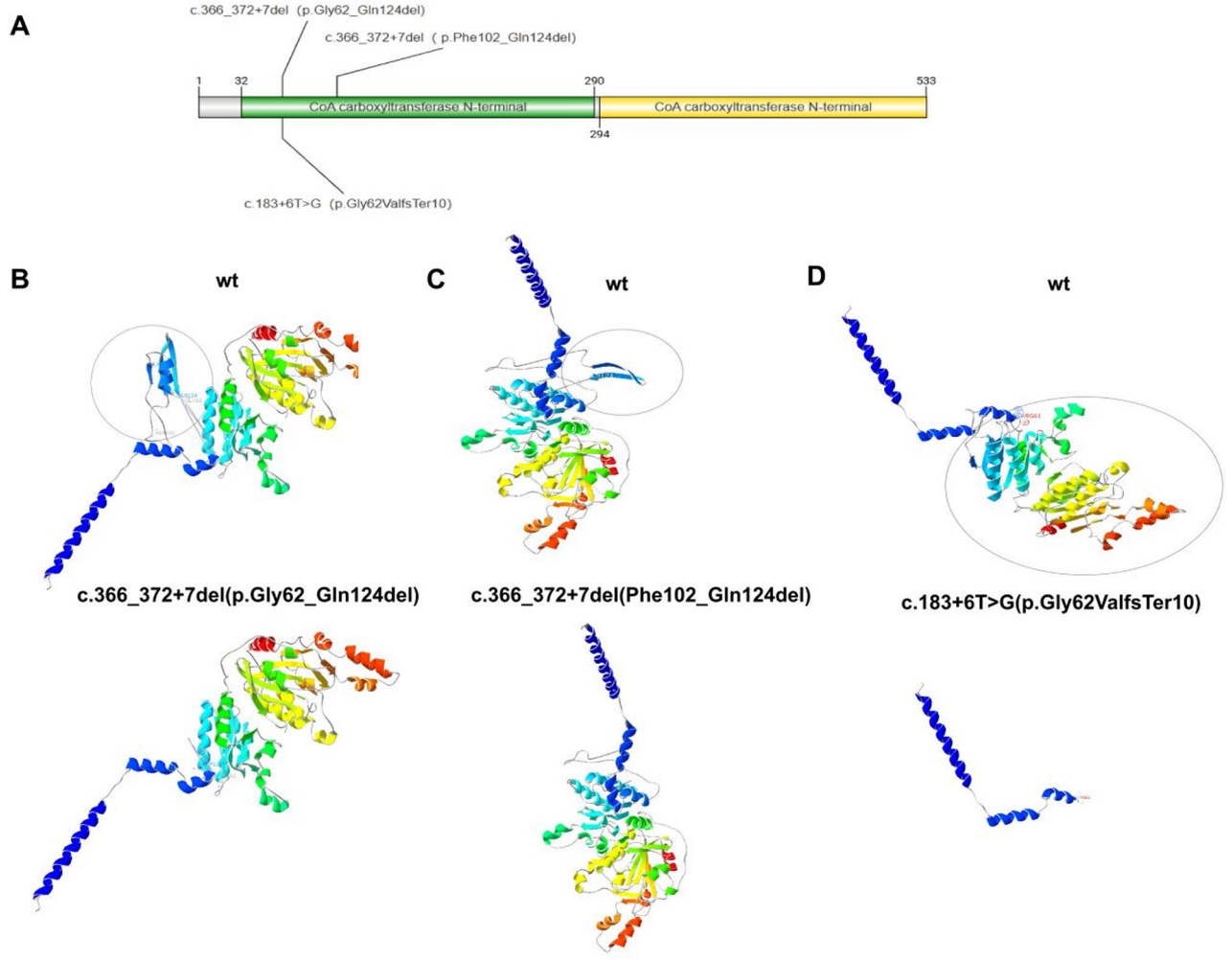

Fig. 6 Schematic representation of *PCCB* protein domain and model. **A** *PCCB* protein sequence representation with fetus variants. **B** Three-dimensional structure diagram of *PCCB* protein. Display of protein caused by p.Gly62_Gln124del. **C** Display of protein caused by Phe102_Gln124del (**D**) Display of protein truncation caused by nonsense p.Gly62ValfsTer10

exon-skipping event on protein structure.The c.183 + 6T > G variant caused a 20 bp retention in intron 1, leading to a frameshift and a truncated protein of 70 amino acids (c.183_184insGTGAGGCCTGAGGGGCCTAA, p.Gly62Valfs10). SWISS-MODEL predicts that the c.183 + 6T > G variant induces significant alterations in protein structure. These truncated proteins likely impair the carboxyltransferase activity of PCC, contributing to the development of PA [11]. The elevated levels of propionylcarnitine (C3) in the amniotic fluid further support the diagnosis of PA.

This study identified novel compound heterozygote variants in the *PCCB* gene in a Chinese fetus with PA using Exome Sequencing and Sanger sequencing. Technical limitations in obtaining fetal tissue samples precluded the direct measurement of the enzymatic activity of the truncated *PCCB* proteins. Moreover, the untimely death of the proband rendered the genetic data unavailable, thus restricting the familial segregation analysis. Despite these limitations, our findings contribute to the understanding of *PCCB* splicing variants and underscore the importance of integrating multiple diagnostic modalities—genetic, biochemical, and functional—for the prenatal diagnosis of propionic acidemia.

Abbreviations

ACMG	The American College of Medical Genetics and Genomics
ES	Exome Sequencing
OMIM	Online Mendelian inheritance in man
DNA	Deoxyribonucleic acid
PA	Propionic acidemia
VUS	Variants of uncertain significance
PCC	Propionyl-CoA carboxylase
RDDC	Rare Diseases Data Center
RT-PCR	Reverse Transcription Polymerase Chain Reaction
MS/MS	Tandem Mass Spectrometry
wt	wild-type

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12920-026-02338-1.

Supplementary Material 1.

Supplementary Material 2.

Acknowledgements
We deeply appreciate the cooperation of all study participants.

Authors' contributions
XF and HX designed the research framework. YH and CX carried out laboratory work including DNA extraction and sequencing. XF and LZ performed variant annotation and interpretation. NM conducted statistical analyses. HY provided pediatric genetics expertise. HX secured funding and ethics approval. All authors contributed to manuscript drafting and critical revision, and approved the final version for publication.

Funding
This study was supported by Hunan Provincial Maternal and Child Health Hospital-High-level Health Talent Cultivation Program.

Data availability
The datasets generated and analysed during the current study are available in the ClinVar repository, SCV006079881 - SCV006079882.

Declarations

Ethics approval and consent to participate
This study complies with the Helsinki Declaration, and the research protocol has been reviewed and approved by the Ethics Committee of the Research Center of Hunan Maternal and Child Health Hospital (protocol number 2024-S052. Participants (parents) gave written informed consent to the use of any data for scientific purposes.

Consent for publication
Written informed consent was obtained from the patient/legal guardian for publication of this case report and any accompanying images. A copy of the consent form is available for review by the Editor of this journal.

Competing interests
The authors declare no competing interests.

Received: 22 May 2025 / Accepted: 23 February 2026
Published online: 03 March 2026

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