



Short Communication

Whole-exome sequencing identifies novel truncating ALPK3 variants in a compound heterozygous state associated with pediatric hypertrophic cardiomyopathy and phenotypic heterogeneity

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ABSTRACT

Background: Pathogenic biallelic variants in *ALPK3* are associated with recessively inherited early-onset and severe cardiomyopathies in children, often accompanied by extracardiac manifestations such as facial deformities and skeletal malformations. However, the number of reported cases remains limited. In this study, we report two additional pediatric patients with hypertrophic cardiomyopathy (HCM) having (likely) pathogenic heterozygous *ALPK3* variants in a compound heterozygous state, together with a comprehensive review of the literature.

Methods: Whole-exome sequencing (WES) was performed to identify potential genetic causes of cardiomyopathy in the patients. Candidate variants were confirmed by Sanger sequencing in the patients and their parents to determine segregation patterns. Clinical evaluations, including echocardiography and physical examinations, were conducted to assess both cardiac and extracardiac features.

Results: Patient 1, a 6-year-old girl, presented with HCM, short stature, webbed neck, joint contractures, pectus carinatum, and scoliosis. Patient 2, a 3-year-old boy, was diagnosed with HCM and exhibited reduced left ventricular systolic function and short stature. Genetic analysis identified novel (likely) pathogenic truncating variants in *ALPK3* in a compound heterozygous state: c.109del, p.(R37Gfs*72) and c.2757dup, p.(T920Hfs*14) in patient 1; and c.3272del, p.(G1091Vfs*43) and c.3517A > T, p.(R1173*) in patient 2. Segregation analysis via Sanger sequencing confirmed that each pair of variants was inherited in trans from unaffected parents, consistent with a compound heterozygous configuration.

Conclusion: We report two pediatric HCM cases with novel *ALPK3* variants, expanding the genetic and phenotypic spectrum of *ALPK3*-associated cardiomyopathies. These findings enhance our understanding of genotype-phenotype correlations and underscore the importance of genetic testing, comprehensive cardiac evaluation, and long-term follow-up for patients with *ALPK3*-related cardiomyopathy.

1. Introduction

Hypertrophic cardiomyopathy (HCM) is a prevalent inherited cardiac disorder characterized by left ventricular hypertrophy, most

notably of the interventricular septum, often leading to left ventricular outflow tract obstruction. It represents a leading cause of sudden cardiac death (SCD) in children and adolescents. Typically inherited in an autosomal dominant pattern, HCM affects approximately 1 in 500

Abbreviations: CMH27, Familial hypertrophic cardiomyopathy-27; HCM, Hypertrophic cardiomyopathy; DCM, Dilated cardiomyopathy; SCD, Sudden cardiac death; WES, Whole-exome sequencing; gnomAD, Genome Aggregation Database; ExAC, Exome Aggregation Consortium; ACMG, American College of Medical Genetics and Genomics; IVS, Interventricular septum; LVPW, Left posterior ventricular wall; LVEF, Left Ventricular ejection fractions; LVFS, Left ventricular fraction shortening; NMD, Nonsense-mediated mRNA decay; ICD, Implantable cardioverter defibrillator; CMR, Cardiac magnetic resonance.

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individuals (Massera et al., 2023). Pathogenic variants have been identified in 30–60 % of cases, most frequently in *MYH7* and *MYBPC3*, with other genes including *TNNT2*, *TNNI3*, *TPM1*, *ACTC1*, *MYL2*, and *MYL3* (Hespe et al., 2025; Ommen et al., 2024). Clinical features vary from exertional dyspnea, chest pain, and syncope to asymptomatic hypertrophy. About 25 % of patients develop atrial fibrillation and 20–30 % have ventricular arrhythmias (Marian and Braunwald, 2017). Diagnosis typically involves electrocardiography (ECG), echocardiography, and cardiac magnetic resonance imaging (CMR); genetic testing aids confirmation and family screening. Management involves β -blockers, calcium channel blockers, septal reduction, or implantable cardioverter-defibrillator (ICD) for SCD prevention, with long-term care focusing on monitoring, lifestyle adjustment, and exercise restriction.

The *ALPK3* gene (OMIM 617608), located on chromosome 15q25.3, spans approximately 56 kb and comprises 14 exons. Identified in 2001 as Midori, it encodes a 201-kDa protein with a nuclear localization signal, two immunoglobulin-like domains, and a C-terminal α -kinase domain. Although initially classified as atypical, The *ALPK3* protein lacks catalytic activity and is the first pseudokinase in the α -kinase family, potentially regulating sarcomeric phosphorylation indirectly (Feng et al., 2023). *ALPK3* produces a single full-length transcript (RefSeq: NM_020778.5) encoding a complete α -kinase-like protein, with high mRNA and protein expression in cardiac and skeletal muscle (Feng et al., 2025). During embryogenesis, it is expressed in the cardiac crescent and promotes cardiomyocyte differentiation, suggesting a role in cardiac transcriptional regulation (Hosoda et al., 2001).

ALPK3 was initially identified as a nuclear protein in 2001 (Hosoda et al., 2001). Later studies revealed predominant M-band localization, critical for sarcomeric stability and contraction. In *ALPK3*-mutant hiPSC-derived cardiomyocytes, the *ALPK3* protein colocalized with myomesin proteins (MYOM1 and MYOM2) at the nuclear envelope and M-band, and is required for the sarcomeric localization of SQSTM1 (Agarwal et al., 2022; McNamara et al., 2023). Recent findings suggest that *ALPK3* regulates sarcomeric protein turnover via MuRF1, an E3 ubiquitin ligase critical for thick filament degradation. Moreover, *ALPK3* deficiency has been reported to drive a progressive transition from dilated cardiomyopathy (DCM) to left ventricular hypertrophy (LVH), and potentially to HCM. (Feng et al., 2025). These findings suggest a potential molecular mechanism underlying *ALPK3* deficiency-induced cardiomyopathy.

