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CLINICAL REPORT

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# Childhood onset nexilin dilated cardiomyopathy: A heterozygous and a homozygous case

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## Abstract

Pathogenic heterozygous NEXN variants are associated with progressive dilated cardiomyopathy (DCM) usually presenting around 50 years of age. We describe an asymptomatic boy who had transient DCM at 3 months of age, that resolved by 4 months. Presently, at 11 years of age, he has normal cardiac function with signs of mild DCM on cardiac MRI. Genetic diagnostics revealed a paternally derived, heterozygous 1949_1951del class 4 variant in *NEXN*. His father had mild DCM with mildly reduced systolic function. The second patient presented with fetal hydrops at 33 weeks gestation requiring emergency caesarian delivery. Postnatally she required ventilation and continuous inotropic support for left ventricle systolic dysfunction. She died after 2 weeks when therapy was withdrawn. Homozygous c.1174C > T,p.(R392*) class 4 variants in the *NEXN* gene were found via WES. Microscopic investigation showed endomyocardial fibroelastosis. Her parents, both heterozygous carriers, had normal cardiac function and the family history was normal. These patients show a new clinical spectrum of pediatric cardiac disease seen in heterozygous and homozygous *NEXN* variants, ranging from mild, transient DCM to a severe, fatal neonatal DCM. These patients support the inclusion of the *NEXN* gene in the investigation of pediatric patients with DCM, even in cases with transient DCM.

**KEYWORDS**
dilated cardiomyopathy, nexilin, NEXN

## 1 | INTRODUCTION

Dilated cardiomyopathy (DCM) is the predominant pediatric cardiomyopathy with an estimated incidence of 0.57 per 10,000 children per year (Towbin et al., 2006). There is a high risk for mortality and morbidity, such as requirement of circulatory support and heart transplantation (Arola et al., 1998). There are numerous causes of DCM, but a familial form can be identified in 30%–35% of the patients (Grünig et al., 1998). In recent years, immense advances have been made to unravel the variants responsible for genetic forms, which mostly encode for structural elements of the myocardium (Haas et al., 2015).

**Abbreviations:** DCM, dilated cardiomyopathy; EFE, endomyocardial fibroelastosis; LV, left ventricle.

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*NEXN* was recently discovered as a DCM-associated gene, encoding for nexilin. The first publication demonstrated that nexilin variants lead to Z-disk instability (Hassel et al., 2009) both in a zebrafish model as well as in human tissue from a myocardial biopsy. In addition, recent research in mouse models showed nexilin to be a component of the junctional membrane complexes essential for Calcium influx and T-tubule formation (Liu et al., 2019). DCM due to *NEXN* variants is rare and mostly described in the heterozygous form in adults with an average age of onset of 50 years (Hassel et al., 2009).

We present two pediatric cases of nexilin DCM. The first is heterozygous for a *NEXN* variant. He had a transient DCM at 3 months and is currently asymptomatic at the age of 11 years although his left ventricle is mildly dilated on cardiac MRI. The second patient, homozygous for *NEXN* variants, presented with fetal hydrops and died 2 weeks postnatally.

## 2 | FIRST PATIENT

This boy, the child of healthy, unrelated parents, presented at 5 days of age at the pediatric cardiology outpatient clinic because of a systolic murmur. Echocardiography showed a structurally and functionally normal heart with normal diameters of the left ventricle (LV). However, a mild tricuspid regurgitation prompted a repeat ultrasound. At 3 months of age, he was in good clinical condition and had grown and developed normally. The echocardiogram now unexpectedly revealed a dilated LV with a left ventricular end-diastolic diameter of 34.8 mm (Z score + 5 [Pettersen et al., 2008]), and a reduced shortening fraction of 19.8% (Figure 1 and video 1: online). Further investigations, including screening for infections, metabolic and neuromuscular diseases, a 12 lead and 24 h Holter electrocardiogram, and a coronary angiography, showed no abnormalities besides evidence for a recent Coronavirus infection. An angiotensin-converting-enzyme inhibitor and diuretics were started and the systolic function and left ventricular dimensions normalized during the following 4 months. All medication could be stopped after 7 months of treatment. The family history was negative for DCM. With the presumptive diagnosis of a recovering myocarditis, genetic testing was not performed at that stage. Further echocardiograms showed normal systolic function and left ventricular dimensions (z score + 1 to +1.5) (Pettersen et al., 2008). A cardiac MRI performed at the age of 9 years, showed a mildly dilated left (end-diastolic volume 132 ml/$m^2$) and right (120 ml/$m^2$) ventricles with normal systolic function and no obvious myocardial fibrosis. The MRI was repeated a year later and showed unchanged dilated ventricles with preserved systolic function. Presently, at the age of 11 years, he is asymptomatic and plays soccer at a high level. His most recent electrocardiogram shows a sinus rhythm and normal AV conduction, but abnormal repolarization in V2, V3, and V4. His last 24-h Holter electrocardiograms showed no arrhythmias.

