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ORIGINAL ARTICLE

Homozygous *TNNI3* frameshift variant in a consanguineous family with lethal infantile dilated cardiomyopathy

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Abstract

Background: Dilated cardiomyopathy (DCM) is characterized by dilatation of the left ventricle, systolic dysfunction, and normal or reduced thickness of the left ventricular wall. It is a leading cause of heart failure and cardiac death at a young age. Cases with neonatal onset DCM were correlated with severe clinical presentation and poor prognosis. A monogenic molecular etiology accounts for nearly half of cases.

Family description: Here, we report a family with three deceased offspring at the age of 1 year old. The autopsy of the first deceased infant revealed a DCM. The second infant presented a DCM phenotype with a severely reduced Left Ventricular Ejection Fraction (LVEF) of 10%. Similarly, the third infant showed a severe DCM phenotype with LVEF of 30% as well, in addition to eccentric mitral insufficiency.

Results: Exome sequencing was performed for the trio (the second deceased infant and her parents). Data analysis following the autosomal dominant and recessive patterns of inheritance was carried out along with a mitochondrial pathways-based analysis. We identified a homozygous frameshift variant in the *TNNI3* gene (c.204delG; p.(Arg69AlafsTer8)). This variant has been recently reported in the ClinVar database in association with cardiac phenotypes as pathogenic or likely pathogenic and classified as pathogenic according to ACMG.

Conclusion: Genetic counseling was provided for the family and a prenatal diagnosis of choronic villus was proposed in the absence of pre-implantation genetic diagnosis possibilities. Our study expands the case series of early-onset DCM patients with a protein-truncating variant in the *TNNI3* gene by reporting three affected infant siblings.

KEYWORDS

exome sequencing, lethal neonatal DCM, Pathogenic *TNNI3* variant

Stéphane Zaffran and Hager Jaouadi are co-last authors.

1 | INTRODUCTION

Dilated cardiomyopathy (DCM) is defined by left ventricular enlargement and systolic dysfunction. The dilation can occur in one or both of the ventricles along with a left ventricular ejection fraction (LVEF) of less than 45% (Mahmaljy et al., 2024). Indeed, the diagnosis is based on cardiac imaging and defined by the presence of two major clinical criteria: LV fractional shortening less than 25% and/or LV ejection fraction less than 45% along with end-diastolic diameter greater than 117% of the predicted value (Faggiano et al., 2021; Sweet et al., 2015). Additional criteria may be found such as increased LV wall thickness, right ventricle, or atrial chamber enlargement.

DCM is the most frequent pediatric cardiomyopathy accounting for nearly 60% of cases, with a high proportion occurring in infants under the age of 1 year (Soares et al., 2017). Moreover, it is a leading cause of heart failure among children and causes 10% of cardiac deaths. Genetic forms of DCM represent almost half of the cases and are characterized by genetic heterogeneity, with more than 100 causative genes having been identified so far (Jammal Addin et al., 2019; McNally & Mestroni, 2017; Mestroni et al., 2014). These genes encode proteins of broad cellular functions such as cytoskeletal, sarcomeric, mitochondrial, desmosomal, nuclear membrane, and RNA-binding proteins (McNally & Mestroni, 2017). Most variants occurred in titin (*TTN*; 15%–25%), lamins A/C (*LMNA*; 6%), beta-myosin heavy chain (*MYH7*; 4%), cardiac troponin T (*TNNC1*, *TNNI3*, and *TNNT2*; 4%), and myopalladin (*MYPN*; 3%) (Hershberger & Jordan, 1993; McNally & Mestroni, 2017). These mutations are associated with different modes of inheritance.