In 2016, homozygous mutations in the *ALPK3* gene were first identified as causative for familial hypertrophic cardiomyopathy-27 (CMH27) (OMIM#618052) consistent with autosomal recessive inheritance pattern. Previous evidence suggested that heterozygous carriers of *ALPK3* mutations may be at increased risk of HCM in adulthood (Chumakova et al., 2022a). This association was later confirmed, as monoallelic truncating variants in *ALPK3* were strongly linked to HCM in subsequent studies (Hespe et al., 2025). To date, 45 patients with biallelic pathogenic variants in *ALPK3* have been reported, exhibiting a broad phenotypic spectrum, according to our latest literature review. We report two Chinese pediatric patients carrying (likely) pathogenic truncating variants in *ALPK3*, confirmed to be in trans and thus forming a compound heterozygous state. In addition, we review previously reported cases to summarize the clinical features and progression patterns associated with *ALPK3*-related cardiomyopathy.

2. Materials and Methods

The study was approved by the ethics committee of the Children's Hospital of Nanjing Medical University. Routine clinical evaluations included a physical examination, ECG, chest radiograph, transthoracic echocardiography, cardiac magnetic resonance imaging (CMR), neurological examination, and biochemical and cardiac enzyme testing. Family history was obtained through structured interviews with the patients' parents and review of medical records. Additionally, the patients' parents and siblings also underwent comprehensive cardiac

evaluations, including clinical assessment, ECG, and echocardiography. Written informed consent was obtained from the parents prior to genetic testing and for the publication of clinical and imaging data.

2.1. Whole-exome sequencing (WES)

Genomic DNA was extracted from peripheral blood samples of the patients and their parents using a DNA Isolation Kit (Tiangen, China), following the manufacturer's instructions. Extracted DNA was randomly fragmented into 180–280 bp segments using ultrasonication. Fragmented DNA was subjected to end repair, A-tailing, and adapter ligation to construct sequencing libraries. Target exonic regions were captured using the Agilent SureSelect Human All Exon V6 (Agilent Technologies, Santa Clara, CA, USA). Following enrichment and quality control assessment, the libraries were sequenced on the Illumina HiSeq 4000 platform (Illumina, San Diego, CA, USA) to generate paired-end reads.

Raw sequencing data underwent quality control to remove adapter sequences and low-quality reads, yielding high-quality clean reads for downstream analysis. Clean reads were aligned to the human reference genome (GRCh37/hg19) using the Burrows-Wheeler Aligner (BWA, version 0.7.17). Post-alignment processing, including duplicate marking and base quality score recalibration (BQSR), was performed following the GATK Best Practices using the Genome Analysis Toolkit (GATK, version 4.1.9.0). Variant calling, including single nucleotide variants (SNVs) and insertions/deletions (InDels), was conducted using the HaplotypeCaller module. Variants were annotated using multiple public databases, including the Consensus Coding Sequence (CCDS, release 20130630), Genome Aggregation Database (gnomAD), 1000 Genomes Project (Chinese population), dbSNP, and ExAC. Common variants with a minor allele frequency (MAF) greater than 0.01 in these databases were excluded. Remaining rare variants (MAF \leq 0.01) were retained for further analysis. Candidate variants were classified according to the guidelines of the American College of Medical Genetics and Genomics (ACMG) into five categories: pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, and benign. Finally, all candidate variants of potential clinical significance were validated by Sanger sequencing in the patients and their parents to confirm variant authenticity and assess segregation within the family (Dai et al., 2019; Han et al., 2020; Zhang et al., 2020). A schematic overview of the data interpretation process is shown in Fig. 1.

2.2. Sanger sequencing

All exonic regions of *ALPK3* (Ensembl Accession: ENSG00000136383) were amplified using polymerase chain reaction (PCR) with primers designed using Primer software (Supplementary Table 1). The PCR reaction mixture (25 μ l total volume) consisted of 1.5 μ l primers, 2.0 μ l genomic DNA, 12.5 μ l 2 \times Taq Master Mix (Vazyme Biotech Co., Ltd., Nanjing, China), and 9 μ l double-distilled water. PCR cycling conditions included an initial denaturation at 94 $^{\circ}$ C for 5 min, followed by 34 cycles of 94 $^{\circ}$ C for 30 s, 59 $^{\circ}$ C for 30 s, and 72 $^{\circ}$ C for 30 s, with a final extension at 72 $^{\circ}$ C for 5 min. PCR products were purified and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Variants were described based on the NCBI reference sequence for *ALPK3* (NM_020778.5) and interpreted according to the standards and guidelines of the ACMG.

Sanger sequencing was performed to validate the variants. For *ALPK3* (NM_020778.5), the following primer pairs were used:

0c.109del, p.(R37Gfs*72): F 5'-CTATAAATAGGGGCGCGCTCAG-3' and R 5'-ATGGATCAGGAGGGGTCACAAG-3';

0c.2757dup, p.(T920Hfs*14): F 5'-CGCAGGTGGATGCTGGGACA-3' and R 5'-TCCCCAGTCGACCAAGCAT-3';

0c.3272del, p.(G1091Vfs*43): F 5'-CGCAGGTGGATGCTGGGACA-3' and R 5'-TCCCCAGTCGACCAAGCAT-3';

0c.3517A > T, p.(R1173*): F 5'-CGGCAGCTACACCTGAGGAAC-3' and R 5'-AGGGCCACCTCTGTGTCCAGACC-3'.