Genetic diagnostics, with an extended cardiomyopathy Next Generation Sequencing panel, (supplemental data) recently revealed the c.1949_1951del p.(Gly650del) heterozygous (class 4) *NEXN* variant. This 4-base pair (bp) deletion is located in exon 13 and encodes for the IGcam domain of the protein. It leads to the loss of an evolutionary highly conserved amino acid glycine at position 650 (G650del) of nexilin. A copy number variation analysis revealed no deletion. Five additional variants of unknown significance were identified (supplemental data). This NEXN variant has been described previously in six DCM patients (Table 1) (Hassel et al., 2009) and was found 29 times in 279,530 reference alleles from gnomAD (0.01%) (online search in gnomAD Database, 2020). However, during functional analyses, zebrafish embryos developed DCM after injection of this specific mRNA supporting a dominant negative effect on cardiac function (Hassel et al., 2009). Cardiac screening in the patient's four siblings was normal and genetic screening has not been performed (yet) but they remain under surveillance. Cardiac examination of his asymptomatic father, carrier of the same variant, revealed a mildly dilated LV. A paternal uncle also has a dilated LV on cardiac evaluation, but he declined genetic testing. His paternal grandmother died of heart failure and his paternal grandfather has no known heart disease.

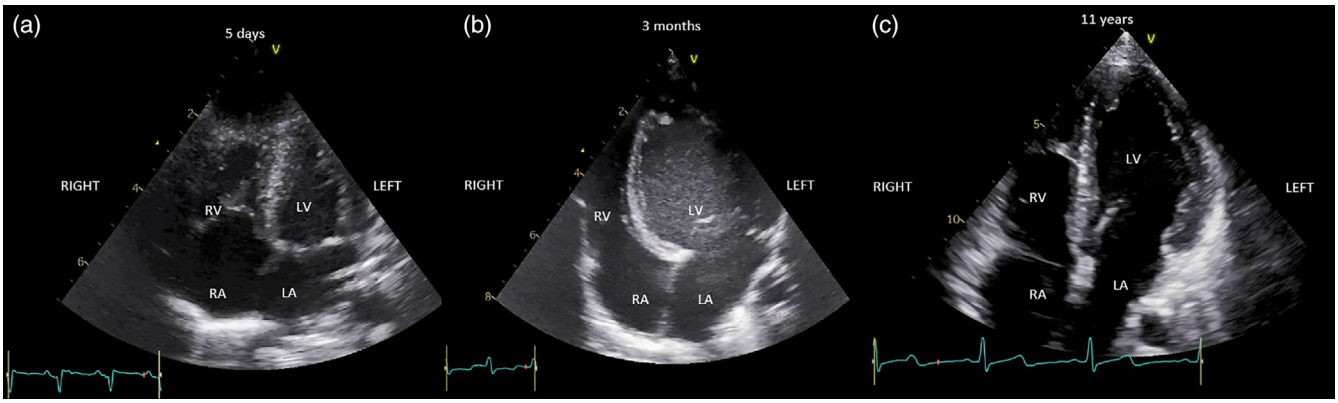


**FIGURE 1** Echocardiograms from patient 1. In panel a the echocardiogram made at 5 days of age is shown. The heart was structurally and functionally normal heart with normal diameters. Panel b shows the severely dilated LV seen at 3 months of age, and panel c the echocardiogram at 11 years of age. LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle [Color figure can be viewed at wileyonlinelibrary.com]