Autosomal recessive DCM is due to mutations in a dozen genes which are *TNNI3* on chromosome 19q13 (Murphy et al., 2004; Sorrentino et al., 2023), *GATAD1* gene on 7q21 (Theis et al., 2006), *PPCS* gene on 1p34 (Iuso et al., 2018), *RPL3L* gene on 16p13 (Al-Hassnan et al., 2020; Ganapathi et al., 2020), *JPH2* gene on 20q13 (Jones et al., 2019; Vasilescu et al., 2018), *BAG5* gene on 14q32 (Hakui et al., 2022), *LMOD2* gene 7q31 (Ahrens-Nicklas et al., 2019; Greenway et al., 2021; Yuen et al., 2022), *GET3* gene on 19p13 (Verhagen et al., 2019), *CAP2* gene on 6p22 (Aspit et al., 2019; Gurunathan et al., 2022), and *FLII* gene on 17p11 (Al-Hassnan et al., 2020; Ruijmbeek et al., 2023) causing CMD2A, CMD2B, CMD2C, CMD2D, CMD2I, CMD2F, CMD2I, and CMD2J, respectively. Thus, the main mode of inheritance of pediatric hereditary forms of DCM is autosomal recessive, unlike adult forms which are often autosomal dominant (56%) with variable expressivity and penetrance (Herman et al., 2012; McNally & Mestroni, 2017; Mestroni et al., 1999). Less frequently, X-linked recessive and mitochondrial transmission have also been reported (Mestroni et al., 1999). As an example, *LMOD2* and *RPL3L* genes were associated with severe forms of neonatal DCM marked by rapid cardiac failure and early mortality. Both homozygous and compound heterozygous variants have been identified in both genes (Ganapathi et al., 2020; Greenway et al., 2021; Yuen et al., 2022).

Here, we report the clinical and genetic investigations of a family with severe DCM in three deceased sisters aged between 12 and 14 months.

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

This study was conducted according to the principles of the Declaration of Helsinki and to the ethical standards of the first author's institutional review board. All patients described in this study (or their parents) gave informed consent to participate in this study.

2.2 | Exome sequencing

A peripheral blood sample was collected after obtaining the written informed consent. Genomic DNA was extracted following standard techniques.

Exome sequencing was performed for the deceased patient (IV-5 Figure 1) and her parents (III-1 and III-2) using Twist Comprehensive Exome kit (Twist Bioscience). The captured libraries were sequenced on the Illumina NovaSeq6000 sequencing platform (Illumina, San Diego, CA, USA). Raw fastQ files were aligned to the hg19 reference human genome (University of California Santa Cruz, UCSC) using BWA software. The alignment quality was assessed using Qualimap 2.2.2. Variant calling workflow was performed according to the GATK best practices. The output files were annotated using ANNOVAR software. Gender and familial relationship quality control steps were performed using somalier (v0.2.16) software packages.

2.3 | Variant prioritization

Variant prioritization was carried out using Variant Annotation and Filtering Tool (VarAFT http://varaft.eu/) version 2.17. To pinpoint candidate causative variants, we adopted the following filtering strategy: firstly, a minimum variant allele frequency of 1% was applied using gnomAD database (http://gnomad.broadinstitute.org). Then,