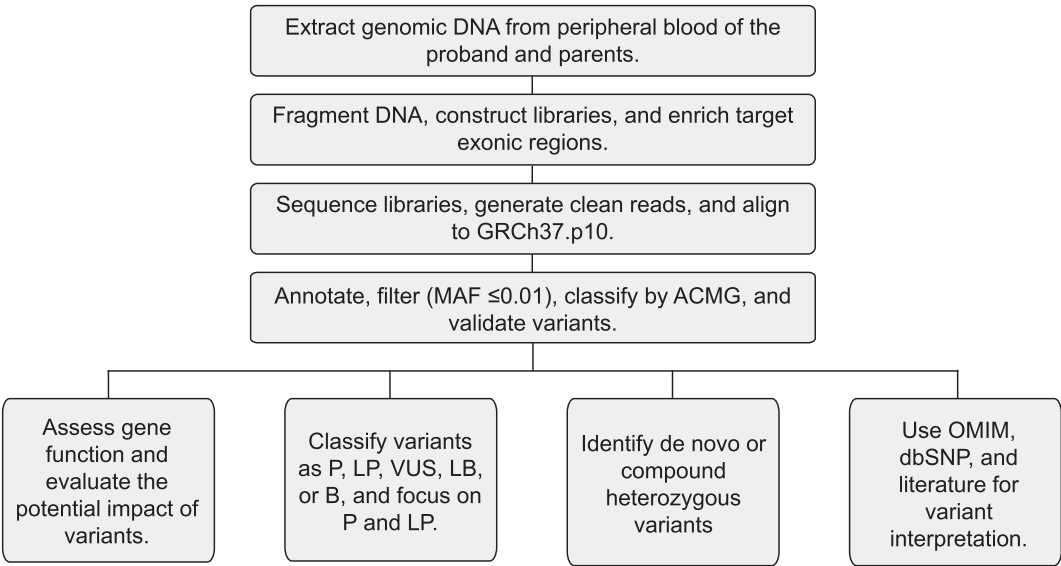


Fig. 1. Schematic overview of the whole-exome sequencing (WES) workflow.

3. Results

3.1. Genetic analysis

WES revealed that patient 1 harbored two novel variants in *ALPK3* (NM_020778.5) in a compound heterozygous state: c.109del, p.(R37Gfs*72); and c.2757dup, p.(T920Hfs*14). Both variants were confirmed by Sanger sequencing, which revealed that the father carried the c.109del, p.(R37Gfs*72) variant, while the mother and the younger brother carried the c.2757dup, p.(T920Hfs*14) variant (Fig. 2A, B). According to the ACMG criteria, the c.109del, p.(R37Gfs*72) variant was classified as pathogenic (PVS1 + PM2 + PM3), and the c.2757dup, p.(T920Hfs*14) variant was classified as likely pathogenic (PVS1 + PM2). The two frameshift variants were predicted in silico analysis to undergo degradation via the nonsense-mediated mRNA decay (NMD) pathway (<https://nmdpredictions.shinyapps.io/shiny/>).

For Patient 2, two additional novel variants in *ALPK3* (NM_020778.5) were also identified in a compound heterozygous state: c.3272del, p.(G1091Vfs*43); and c.3517A > T, p.(R1173*). Sanger sequencing confirmed that the mother harbored the c.3272del, p.(G1091Vfs*43) variant, while the father harbored the c.3517A > T, p.(R1173*) variant (Fig. 2A, B). According to the ACMG guidelines, the c.3272del, p.(G1091Vfs*43) was classified as pathogenic (PVS1 + PM2 + PM3), while the c.3517A > T, p.(R1173*) was classified as likely pathogenic (PVS1 + PM2), respectively. Both truncating variants were predicted in silico to undergo nonsense-mediated decay (NMD) using the NMD prediction tool (<https://nmdpredictions.shinyapps.io/shiny/>) (Fig. 2C).

3.2. Clinical manifestations

Patient 1, a 6-year-old girl, was born with bilateral fifth finger flexion contractures, limited extension of the left ring finger, and bilateral interphalangeal joint contractures. Motor milestones were delayed (independent walking at 18 months), while language development was normal. At age 2, she showed a webbed neck, widely spaced nipples, and pectus carinatum; scoliosis (Cobb angle: 56°) was diagnosed at age 3 with thoracic hemivertebrae on imaging (Fig. 2D). Severe pneumonia at age 3 required fiberoptic bronchoscopy with alveolar lavage, which revealed airway compression and localized collapse due to vertebral deformities; resting oxygen saturation ranged between 92 % and 96 %. Preoperative cardiac evaluation in 2024 showed interventricular septal

thickness in diastole (IVSd: 11.8 mm), increased trabeculations, and preserved systolic function (LVEF: 67 %, LVFS: 36.3 %) (Fig. 2D). ECG revealed biventricular hypertrophy with diffuse ST-T abnormalities and a prolonged QTc interval of 492 ms; 24-hour Holter monitoring showed no ventricular arrhythmia. CMR demonstrated symmetric left ventricular hypertrophy without delayed enhancement (Fig. 2D). Laboratory testing revealed elevated blood lactate (1.8 mmol/L; ref: 0.36–0.75), mildly elevated liver enzymes (ALT: 48 U/L, AST: 55 U/L), with normal creatine kinase and NT-proBNP. Treatment included oral L-carnitine, coenzyme Q10, and metoprolol. At 1-year follow-up, no progression of myocardial hypertrophy or cardiac symptoms was observed. Posterior spinal fusion with growing rod implantation were completed in February 2025. Family history was unremarkable for cardiomyopathy, scoliosis, or thoracic cage abnormalities; cardiac evaluations of her parents and younger brother were normal.

Patient 2, a 4-year-old boy, was born in August 2020 via full-term spontaneous vaginal delivery (G1P1, non-consanguineous parents). Prenatal examinations were unremarkable, and the mother had no history of infection, chronic illness, or medication exposure during pregnancy. At birth, he weighed 2.85 kg and measured 50 cm in length, with Apgar scores of 9. Neonatal vital signs were stable. Postnatally, he exhibited persistent growth retardation, and at 4 years and 8 months of age, his height was 99 cm (−1.83 SD) and weight 15 kg (−1.18 SD). Mild developmental delay was observed, with independent walking achieved at 2 years of age. He frequently experienced fatigue during daily activities and was unable to sustain vigorous physical exertion. There was no history of significant infections, trauma, surgery, or drug allergies, and no family history of cardiomyopathy. In 2023, he presented with a cough, and chest X-ray revealed cardiomegaly (cardiothoracic ratio = 0.52). Echocardiography demonstrated symmetric hypertrophy of the interventricular septum and left ventricular posterior wall (IVSd: 8.7 mm; LVPWd: 8.9 mm), along with reduced left ventricular systolic function (LVEF: 45.5 %, Simpson method) (Fig. 2D). ECG showed sinus rhythm, left ventricular high voltage, diffuse ST-T abnormalities, and prolonged QTc interval (maximum QTc interval reaching 520 ms). Physical examination revealed a high-arched palate and webbed neck; muscle tone was normal, and strength was grade 4–5. Laboratory testing indicated elevated arterial blood lactate (3.12 mmol/L; reference: 0.36–0.75 mmol/L), while serum creatine kinase and NT-proBNP levels were repeatedly normal. Liver and kidney function tests, along with blood and urine metabolic screening, were unremarkable. CMR revealed symmetric thickening of the left ventricular wall and interventricular