**TABLE 1** Summary of published cases with Nexilin dilated cardiomyopathy

| Number | Author | Sex | Age at onset | DNA sequence change | Amino acid change | Clinical outcome | Remark |
|---|---|---|---|---|---|---|---|
| Homozygous | | | | | | | |
| 1 | Patient 2 | Female | Fetal | 1174C > T | R392* | Died after 2 weeks | |
| 2 | Al-Hassnan et al. | ? | 1 month | 1582-1584del | GAA528del | Severe DCM | Sibling of patient 3 |
| 3 | | ? | ? | 1582-1584del | GAA528del | Severe DCM | Sibling of patient 2 |
| Heterozygous | | | | | | | |
| 4 | Kean et al. | Male | 2 months | 1723G > T | E575X | Severe DCM, improved later, in good clinical condition at 21 years | Twin of patient 5, additional SCN5A mutation |
| 5 | | Male | 2 months | 1723G > T | E575X | Mildly reduced LV systolic function at 21 years | Twin of patient 4, additional SCN5A mutation |
| 6 | Patient 1 | Male | 3 months | 1949_1951del | G650del | Severe DCM, improved later, in good clinical condition at 11 years | |
| 7 | Klauke et al. | Male | 5 years | 1955A > G | Y652C | Severe DCM, ventricular assist device implantation at 22 years | |
| 8 | Hassel et al. | Female | 35 years | 1948-1950del | G650del | DCM, NYHA I | |
| 9 | | Female | 40 years | 1955A > G | Y652C | Severe DCM, NYHA II | |
| 10 | | Female | 49 years | 1948-1950del | G650del | Severe DCM, NYHA III | |
| 11 | | Male | 51 years | 1831C > A | P611T | Severe DCM, NYHA III | |
| 12 | | Male | 51 years | 1948-1950del | G650del | Severe DCM, NYHA II | |
| 13 | | Male | 52 years | 1948-1950del | G650del | Severe DCM, NYHA II | |
| 14 | | Male | 56 years | 1948-1950del | G650del | Severe DCM, NYHA II | |
| 15 | | Female | 58 years | 1955A > G | Y652C | Severe DCM, received heart transplantation | |
| 16 | | Female | 65 years | 1948-1950del | G650del | Severe DCM, NYHA II | |
| 17 | Haas et al. | Male | ? | 1174C > T | R392* | ? | |

Abbreviation: DCM, dilated cardiomyopathy.

## 3 | SECOND PATIENT

During an ultrasound at 33 weeks of gestational age, severe fetal hydrops was identified. Fetal echocardiography showed a severely dilated LV with reduced function (Figure 2, video 2: online), prompting emergency caesarean section. The girl, weighing 2730 grams, was intubated directly after birth and admitted to the neonatology intensive care unit for cardiorespiratory support. A 12 lead electrocardiogram showed sinus rhythm, normal AV conduction, small R waves, and flat T waves, especially in the inferior leads. Continuous telemetry revealed no arrhythmias. Postnatal echocardiography (video 3: online) showed a severe DCM (left ventricular end-diastolic diameter 25 mm, Z score + 3.6), with extremely poor left ventricular function, pleural effusions, and ascites. Maximal therapy was initiated including diuretics, inotropes, and bilateral chest drains. Extensive diagnostics revealed no infectious, metabolic, endocrine, or neuromuscular cause for her cardiac failure. Trio whole exome sequencing (supplemental data) revealed a homozygous c.1174C > T p.(R392*) (Class 4) nonsense variant in *NEXN* and confirmed a heterozygous carrier status in both parents. This variant is located in exon 10, encoding for the actin binding domain (ABD) of the protein. It leads to a premature stop codon and is predicted to translate into a nonfunctional nexilin protein. It has been described previously in a heterozygous state in an adult patient affected with DCM (Table 1) (Haas et al., 2015) and was found heterozygously seven times in 280,056 (0.002%) reference alleles from gnomAD (online search in gnomAD Database, 2019).

Due to persistent cardiorespiratory failure despite maximal therapy, and a very poor prognosis, treatment was discontinued and she died, little over 2 weeks after birth. Macroscopic postmortem examination confirmed an extremely dilated heart mostly affecting the LV. Microscopic examination showed endomyocardial fibroelastosis (EFE) (Figure 3 online; panels a–c). On electron microscopic images Z-disks were normally developed (Figure 3 panel d); however, due to autolysis the T-tubuli could not be evaluated. Compared to an age-matched control heart (E), immunostaining with a nexilin-specific