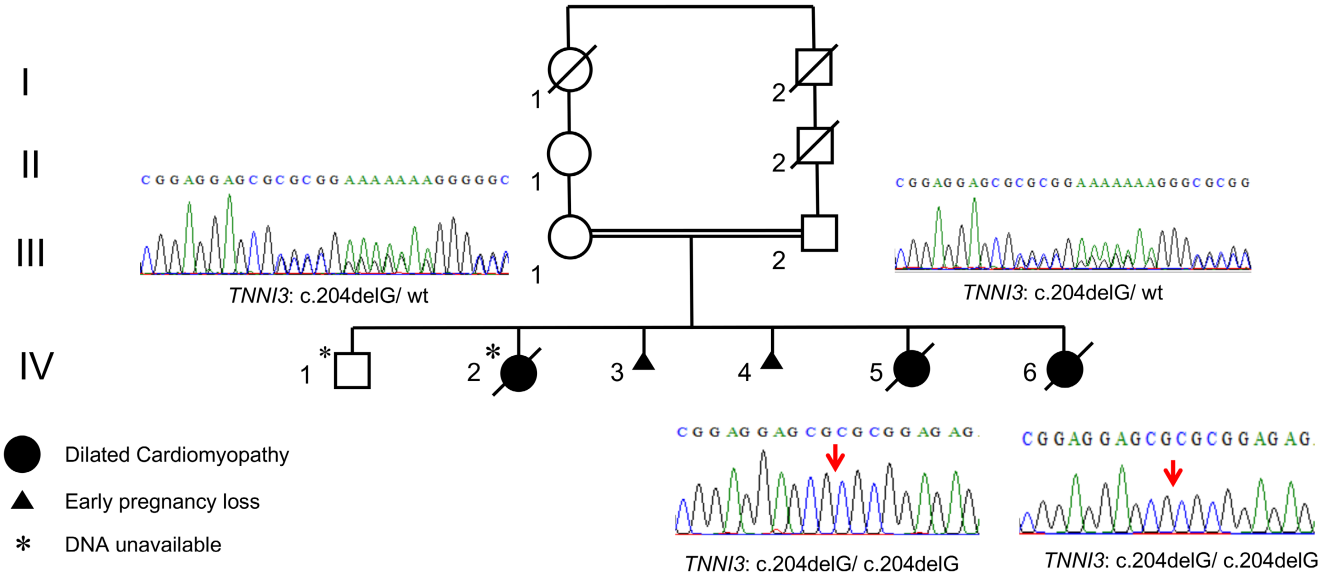

FIGURE 1 Family pedigree and Sanger sequencing results showing the homozygous deletion*TNNI3 (NM_000363.5)* : c.204delG; p.(Arg69AlafsTer8) in patients IV-5 and IV-6 and her heterozygous parents (III-1 and III-2).

non-coding and synonymous variants were removed. The remaining high-impact variants including missense and protein-truncating variants (PTV) were filtered based on their in-silico pathogenicity prediction. Prioritized variants were reviewed in concert with the clinical description of the three cases and considered relevant based on adequate phenotype–genotype correlation in genes and literature.

3 | RESULTS

3.1 | Clinical findings

The three siblings' cases were born to consanguineous parents. They had DCM and died from decompensated heart failure around the age of 12 months (Figure 1).

3.1.1 | First affected child

The oldest girl (IV-2) was born at term from an uneventful pregnancy. She presented sudden health deterioration with whining and died at the age of 12 months. Autopsy findings revealed a decompensated DCM. DNA sampling was not carried out.

3.1.2 | Index case

The second daughter (IV-5) is the index case. She was born from a full-term and uneventful pregnancy by vaginal delivery. Nuchal translucency was 1.7 mm. Second-trimester maternal serum markers were 1/1162 for trisomy 21. Psychomotor development was normal.

Medical history revealed recurrent episodes of dyspnea occurring once a month since the age of 4 months. At the age of 11 months, the patient was diagnosed with infant asthma and was placed on inhaled corticosteroids.

At the age of 13 months, she presented at the pediatric emergency department with dyspnea, a productive cough, and whining. Despite her inhaled treatment, there was no improvement. The dyspnea worsened with the onset of wheezing breath sounds. Considered asthma exacerbation, the patient was admitted to the hospital and was placed on nebulized beta 2 mimetics, intravenous corticosteroids, and magnesium sulfate. As her condition deteriorated, she was transferred to the intensive care unit.

Clinical examination showed weight, height, and head circumference at average, heart rate at 172 beats per minute, blood pressure at 88/64 mmHg, and hepatomegaly. The patient was in respiratory distress with pulsed O2 saturation at 75% and metabolic acidosis at blood gas. The electrocardiogram was normal with no sign of ischemia (Figure 2). Cardiac ultrasound showed LVEF at 10%, and DCM with mitral and tricuspid regurgitation.

The biological assessment including the levels of serum calcium, lactates, transaminases, urea, creatinine, and troponin was normal. The blood count showed hyperleukocytosis at 29660/mm^3 and hemoglobin at 10.5 g/dL. Urinary organic acid chromatography, amino acid, acylcarnitine dosage, and very long-chain fatty acids pattern were normal. The patient was intubated and ventilated and administered adrenaline, norepinephrine, and prednisolone. She also received a supplementation with