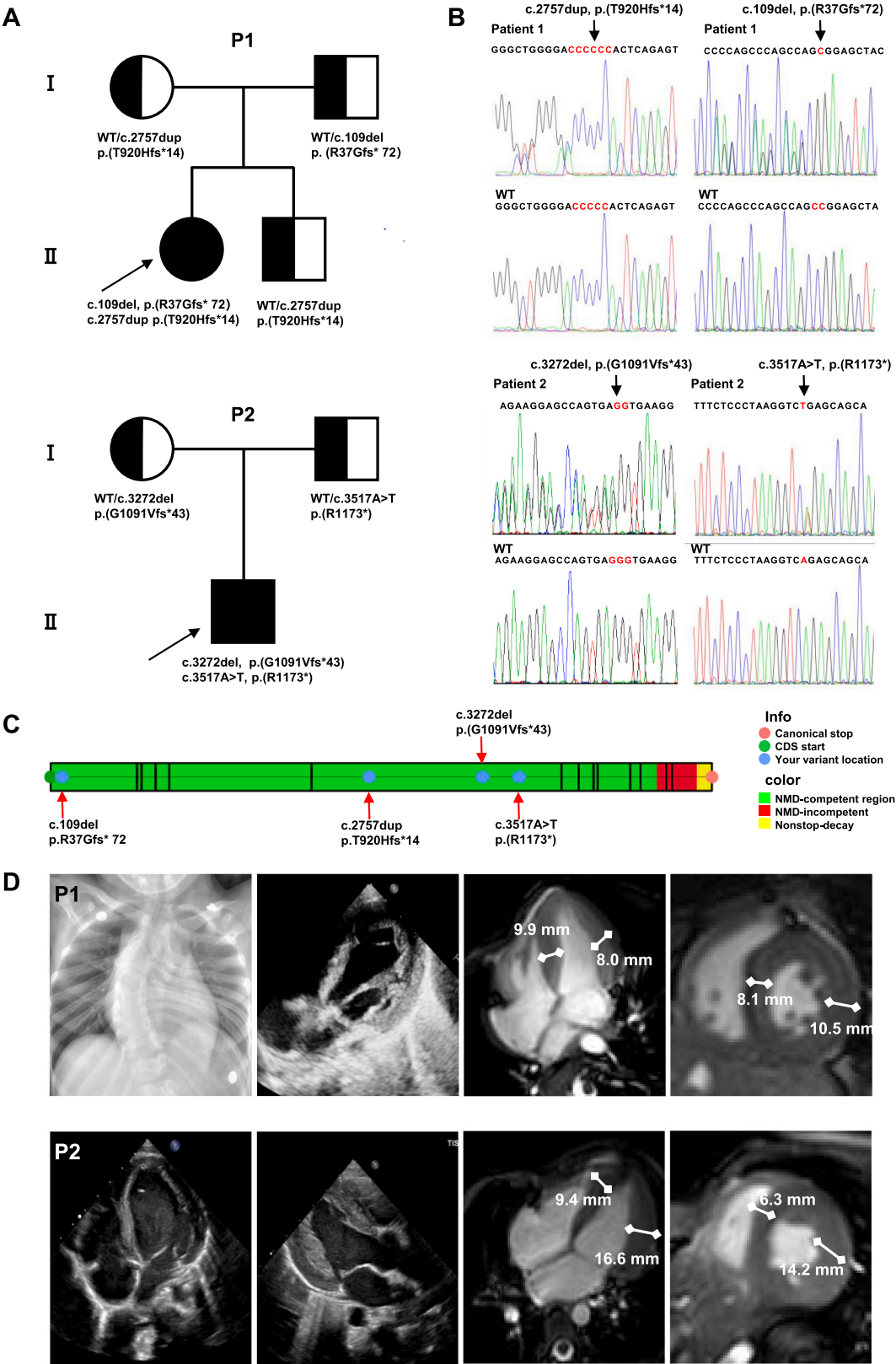


Fig. 2. (A) Pedigree of patient 1 and patient 2. (B) Sanger sequencing demonstrating the ALPK3 variants of patient 1 and patient 2 (C) All variants were fully degraded by the NMD pathway as predicted by the silico. (D) Chest radiograph, echocardiogram and CMR in patient 1 and patient 2. CMR: Cardiac magnetic resonance; IVS: interventricular septum; LVPW: left ventricular posterior wall.

septum without evidence of delayed enhancement (Fig. 2D). During hospitalization, the patient received intravenous furosemide and dobutamine. After discharge, long-term oral treatment with coenzyme Q10, L-carnitine, metoprolol, and captopril was initiated. Over a 2-year follow-up period, no chest pain or syncope occurred. QTc interval fluctuated between 460–520 ms, and no significant progression of myocardial hypertrophy was observed. Following regular pharmacologic therapy, lactate levels normalized (1.18 mmol/L), with marked improvement in exercise tolerance and muscle strength. Cognitive development was normal, though the patient continued to experience fatigue during daily activities and remained unable to sustain high-intensity exercise. Echocardiography and 12-lead ECGs in both parents were unremarkable. The follow-up cardiac ultrasound data of the two patients, from the initial consultation to the present, are provided in **Supplementary Material**.