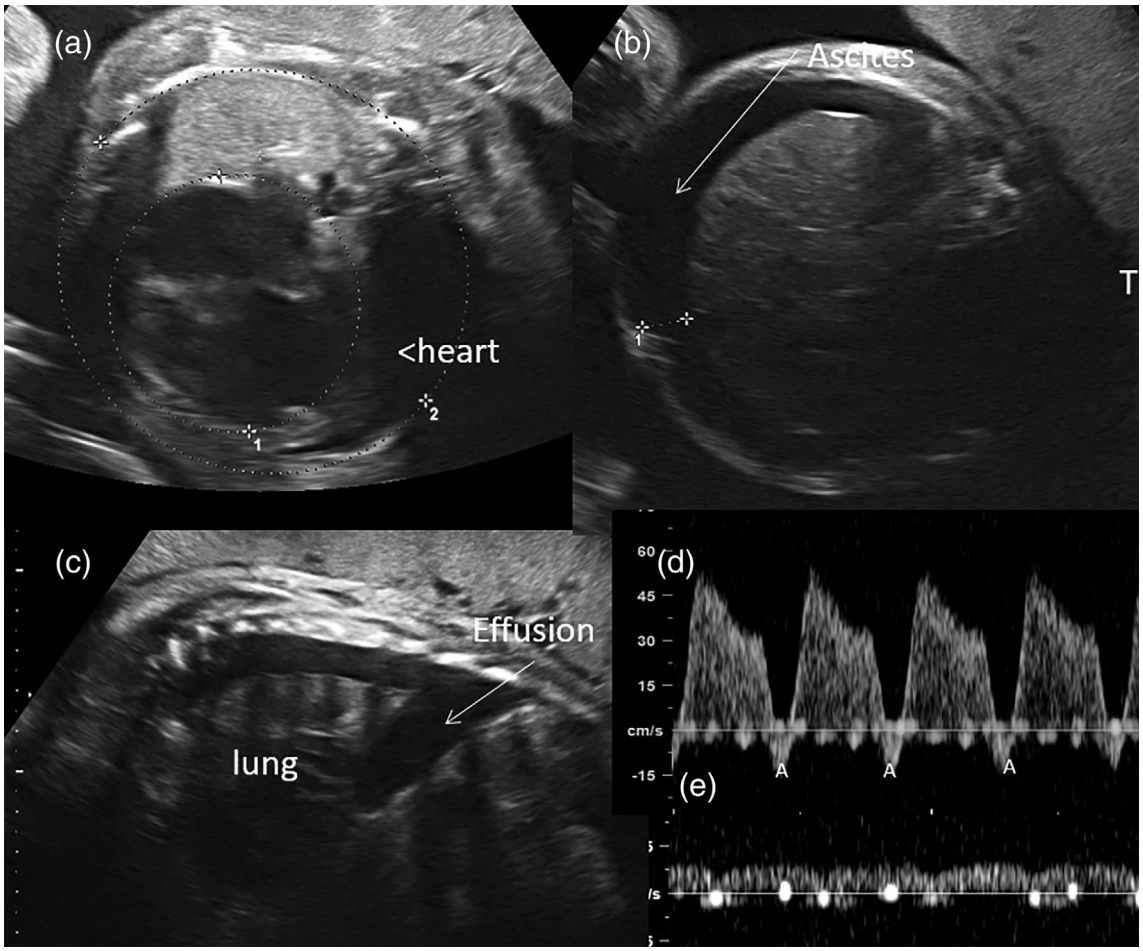


**FIGURE 2** Prenatal echocardiogram: 33 + 1 weeks gestation. (a) Cardiomegaly (cardiothoracic ratio 62.5%, normally <50%); (b) Ascites; (c) Pleural effusion; (d) Abnormal ductus venosus Doppler with retrograde "a"-wave; (e) Pulsatile flow in umbilical vein and holosystolic regurgitation of both atrioventricular valves giving a cardiovascular profile score of 5, consistent with a poor prognosis

antibody shows diffuse downregulation of protein expression in the patient's cardiomyocytes (F). Macroscopic and microscopic analyses of the other organs were normal. Echocardiography was normal in both parents. Testing of the paternal grandparents showed carriership in the paternal grandfather. He is healthy and cardiac examination is planned. The family history did not identify other affected family members.

## 4 | DISCUSSION

We have described two cases of *NEXN* related DCM in pediatric patients: a heterozygous form with an initial transient reduction in systolic function, followed by a chronic mild DCM, and a homozygous genotype with the first description of an antenatal presentation of DCM. DCM caused by *NEXN* variants is rare and mainly described in the heterozygous state associated with progressive DCM presenting at a mean age of 50 years. We only found 15 additional cases in the literature and 13 of these were associated with a heterozygous genotype (Table 1).