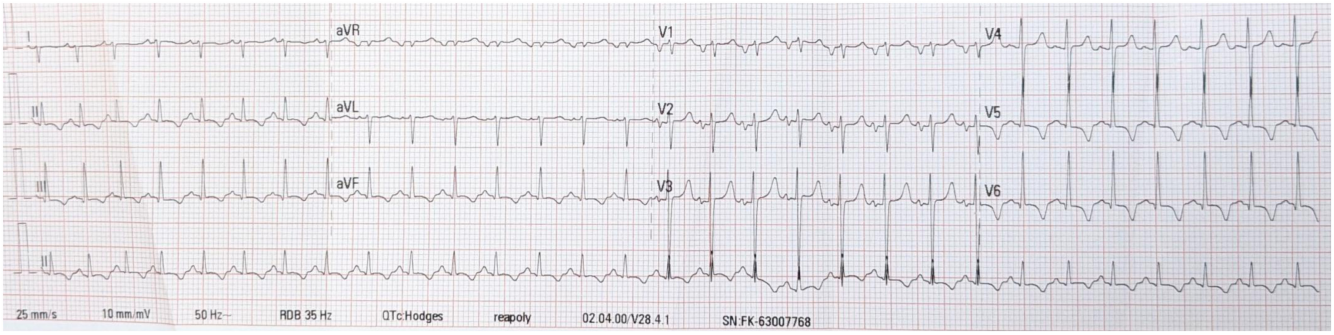

FIGURE 2 Electrocardiogram of patient IV-5 showing normal tracing at admission to the intensive unit care.

L-carnitine. A second inodilator (Levosimendan) was associated due to the persistence of heart dysfunction.

A first extubation was attempted on the 6th day of hospitalization with high-flow nasal cannula (HFNC) oxygen therapy. Three days later, the patient developed respiratory distress due to hospital-acquired pneumonia, necessitating mechanical ventilation in assist control mode. A day later, the patient was switched to pressure support mode for 48 h. Another extubation ended in respiratory failure despite HFNC. The patient was mechanically ventilated again. The mechanical ventilation was realized under sedation with midazolam and morphine.

After hemodynamic stabilization, an attempt to reduce vasoactive medication resulted in a decompensation of her DCM. The follow-up was complicated by ventricular fibrillation, which rapidly progressed to asystole.

Despite effective cardiopulmonary resuscitation, including cardiac compressions and adrenaline boluses, the patient died at the age of 14 months.

3.1.3 | Third affected child

The third affected sister (IV-6) was born a month prior to the passing of her sister (index case) from an uneventful pregnancy and full-term delivery.

Nuchal translucency was 1.7 mm. Second-trimester maternal serum markers were 1/354 for trisomy 21. Her birth weight was 2900 g, height was 46 cm, and head circumference was 32.5 cm.

Psychomotor development was normal. The patient presented three apyretic tonic–clonic convulsive seizures at the age of 12 months and was hospitalized for status epilepticus. The electroencephalogram was normal. Brain MRI was indicated but not performed. The patient was placed on Valproate of sodium. Subsequently, she was admitted to the hospital due to the onset of dyspnea at the age of 13 months.

Cardiac ultrasound showed a dilated non-hypertrophied left ventricle, an end-systolic diameter of 30 mm, an ejection fraction of 30%, a dilated left atrium, and severe eccentric mitral regurgitation. L-carnitine was prescribed for the patient. The Holter showed a supraventricular arrhythmia and amiodarone was administered. The alpha-glucosidase activity assay was normal. Renal and liver tests were normal. LDH and CPK were elevated at 583 and 598 IU/L, respectively. The complete blood count revealed anemia with a hemoglobin level of 9.3 g/dL and a mean corpuscular volume of 82 fl. L-carnitine assay showed an elevation in free carnitine and total carnitine related to L-carnitine supplementation. The patient died after 20 days of hospitalization despite medical management due to decompensation of her heart failure.

3.2 | Genetic findings

Given the consanguinity in the family and the high rate of cardiac deaths among the siblings we focused our trio-exome analysis on the autosomal recessive pattern of inheritance. Moreover, a mitochondrial pathways-based analysis was performed using the following Human Phenotype Ontology terms: “cardiomyocyte mitochondrial proliferation” or “abnormal mitochondria in muscle tissue” or “mitochondrial myopathy” or “depletion of mitochondrial DNA in muscle tissue” or “abnormality of mitochondrial metabolism” or “abnormal activity mitochondrial respiratory chain” or “decreased activity of mitochondrial complex I, II, III, and IV”.