4. Discussion

This study identified two pediatric patients harboring (likely) pathogenic truncating variants in *ALPK3* (NM_020778.5) in a compound heterozygous state, including three frameshift variants (p.(R37Gfs*72), p.(T920Hfs*14), and p.(G1091Vfs*43)) and one nonsense variant (p.(R1173*)). Both patients presented primarily with HCM. Patient 1 exhibited additional dysmorphic features, including a webbed neck, widely spaced nipples, joint contractures, pectus carinatum, and scoliosis. She recently underwent successful posterior spinal fusion with growing rod implantation and is recovering well. Ongoing monitoring will focus on the progression of myocardial hypertrophy. Patient 2 showed reduced left ventricular systolic function in the absence of significant extracardiac malformations. Following pharmacologic treatment, exercise tolerance and muscle strength improved, but fatigue persisted, limiting high-intensity activity. Ongoing monitoring will focus on potential neuromuscular involvement. Genetic analysis confirmed a diagnosis of cardiomyopathy-27 (CMH27), a rare autosomal recessive disorder caused by biallelic *ALPK3* variants.

To date, 45 patients from 33 families with *ALPK3*-related cardiomyopathy have been reported (Table 1) (Ader et al., 2024; Al Senaidi et al., 2019; Almomani et al., 2016; Çağlayan et al., 2017; Chumakova et al., 2022b; Ding et al., 2021; Grutters et al., 2023; Herkert et al., 2020; Jaouadi et al., 2018; Jorholt et al., 2020; Li et al., 2023; Papadopoulos et al., 2022; Phelan et al., 2016; Poleg et al., 2023).

The most commonly observed cardiac phenotypes among *ALPK3*-related cases include HCM and DCM. Among 45 reported cases, more than half (25/45, 55.6 %) were associated with extracardiac dysmorphic features, most frequently scoliosis (13/25) and joint contractures (10/25). Growth retardation was reported in 13 of 45 individuals. Although age of onset ranged from the fetal period to adulthood, individuals with disease onset before one year of age ($n = 25$, including 8 fetal cases) tended to exhibit more severe clinical presentations and poorer outcomes. Notably, all five reported deaths occurred within this early-onset group, attributed to progressive heart failure ($n = 3$) or malignant arrhythmias ($n = 2$). In addition, five patients underwent ICDs for life-threatening arrhythmias at a median age of 14.8 years, including one who subsequently required heart transplantation. Three other patients received HT due to refractory cardiac failure at a median age of 16 years. Longitudinal phenotypic progression was observed in 14 of these 25 early-onset cases. In contrast, childhood-onset ($n = 9$) and adolescent-onset ($n = 5$) cases demonstrated heterogeneity in disease severity, generally manifesting as progressive concentric myocardial hypertrophy with declining left ventricular systolic function. Adult-onset cases ($n = 6$) were generally milder, with most remaining asymptomatic and without extracardiac involvement. The oldest surviving individual was 53 years old. However, delayed deterioration was reported in one adult patient, who developed worsening cardiac symptoms following her second pregnancy and cesarean section.

Notably, pronounced intrafamilial phenotypic heterogeneity has

been observed, even among individuals sharing identical *ALPK3* genotypes. In a consanguineous family, two affected fetuses died of progressive heart failure—one at 35 weeks of gestation and the other within two hours after birth. A third sibling, despite presenting with severe neonatal-onset HCM, survived into adolescence (Almomani et al., 2016). Similarly, in one family with six affected individuals, two neonates exhibited a mixed phenotype comprising left ventricular non-compaction, myocardial hypertrophy, and ventricular dilatation at birth. Among the remaining four members, one developed DCM later in life, one had no evidence of cardiomyopathy at birth, and two remained asymptomatic in adulthood (Al Senaidi et al., 2019).

This study included two pediatric patients: Patient 1 (6 years old), who exhibited cardiomegaly and extracardiac manifestations, and Patient 2 (3 years old), who primarily presented with myocardial hypertrophy and reduced left ventricular systolic function. Both patients showed growth delay, consistent with previously reported phenotypes. Nevertheless, no phenotypic transition was observed in our patients. However, it is important to note that both patients had not undergone any specialized cardiac evaluations, such as echocardiography, prior to their initial consultation. As a result, it is unclear whether there was any prior presence of DCM before the onset of HCM. Additionally, no evidence of a concurrent phenotype characterized by both myocardial hypertrophy and cardiac dilation was observed. Despite pharmacologic treatment preventing progressive myocardial hypertrophy and left ventricular outflow tract obstruction, and multiple Holter monitoring sessions showing no evidence of malignant arrhythmias, both patients exhibited prolonged QT intervals. Notably, Patient 2, was found to have a QTc of 520 ms during the most recent follow-up, highlighting the need for continued vigilance regarding the potential development of malignant arrhythmias and the importance of long-term monitoring.

To date, a total of 50 pathogenic variants in the *ALPK3* gene associated with CMH27 have been identified, with frameshift (20/49) and nonsense (16/49) variants being the most common, followed by missense variants (10/49), splice-affecting variants (2/49), and a whole gene deletion (1/49) (Table 1). Truncating variants have been proposed to associate with more severe clinical outcomes, whereas missense variants tend to result in milder phenotypes. However, review of 45 published cases has not established a consistent correlation between variant type and disease severity or prognosis (Li et al., 2023).

In the present cohort, four novel truncating variants in *ALPK3*—p.(R37Gfs*72), p.(T920Hfs*14), p.(G1091Vfs*43), and p.(R1173*)—were identified across two patients. These variants were classified as pathogenic or likely pathogenic based on clinical presentation and supporting genetic evidence. According to the ACMG guidelines, p.(R37Gfs*72) and p.(G1091Vfs*43) met the criteria for pathogenic classification, while p.(T920Hfs*14) and p.(R1173*) were deemed likely pathogenic.