In case 1 the echocardiogram was completely normal at 5 days of age, but unexpectedly a dilated left ventricle was seen at 3 months of age. Pediatric cased described previously (Kean et al., 2019; Klauke et al., 2017) also presented at a young age (patients 4, 5, and 7 in Table 1). The known adult cases presented much later (starting at 35 years). The G650del variant described in our first patient and his father with a mild DCM was also found in six of these adult patients, possibly suggesting a slightly milder phenotype. The reversible left ventricular dilatation seen in the first 6 months of life in these patients is intriguing. Possibly, the myocardial dysfunction at 3 months may have been exacerbated by an intercurrent viral infection/myocarditis as he had evidence of a Coronavirus infection. However, improved left ventricular function was also described in one of the twins (Kean et al., 2019) (patient 4 in Table 1). In addition, heterozygous mice with knock-out of just one *NEXN* allele displayed a DCM phenotype with mild dilatation and mildly reduced systolic function up to 6 days postnatally (Aherrahrou et al., 2016). However, after 3 months, no significant differences could be noted compared to the wild-type mice, similar to our patient. A transient infection in these mouse models is highly

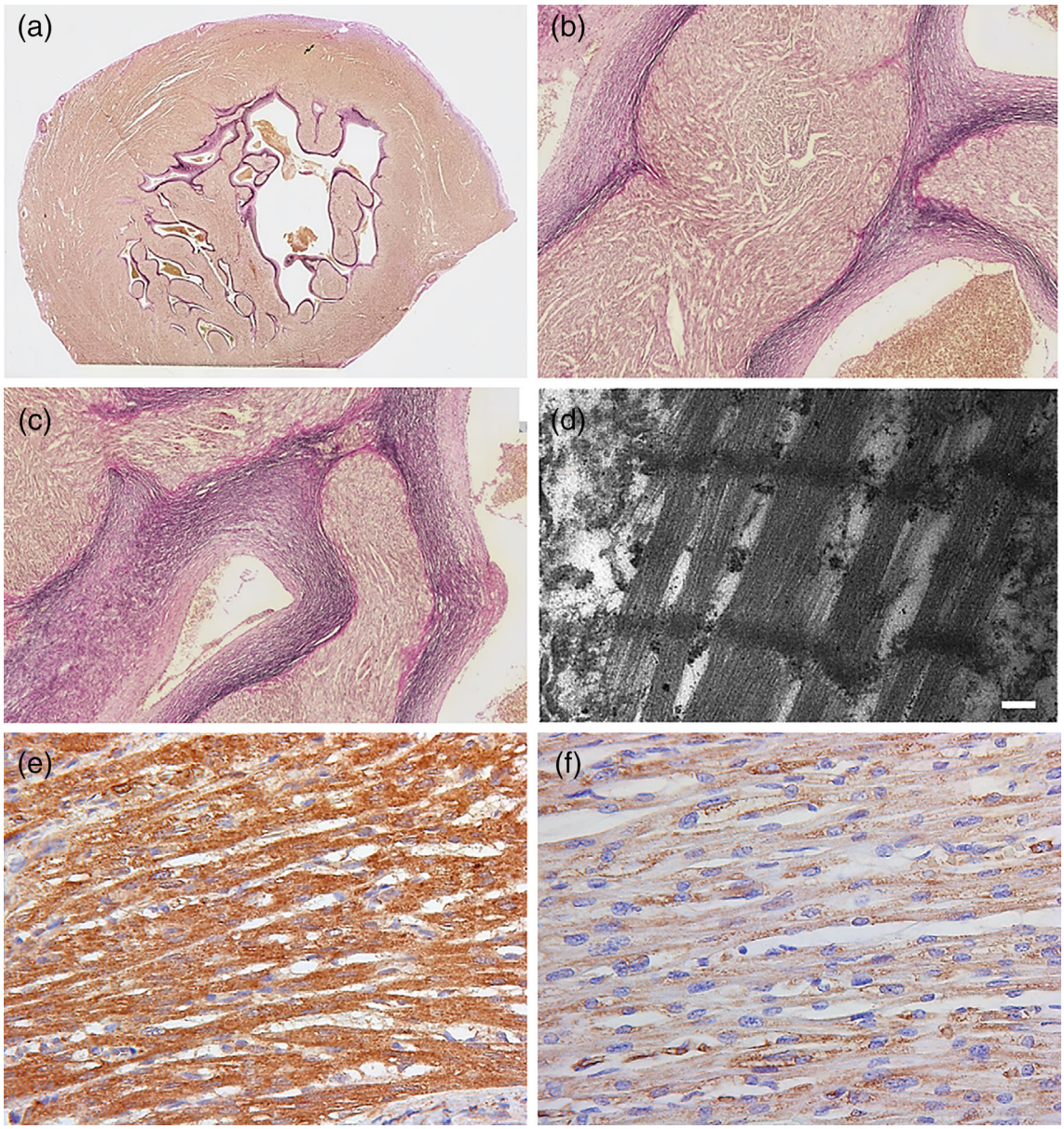


**FIGURE 3** Histopathology of the heart of patient 2 showed normally arranged cardiomyocytes without disarray and without cytoplasmic vacuolization (a). There was a pronounced trabecular aspect of the myocardium at the endocardial side (b and c). The blood vessels were normal. A single papillary muscle showed a small focus of dystrophic calcification. The endocardium was broadened. No interstitial or replacement fibrosis was present. No iron or glycogen accumulation was seen. The cardiomyocytes contained abundant glycogen especially subendocardially. (d) Electron microscopy showed autolysis, precluding the assessment of mitochondria and T-tubuli. The myofibrils looked ultrastructurally normal. No changes in the Z lines were seen. (e and f) compared to an age-matched control heart (e), staining against nexilin shows diffuse downregulation of protein expression in the patient's cardiomyocytes (f) (a, b, c, e, and f): Stained with elastin van Gieson. (d) Bar = 200 nm [Color figure can be viewed at wileyonlinelibrary.com]

unlikely. Nexilin is a component of the junctional membrane complexes essential for Calcium influx and T-tubule formation. Potentially the immature sarcoplasmic reticulum of infants leads to a vulnerable time frame for transient left ventricular dilation and dysfunction.