The latter analysis did not identify any relevant variants. However, two homozygous variants were prioritized in cardiac-associated genes (Table 1).

The variant in the *LMOD3* gene was reported in the ClinVar database as benign in association with Nemaline myopathy (RCV000555540.7).

Nonetheless, the frameshift *TNNI3* variant is the likely causative variant considering its type and the clinical description of the patients. By checking ClinVar database, the *TNNI3* prioritized variant (rs727504872) has been recently reported as pathogenic for hypertrophic cardiomyopathy (RCV001237455.5), DCM type 2A (RCV001254648.1), and myocarditis primary DCM (RCV002056120.1) and as

TABLE 1 Homozygous prioritized variants in the index case.

The homozygous identified variants	Associated phenotype
TNNI3 (NM_000363.5): c.204delG;p.(Arg69AlafsTer8)	Cardiomyopathy, dilated, 2A
LMOD3 (NM_198271.5): c.1493G>A;p.(Arg498Gln)	Nemaline myopathy 10

likely pathogenic for primary familial DCM. Its allele frequency in gnomAD (v4.0.0) is 0.00001676 with no homozygous variant carriers.

Two assessment classification tools were queried to apply the American College of Medical Genetics and Genomics (ACMG) criteria, Franklin (https://franklin.genoox.com/clinical-db/home) and Varsome (https://varsome.com/). Both tools classified the *TNNI3*: c.204del variant as pathogenic. The ACMG criteria assigned by Franklin are PVS1 (Null variant in a gene where loss of function is a known mechanism of disease), PM3 (For recessive disorders, detected in trans with a pathogenic variant, or in a homozygous or compound heterozygous state in affected cases), and PM2 (low frequency in gnomAD population databases). According to Varsome, the variant met the following criteria: PVS1, PM2, and PP5 (Richards et al., 2015).

Subsequently, Sanger sequencing was performed for variant confirmation and segregation. Thus, the index case (IV-5) and her deceased sister (IV-6) carried the *TNNI3*: c.204delG; p.(Arg69AlafsTer8) variant in a homozygous state and the parents (III-1 and III-2) carried the variant at the heterozygous state (Figure 1).

The parents declined the segregation analysis in the apparently healthy brother aged 8 years (IV-1).

4 | DISCUSSION

We report three siblings with severe DCM and the exome sequencing result of the second deceased case and her parents which revealed the homozygous *TNNI3*: c.204delG; p.(Arg69AlafsTer8) variant.

The first child presented sudden health deterioration at the age of 1 year with no particular medical history. The second case had also recurrent episodes of dyspnea with a decline in health status at 1 year of age. The third sister had seizures in addition to dyspnea and DCM. LV ejection fractions were at 10% and 30% for patients IV-5 and IV-6, respectively. DCM can be syndromic or isolated with no systemic involvement. The three sisters had no additional clinical features, such as growth retardation, hypotonia, facial dysmorphism, or muscular weakness. Moreover, infant DCM cases usually develop symptoms of heart failure such as dyspnea, tachycardia, and feeding difficulty as observed in our patients (Daubeney et al., 2006; Soares et al., 2017).

The youngest child had seizures which were the initial symptoms that prompted consultation of the pediatric emergency department. The association of epilepsy and DCM could be observed in mitochondrial disorders but no variant in nuclear genes associated with mitochondrial function was found. To our knowledge, the association of epilepsy and mutations in the *TNNI3* gene has never been reported before. This association could be fortuitous, or the seizures might be secondary to ischemia caused by DCM. The latter hypothesis cannot be verified, as no brain MRI has been performed.