These findings expand the known mutational spectrum of *ALPK3* and highlight the pathogenic relevance of truncating variants in the development of *ALPK3*-related cardiomyopathy. Mechanistically, these variants are predicted to introduce premature termination codons, potentially leading to nonsense-mediated mRNA decay (NMD) or the translation of truncated, nonfunctional proteins, ultimately resulting in reduced or absent *ALPK3* protein expression.

Management of *ALPK3*-associated cardiomyopathy remains largely symptomatic and supportive, with limited therapeutic efficacy. Among the 45 reported cases, 12 (26.7 %) developed congestive heart failure and 12 (26.7 %) experienced fatal arrhythmias (Table 1), with poor outcomes frequently necessitating ICD implantation or heart transplantation. Of the 12 patients with deadly arrhythmias, Nine patients received ICDs and achieved long-term survival, with the longest reported survivor reaching 38 years of age (Ader et al., 2024; Almomani et al., 2016; Herkert et al., 2020; Phelan et al., 2016). In contrast, among the three patients who did not receive ICDs, two died suddenly and one experienced recurrent cardiac arrest (Herkert et al., 2020; Jorholt et al., 2020). In the present cohort, both patients were treated with cardiac-

Table 1
Clinical and genetic characteristics of patients with *ALPK3* biallelic pathogenic variants.

Family	Patient	Sex	Age at onset	Cardiac Phenotype at Presentation	Cardiac Outcome	Deformity Characteristics	Growth	Genotype	Variant 1	Variant 2
F1	P1	M	At birth	Severe biventricular dilatation, DCM	5 days of post-partum: Died of progressive HF	NM	Normal	Hom	0c.4736-1G > A, p. Val1579Glyfs*30	0c.4736-1G > A, p. Val1579Glyfs*30
F2	P2	F	33 weeks of gestation	Cardiomegaly, HCM	35 weeks of gestation: Died of progressive HF	NM	NM	Hom	0c.3781C > T, p. Arg1261*	0c.3781C > T, p. Arg1261*
	P3	F	20 weeks of gestation	Severe biventricular hypertrophy and dilation, DCM + HCM	2 h post-partum: Died of progressive HF	NM	Normal	Hom	0c.3781C > T, p. Arg1261*	0c.3781C > T, p. Arg1261*
	P4	F	At birth	Severe concentric LVH, HCM	11y: Status quo; 13y: LVH 23 mm, LVFS 27 %, PVCs	NM	Short stature (−2.8SD)	Hom	0c.3781C > T, p. Arg1261*	0c.3781C > T, p. Arg1261*
F3	P5	M	4y	Severe concentric LVH, RVH, HCM	7y: VF, SCD, ICD; 14y: increased LVH, LVFS 26 %	Cleft palate, Ptosis, Low set ears, Knee contractures, Kyphoscoliosis	Short stature (−2.0SD)	Hom	0c.5294G > A, p. Trp1765*	0c.5294G > A, p. Trp1765*
F4	P6	F	6 m	DCM + LVNC with rapid transformation into significant LVH	18y: ICD;30y: severe LVH, mild systolic dysfunction (DCM evolution to HCM)	Pterygia, Cleft palate, Knee and shoulder contracrures, Camptodactyly, Webbed neck	NM	Hom	0c.3792G > A, p. Trp1264*	0c.3792G > A, p. Trp1264*
	P7	M	7 weeks after birth	DCM with transformation into severe HCM at 8 m	17y: LVH 27 mm 19y: mild systolic dysfunction, NSVT (DCM evolution to HCM)	Pterygia, Cleft palate, Knee and shoulder contracrures , Camptodactyly, Webbed neck, Kyphoscoliosis	NM	Hom	0c.3792G > A, p. Trp1264*	0c.3792G > A, p. Trp1264*
F5	P8	M	21 weeks of gestation	DCM progressed to diffuse HCM at 4 m	5y: ICD (DCM evolution to HCM)	High arched palate, Low set ears	Normal	Hom	0c.2023delC, p. Gln675Serfs*30	0c.2023delC, p. Gln675Serfs*30
F6	P9	M	1 week after birth	HCM/DCM; LVEF 35 % at 3 m	6 m: LVEF 50 % 11 m: biventricular HCM, LVEF 74 % 3y: alive (DCM + HCM evolution to HCM)	Cleft palate, VPI, Ptosis, Low set ears, Micrognathia, Camptodactyly, Webbed neck, pectus excavatum	Normal	Hom	0c.1531_1532delAA, p. Lys511Argfs*12	0c.1531_1532delAA, p. Lys511Argfs*12
F7	P10	M	At birth	DCM, LVEF 12 %; progressed to LVH + LVNC + RVH at 6 m	5y: PH 6y: SCD (DCM evolution to HCM, LVNC)	VPI, Ankyloglossia, Ptosis, Hypertelorism, Low set ears, Micrognathia, Knee contractures, Kyphoscoliosis, Webbed neck	Short stature (−2.0SD)	Comp het	0c.1018C > T, p. Gln340*	0c.2434G > A, p. Val812Met
	P11	M	Fetal	Biventricular dysfunction; LVEF 37 % at post-partum; DCM at 6 m; severe LVH at 12 m	3.5y: severe HCM with normal LVEF; 4y: RVH, HT (DCM evolution to HCM)	Hypertelorism, Low set ears, Micrognathia, Knee contractures, Kyphoscoliosis, Webbed neck	Normal	Comp het	0c.1018C > T, p. Gln340*	0c.2434G > A, p. Val812Met
	P12	F	4 m	Biventricular hypertrophy, HCM	11y: VF, ICD;28y: biventricular hypertrophy and dilatation, HT	Hypertelorism, Webbed neck	Short stature	Comp het	0c.1018C > T, p. Gln340*	0c.4332delC, p. Lys1445Argfs*29
F8	P13	M	31y	Biventricular dilation, preserved EF, DCM	35y: IVS 11 mm	Hypertelorism	Normal	Comp het	0c.541delG, p. Ala181Profs*130	0c.3439C > T, p. Arg1147Trp
F9	P14	M	53y	HCM, LVEF 65 %	Alive	Spondylolysis	NM	Comp het	0c.4997delA, p. Asn1666Thrfs*14	0c.4091G > C, p. Gly1364Ala
F10	P15	F	3 m	DCM, LVFS 9.4 %	5 m: dilated LV with FS 27 % ; 6m: concentric LVH, LVFS 41 % ; 9y: LVH 23 mm, PH (DCM evolution to HCM)	NO	Short stature (−2.0SD)	Comp het	c.5105 + 5G > C	0c.597G > T, p. Glu199Asp