Our second patient is the first patient documented with a prenatal presentation of Nexilin DCM. Only two other patients have been described who are homozygotic for a *NEXN* variant, more precisely the *c.1582-1584d p.(GAA528del)* variant. These two young children of consanguineous Saudi parents also developed severe DCM (Al-Hassnan et al., 2013). Unfortunately, no information is given about the fetal findings. Patients with homozygous *NEXN* variants closely resemble animal knock out models: after inactivating *NEXN* in zebrafish embryos (Hassel et al., 2009), the embryos initially show normal morphogenesis of the heart, but 24 h after fertilization their hearts start to dilate and significant

systolic dysfunction develops leading to heart failure, similar to our second patient. The functional role of nexilin was further investigated in a *NEXN* knock-out mice model (Aherrahrou et al., 2016). These knock-out mice have severely reduced survival, with most dying within 8 days after birth. At birth, the cardiac structure and function appear normal, but after 4 days they have progressive dilation of the LV and EFE develops. EFE was also seen in the microscopic evaluation of the heart of patient 2. EFE is rare in adults, but is seen in 25% of all pediatric DCM patients requiring heart transplantation (Seki et al., 2013) and is associated with a younger median age of transplantation, (10 vs. 142 months in non-EFE heart transplant patients). Without a cardiac transplant, children with EFE do not survive beyond 2 years (Seki et al., 2013). Electron microscopy of this patient confirmed data of mouse models that nexilin does not affect Z disks, but unfortunately autolysis did not allow evaluation of the T-tubuli.

Both the G650del as well as the R392* variant have also been described heterozygously in "asymptomatic" controls in the gnomAD database impeding a class 5 classification. Given the variable age of presentation of heterozygous carriers however, these patients may have still been in the presymptomatic phase of their illness.

In summary, the presented patients show the clinical spectrum of pediatric cardiac disease seen in heterozygous and homozygous NEXN variants, ranging from mild, transient DCM to a severe, fatal neonatal DCM. These patients support the inclusion of the NEXN gene in the investigation of pediatric patients with DCM, even in cases with transient DCM.

## CONFLICT OF INTEREST

The authors declare no real or perceived conflicts of interest related to this work.

## AUTHOR CONTRIBUTIONS

Luc Bruyndonckx and Judith L. Vogelzang: analyzed clinical data and drafted manuscript. Marianna Bugiani, Bart Straver, Irene M. Kuipers, Wes Onland, Eline A. Nannenberg, and Sally-Ann Clur: contributed to clinical data collection, analysis, and manuscript editing. Saskia N. van der Crabben: supervised the work. All authors have read and approved the final manuscript.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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