The *TNNI3*: c.204delG; p.(Arg69AlafsTer8) variant has been previously identified in nine unrelated cases of autosomal recessive DCM with clinical features similar to those observed in our cases (Table 2) (Kühnisch et al., 2019; Sorrentino et al., 2023). The phenotype associated with homozygous mutations of the *TNNI3* gene varies depending on the type of mutation. Indeed, missense mutations are most likely associated with a less severe clinical presentation, and linked to different adult-onset cardiomyopathies. However, the *TNNI3*: c.204delG variant was associated with infantile DCM (Seidel et al., 2021; Sorrentino et al., 2023). The median age of diagnosis for the previously reported DCM cases was 9.7 months (range: 2–16.8 months) (Pezzoli et al., 2021; Seidel et al., 2021; Sorrentino et al., 2023). In the present family, our patients were diagnosed with DCM at the age of 12 and 13 months, further supporting the involvement of the *TNNI3*: c.204delG variant in early onset DCM. Other common clinical features among *TNNI3*: c.204delG carriers include sudden onset of dyspnea, rapid and global health deterioration, and mitral regurgitation (Janin et al., 2022; Sorrentino et al., 2023). Of note, the majority of the reported patients had undergone heart transplantation, without which the outcome is fatal, as observed in our patients (Table 2) (Pezzoli et al., 2021).

Sarcomeric genes account for nearly 35% of DCM cases with a major contribution of the *TTN* gene (25%), mostly in adult patients (Herman et al., 2012; McNally & Mestroni, 2017; Sorrentino et al., 2023). Genes encoding myosin proteins (*MYH6*, *MYH7*, and *MYBPC3*), actin proteins (*ACTC1* and *ACTN2*), tropomyosin protein (*TPM1*), and troponin complex proteins (*TNNC1*, *TNNT2*, and *TNNI3*) are instead involved in neonatal and infantile forms of the disease (Jordan et al., 2021; Sorrentino et al., 2023).

TNNI3 gene variants account for less than 1% of DCM cases with both autosomal dominant and autosomal recessive inheritance patterns. Indeed, *TNNI3* missense variants at a heterozygous state have been implicated in different cardiomyopathies: HCM (MIM #613690), RCM (MIM #115210), DCM (MIM #613286), and mixed

TABLE 2 Clinical description of cases carrying the *TNNI3 (NM_000363.5)*: c.204delG; p.(Arg69AlafsTer8) homozygous mutation.

Patient	Sex	Ethnicity	Age at onset	Cardiac phenotype	Personal and familial medical history	Parents' phenotype		Reference
						Father	Mother	
1	M	Caucasian	15 months	DCM	Symptomatic with CHD; suspected myocarditis; implantation of LVAD at 15 months, transplanted at 22 months	NS	NS	Kühnisch et al., (2019)
2	–	–	Median age of the cohort: 17 months	DMC-Myocarditis	NS	NS	NS	Seidel et al., (2021)
3	F	Moroccan	9 months	DCM	Isolated DCM, transplanted, tissue level of cTnI <0.01 ng/mL, myoglobin (MYO) = 1026 ng/mL, muscular isoforms of creatine kinase (CK-MB) = 32 ng/mL and aspartate aminotransferase (AST) = 134 U/L in myocardial specimens from explanted frozen LV. The patient and her sister were transplanted	NS	NS	Pezzoli et al., (2021)
4	F	Italian	10 months	DCM	Isolated DCM, 3 patients with *TNNI3* variant (one patient with c.24+2T>A variant and 2 patients with c.204delG), 2 transplanted and one died	NS	NS	Pezzoli et al., (2021)
5	F	French	11 months	DCM	Consanguinous parents. Global deterioration with dyspnea and vomiting. Clinical examination showed tachycardia associated with hepatomegaly and bilateral pleural effusions. TTE: DCM with LVEF at 15%. Transplanted at 3 years old	NS	NS	Janin et al., (2022)
6	F	French	2 months	DCM	Consanguinous parents. The patient had a brother with spina bifida and another brother died at 10 months of age of DCM under circulatory support. TTE in utero: normal. TTE at 6 weeks: early cardiomyopathy, LVEF at 28%. At 10 months: polypnea, tachycardia, LVEF at 20%, and functional mitral leak. Transplanted at 18 months	53 years, 24-h Holter monitor test: frequent Monomorphic ventricle extrasystoles (850 per 24 h) Cardiac MRI: subnormal LVEF (52%) and increased extracellular volume (45%)	43 years, Unaffected	Janin et al., (2022)