(continued on next page)

Table 1 (continued)

Family	Patient	Sex	Age at onset	Cardiac Phenotype at Presentation	Cardiac Outcome	Deformity Characteristics	Growth	Genotype	Variant 1	Variant 2
	P16	F	3 m	DCM, LVFS 14 %	3.5y: concentric LVH, LVFS 33 % 9y: moderate LVH, LVFS 36 %, PH (DCM evolution to HCM)	NO	Short stature (−2.0SD)	Comp het	c.5105 + 5G > C	0c.597G > T, p. Glu199Asp
F11	P17	F	14y	Severe concentric HCM	15y: ICD	Cleft palate, VPI, High arched palate, Low set ears, Camptodactyly, Kyphoscoliosis, Webbed neck, Spondylolysis	Short stature (−2.0SD)	Comp het	0c.2023delC, p. Gln675Serfs*30	0c.4888G > T, p. Val1630Phe
F12	P18	F	10y	Concentric HCM with trabeculation	44y: LVNC (DCM/HCM evolution to LVNC)	Cleft palate, Camptodactyly, Kyphoscoliosis	Short stature (−6.0SD)	Hom	0c.3418C > T, p. Gln1140*	0c.3418C > T, p. Gln1140*
F13	P19	F	35 weeks of gestation	Mild LVH; biventricular HCM with slightly decreased LV function at birth	2d: poor biventricular function 7d: HCM + DCM 2y: moderate concentric LVH (DCM + HCM evolution to HCM)	Cleft palate, VPI, Hypertelorism, Low set ears, Micrognathia, Webbed neck	Short stature (−2.0SD)	Hom	0c.5155G > C, p. Ala1719Pro	0c.5155G > C, p. Ala1719Pro
F14	P20	M	31 weeks of gestation	LVNC, mild ventricular dysfunction; biventricular dilatation and hypertrophy, LVEF 40 %, LVNC at birth; 4 day after birth, HF	30 m: apical LVNC, LVH, no dilatation, LVEF 62 % (HCM, DCM, LVNC evolution to HCM, LVNC)	High arched palate, Low set ears	Normal	Hom	0c.639G > A, p. Trp213*	0c.639G > A, p. Trp213*
	P21	M	At birth	DCM, LVEF 30 %, HF	12y: HCM, LVEF 57 % (DCM evolution to HCM)	NO	Normal	Hom	0c.639G > A, p. Trp213*	0c.639G > A, p. Trp213*
	P22	F	At birth	LV trabeculation, VSD	10y: HCM, LVEF 70 %, no VSD	NO	Normal	Hom	0c.639G > A, p. Trp213*	0c.639G > A, p. Trp213*
	P23	M	38y	HCM, LVEF 60 %, asymptomatic	NA	NO	NM	Hom	0c.639G > A, p. Trp213*	0c.639G > A, p. Trp213*
	P24	M	28y	HCM, LVEF 58 %, asymptomatic	NA	NO	NM	Hom	0c.639G > A, p. Trp213*	0c.639G > A, p. Trp213*
	P25	F	At birth	LVH + LV dilatation + LVNC, LVEF 26 %	3w: HF; 42 m: HCM, LVEF 49 % (HCM, DCM, LVNC evolution to HCM)	NM	Normal	Hom	0c.639G > A, p. Trp213*	0c.639G > A, p. Trp213*
F15	P26	M	At birth	Severe HCM	4y: marked LVH, LVEF 49 %, LV trabeculation 9y: IVS 33 mm; 12y: SCD	Cleft palate, Short neck, Pectus excavatum, Scoliosis	NM	Comp het	0c.2033delG, p.R687fs*	0c.3558delG, p. V1186fs*
F16	P27	F	21y	HCM, LVEF 78 %, asymptomatic	25y: HCM, LVEF 38 %, LV trabeculation, NSVT 30y: prolonged QT, syncope, ICD 33y: HT	NM	NM	Hom	0c.4897G > A, p. Gly1633Arg	0c.4897G > A, p. Gly1633Arg
F17	P28	M	38y	LVEF 30 %; LVH 16 mm; restrictive diastole, LV trabeculation; HCM + DCM	NA	NO	Normal	Comp het	0c.1958C > G, p. Ser653*	0c.3491G > A, p. Arg1164Gln
F18	P29	M	9y	HCM	26y: LVEF 26 %, DCM, wait for HT (HCM evolution to DCM)	NO	Normal	Hom	0c.2107del, p. Ser703Leufs*2	0c.2107del, p. Ser703Leufs*2
F19	P30	M	21 weeks of gestation	DCM	4 m: Diffuse thickening, LVEF normal (DCM evolution to HCM)	High arched palate, Low set ears	Normal	Hom	0c.2018 delC, p. Gln675Serfs*30	0c.2018 delC, p. Gln675Serfs*30

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Table 1 (continued)