TABLE 2 (Continued)

Patient	Sex	Ethnicity	Age at onset	Cardiac phenotype	Personal and familial medical history	Parents' phenotype		Reference
						Father	Mother	
7	M	French	7 months	DCM	Consanguinous parents. The patient was diagnosed with DCM at 6 months of age after acute heart failure ECG: sinus tachycardia TTE: impaired systolic function (LVEF 20%–25%) associated with left atrium and ventricle enlargement and global hypokinesia. Interventricular septum thickness and LV end-diastolic diameter were 5 and 40 mm, respectively. LVAD was implanted at 8 months of age for 40 days. The patient died at 10 months of age. Another affected case in the same family carried the same c.204delG variant: DCM at 7 months of age after acute heart failure due to atrioventricular nodal re-entrant tachycardia. TTE: Impaired systolic function (LVEF 25–35%) associated with left atrium and ventricle enlargement and global hypokinesia. Interventricular septum thickness and left ventricular end-diastolic diameter were 3 and 38 mm, respectively. Pulmonary artery systolic pressure: 45 mmH Right ventricular function was impaired (TAPSE 10 mm) Despite optimal treatment, the patient was admitted to the intensive care unit for a second cardiogenic shock associated with multivisceral failure. The patient died during hospitalization at 13 months of age	30 years Unaffected (clinical examination, ECG, and TTE)	28 years, Unaffected (clinical examination, ECG and, TTE)	Janin et al., (2022)

(Continues)

TABLE 2 (Continued)

Patient	Sex	Ethnicity	Age at onset	Cardiac phenotype	Personal and familial medical history	Parents' phenotype		Reference
						Father	Mother	
8	F	French	10 months	DCM	Consanguinous parents. At the age of 11 months: cardiogenetic shock associated with global heart failure. TTE: LVEF at 20%, dilated LV, and severe mitral insufficiency. The evolution was unfavorable with persistent low blood flow, ventilator-associated pneumonia, and a urinary tract infection with multiresistant Klebsiella leading to severe acute renal insufficiency. The patient died at 14 months of age	NS	NS	Janin et al., (2022)
9	M	Moroccan	7 months	DCM	Consanguinous parents An affected sister died at 8 months Feeding difficulties followed by sudden onset of dyspnea at 6 months X-ray: enlarged cardiac silhouette ECG: sinus tachycardia and signs of LV overload TTE: severe dilation of the left chambers with LVEF of 25% and mitral and tricuspid insufficiency Transplanted at 8 months with favorable outcome	Unaffected	Unaffected	Sorrentino et al., (2023)
10	F	Tunisian	13 months	DCM	Consanguinous parents. Family history of an older sister deceased at 12 months due to decompensated DCM Recurrent episodes of dyspnea since the age of 4 months with aggravation at the age of 11 months. Admitted at Intensive care unit at 13 months TTE: LVEF of 10%, DCM with mitral and tricuspid regurgitation Anemia The patient died at 14 months due to DCM decompensation Heart transplantation was not possible	42 years; Clinical evaluation did not report any cardiac symptoms. No TTE evaluation	40 years; Clinical evaluation did not report any cardiac symptoms. No TTE evaluation	Present report

TABLE 2 (Continued)

Patient	Sex	Ethnicity	Age at onset	Cardiac phenotype	Personal and familial medical history	Parents' phenotype		Reference
						Father	Mother	
11	F	Tunisian	12 months	DCM	(Sister patient 10) Apyretic tonic–clonic convulsive seizures begin at the age of 12 months complicated by status epilepticus EEG: normal Dyspnea at the age of 13 months TTE: dilated non-hypertrophied LV, end-systolic diameter of 30 mm, LVEF of 30%, dilated left atrium, and severe eccentric mitral regurgitation Holter: supraventricular arrhythmia LDH and CPK elevated at 583 and 598 IU/L. Anemia The patient died at 14 months due to DCM decompensation. Heart transplantation was not possible	42 years; Clinical evaluation did not report any cardiac symptoms. No TTE evaluation	40 years; Clinical evaluation did not report any cardiac symptoms. No TTE evaluation	Present report