Family	Patient	Sex	Age at onset	Cardiac Phenotype at Presentation	Cardiac Outcome	Deformity Characteristics	Growth	Genotype	Variant 1	Variant 2
F20	P31	F	10 m	DCM; 12 m, HCM	Alive at 11y (DCM evolution to HCM)	Low set ears, Webbed neck, Hypertelorism, Joint contractures	Short stature	Comp het	0c.721dup, p. Try241Leufs*42	0c.4840C > T, p. Arg1614*
F21	P32	F	14y	HCM	Repeated cardiac arrest, parents refuse ICD	Low set ears, Scoliosis	NM	Comp het	0c.3907_3922del, p. Gly1303Leufs*28	0c.2200A > T, p. Arg734*
F22	P33	F	Infancy	significant left ventricular hypertrophy, >30 mm	20y: VF, ICD	Webbed neck, long nasal bridge with a bulbous tip	NM	Comp het	0c.4234C > T, p. Arg1412*	0c.4500-12A > G
	P34	F	Infancy	significant left ventricular hypertrophy, >30 mm	20y: ICD	Webbed neck, long nasal bridge with a bulbous tip	NM	Comp het	0c.4234C > T, p. Arg1412*	0c.4500-12A > G
F23	P35	M	4y	significant left ventricular hypertrophy	NA	Cleft palate, Joint contractures, Scoliosis	Short stature (−2.53SD)	Comp het	0c.4736-1G > A, p. Val1579Glyfs*30	15q25.2q25.3 microdeletion
F24	P36	NM	2nd trimester of pregnancy	LVNC; 4y: HCM, HF	Alive at 10y	NM	NM	Comp het	0c.1055dup, p.Asp353*	0c.4838 G > T, p. Cys1613Phe
F25	P37	NM	At birth	HCM	16y: HT	NM	NM	Comp het	0c.3175C > T, p. Arg1059*	0c.3186 G > A, p. Trp1062*
F26	P38	NM	9y	HCM, IVS24-26 mm	Alive at 12y	NM	NM	Comp het	0c.690-697 dup, p. Pro233Leufs*36	0c.4025-4035del, p. Gly1342Valfs*6
F27	P39	NM	17y	Concentric HCM	17y SCD, ICD; 38y DCM (HCM evolution to DCM)	NM	NM	Comp het	0c.4234C > T, p. Arg1412*	0c.4500-12A > G
F28	P40	NM	15y	HCM, IVS30mm	Alive at 18y	NM	NM	Hom	0c.4776C > G, p. Tyr1592*	0c.4776C > G, p. Tyr1592*
F29	P41	F	6y	HCM	NA	Low set ears, Webbed neck, Scoliosis, Joint contractures	NM	Comp het	0c.5338-5339delTG, p. Trp1780Glu fs*4	0c.1148G > T, p. Trp383*
F30	P42	F	7y	HCM	NA	Low set ears, Webbed neck, Scoliosis, Joint contractures	NM	Comp het	0c.4840C > T, p. Arg1614*	0c.3061C > T, p. Arg1021*
F31	P43	F	16y	HCM	NA	Webbed neck, Scoliosis	Normal	Comp het	0c.2200A > T, p. Arg734*	0c.5330G > A, P. Gly1777Glu
F32	P44, presented study	F	6y	HCM: LVPW, 10.5 mm; IVS, 9.9 mm	Alive	Webbed neck, Joint contractures, Pectus excavatum, Kyphoscoliosis	Short stature (−3.2SD)	Comp het	0c.109delC, p. R37Gfs*72	0c.2757dup, p. T920Hfs*14
F33	P45, presented study	M	3y	HCM: LVPW, 16.6 mm; IVS, 9.4 mm	Alive	NO	Short stature (−2.0SD)	Comp het	0c.3878delG, p. G1293fs*43	0c.4123A > T, p. R1375*

Comp het: Compound heterozygous; Hom: homozygous; NM: Not mentioned; HCM: Hypertrophic cardiomyopathy; DCM: Dilated cardiomyopathy; IVS: Interventricular septum; LVPW: Left posterior ventricular wall; LVEF: Left ventricular ejection fractions; LVFS: Left ventricular fraction shortening; LVH: Left ventricular hypertrophy; RVH: Right ventricular hypertrophy; LVNC: Left ventricular noncompaction cardiomyopathy; ICD: Implantable cardioverter defibrillator; HT: Heart transplantation; SCD: Sudden cardiac death; PH: Pulmonary hypertension; NVST: Non-sustained ventricular tachycardia; VF: Ventricular fibrillation; HF: Heart failure.

enhancing drugs, including coenzyme Q10, leucovorin, and beta-blockers. At one-year follow-up, cardiac function remained normal, with no episodes of syncope or ventricular tachyarrhythmias recorded during ambulatory monitoring. However, ongoing observation and timely follow-up remain crucial to detect potential disease progression in these patients.

5. Conclusion

In this study, two pediatric patients with HCM were identified as harboring (likely) pathogenic *ALPK3* variants in a compound heterozygous state, thereby expanding the known genetic and phenotypic spectrum of *ALPK3*-related cardiomyopathy. Early-onset cases are frequently associated with severe phenotypes, whereas later-onset cases tend to present with milder clinical features; however, genotype–phenotype correlations remain inconclusive. Given the marked clinical heterogeneity, genetic testing remains essential for accurate diagnosis. Current management is primarily supportive and requires vigilant monitoring for heart failure and arrhythmias. These findings underscore the importance of long-term follow-up and individualized care in patients with *ALPK3*-associated cardiomyopathy.

Consent for publication

Written informed consent was obtained from the minor's legal guardian, for the publication of any potentially identifiable images or data included in this article.

Ethics approval and consent to participate

The studies involving human participants were reviewed and approved by the institutional ethical committee of the Children's Hospital of Nanjing Medical University. Written informed consent to participate in this study was provided by the participant's legal guardian/next of kin.

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CRediT authorship contribution statement

Yuqi Wang: Writing – original draft, Methodology, Data curation, Conceptualization. **Ziwei Wang:** Writing – original draft, Methodology, Conceptualization. **Ningning Sun:** Writing – review & editing, Methodology. **Jie Yin:** Formal analysis, Data curation. **Yi Chen:** Methodology, Investigation. **Chunli Wang:** Project administration, Methodology, Formal analysis, Conceptualization. **Shiwei Yang:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition.

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.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.gene.2025.149597>.

Data availability

The data presented in this study are available on request. The data

are not publicly available due to privacy restrictions.

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