Abbreviations: DCM, dilated Cardiomyopathy; NS, not specified; CHD, congenital heart disease; LV, left ventricle; LVAD, left ventricular assist device; TTE, transthoracic echocardiogram; LVEF, left ventricular ejection fraction.

cardiomyopathy phenotype (Kühnisch et al., 2019; van den Wijngaard et al., 2011). Nevertheless, homozygous *TNNI3* variants are rare and were associated with dilated cardiomyopathy 2A (DCM2A, MIM #611880). According to the clinical genome resource (ClinGen/https://clinicalgenome.org/), the *TNNI3* gene is classified as a definitive gene for HCM, supported by moderate evidence for DCM, and with limited evidence suggesting haploinsufficiency leading to RCM. Troponin I (TnI), along with troponin T (TnT) and troponin C (TnC), is one of 3 subunits that form the troponin complex of the thin filaments of striated muscle. TnI is the inhibitory subunit, blocking actin-myosin interactions, thus mediating striated muscle relaxation. The *TNNI3* gene encodes the TnI-cardiac protein and is exclusively expressed in the heart (Farah & Reinach, 1995).

While heterozygous *TNNI3* missense mutations have been associated with autosomal dominant hypertrophic and restrictive cardiomyopathies, the role of *TNNI3* null variants has been more debated due to the lack of characterization of the reported cases and the low penetrance of heterozygous genotypes (Sorrentino et al., 2023). Nevertheless, homozygous variants associated with DCM2A were first reported by Murphy et al. (2004) in a brother and sister with DCM harboring a missense variant in exon 1 of the *TNNI3* gene (c. 4C>T; p.(Ala2Val)). Functional studies showed significant impairment of *TNNI3* mutant, controversially contrasted with the study by Carballo et al. (2009), which asserts that troponin function is not significantly impaired (Carballo et al., 2009; Sorrentino et al., 2023). Subsequently, several cases of homozygous *TNNI3* variants have been reported with severe forms of neonatal DCM.

In the present study, exome analysis revealed in the two affected patients (IV-5 and IV-6) a homozygous *TNNI3* truncating variant: NM_000363.5: c.204delG; p.(Arg69AlafsTer8) in exon 5. Segregation analysis showed the presence of this variant in the heterozygous state in the parents. Functional studies performed by Kühnisch et al. (2019) on the heart biopsy samples from affected patients with *TNNI3* c. 204delG, demonstrated a markedly reduced levels of *TNNI3* mRNA and the complete absence of the protein, supporting the hypothesis of degradation by nonsense-mediated decay (Kühnisch et al., 2019; Sorrentino et al., 2023).

Genetic testing by identification of the causative mutation can guide therapeutic strategy and predict prognosis, making the prioritization of patients for transplantation versus palliative care or medical treatment (Vasilescu et al., 2018). Genetic counseling was provided for the present family and a prenatal diagnosis on choronic villus was proposed in the absence of pre-implantation genetic diagnosis possibilities.

In conclusion, our report of three affected cases further confirms the association of the homozygous *TNNI3*: c.204delG; p.(Arg69AlafsTer8) variant with early onset and severe DCM, and highlights the importance of genetic

testing which should be performed promptly to identify the underlying cause and to guide management and genetic counseling for infantile DCM. Additional functional studies using patient-specific iPSC-derived cardiomyocytes such as oxygen consumption rates, contractile and relaxation velocities, and their duration, may provide further insights into *TNNI3*: c.204delG pathophysiology and could guide personalized treatment.

AUTHOR CONTRIBUTIONS

Study concept and design: HJ, SZ, LK; Clinical Investigation of the patients: LK, AL, SBA, NA, MK, KM; Analysis and interpretation of data: LK, HJ. Molecular investigation: LK, HJ; Writing—Original draft preparation: LK, HJ; Critical—review & editing: SZ, HJ. Validation: RM, SZ, HJ.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article. Raw data were generated at Genomics & Bioinformatics Platform at MMG and are available from the corresponding authors [SZ, HJ] upon reasonable request.

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