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ORIGINAL ARTICLE

NEXN Gene in Cardiomyopathies and Sudden Cardiac Deaths: Prevalence, Phenotypic Expression, and Prognosis

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BACKGROUND: Few clinical data are available on *NEXN* mutation carriers, and the gene's involvement in cardiomyopathies or sudden death has not been fully established. Our objectives were to assess the prevalence of putative pathogenic variants in *NEXN* and to describe the phenotype and prognosis of patients carrying the variants.

METHODS: DNA samples from consecutive patients with cardiomyopathy or sudden cardiac death/sudden infant death syndrome/idiopathic ventricular fibrillation were sequenced with a custom panel of genes. Index cases carrying at least one putative pathogenic variant in the *NEXN* gene were selected.

RESULTS: Of the 9516 index patients sequenced, 31 were carriers of a putative pathogenic variant in *NEXN* only, including 2 with double variants and 29 with a single variant. Of the 29 unrelated probands with a single variant (16 males; median age at diagnosis, 32.0 [26.0–49.0] years), 21 presented with dilated cardiomyopathy (prevalence, 0.33%), and 3 presented with hypertrophic cardiomyopathy (prevalence, 0.14%). Three patients had idiopathic ventricular fibrillation, and there were 2 cases of sudden infant death syndrome (prevalence, 0.46%). For patients with dilated cardiomyopathy, the median left ventricle ejection fraction was 37.5% (26.25–50.0) at diagnosis and improved with treatment in 13 (61.9%). Over a median follow-up period of 6.0 years, we recorded 3 severe arrhythmic events and 2 severe hemodynamic events.

CONCLUSIONS: Putative pathogenic *NEXN* variants were mainly associated with dilated cardiomyopathy; in these individuals, the prognosis appeared to be relatively good. However, severe and early onset phenotypes were also observed—especially in patients with double *NEXN* variants. We also detected *NEXN* variants in patients with hypertrophic cardiomyopathy and sudden infant death syndrome/idiopathic ventricular fibrillation, although a causal link could not be established.

Key Words: dilated cardiomyopathy ■ hypertrophic cardiomyopathy ■ mutation ■ phenotype ■ prognosis

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Nonstandard Abbreviations and Acronyms

DCM	dilated cardiomyopathy
HCM	hypertrophic cardiomyopathy
IVF	idiopathic ventricular fibrillation
LP	likely pathogenic
LVEF	left ventricular ejection fraction
LVNC	left ventricular noncompaction
MRI	magnetic resonance imaging
SCD	sudden cardiac death
SIDS	sudden infant death syndrome
VUS	variant of uncertain significance

In 1998, Ohtsuka et al[1] isolated the actin-filament-binding protein nexilin for the first time. In 2009, Hassel et al[2] discovered that nexilin was a Z-disk protein in cardiac and skeletal muscle and showed that loss of nexilin led to dilated cardiomyopathy (DCM) in zebrafish. They also identified 3 variants p.(Gly650del), p.(Tyr652Cys), and p.(Pro611Thr) in the nexilin gene (*NEXN)* which led to DCM in humans. In 2016, Aherrahrou et al[3] showed that knockout of nexilin in mice led to DCM. It was subsequently found that nexilin is required for the initiation and formation of T-tubules[4] and the maintenance of the T-tubule network.[5] Liu et al[6] reported that the homozygous p.(Gly650del) *NEXN* variant causes DCM in mice. In line with these clinical and functional data and a few case reports,[7,8] *NEXN* was classified as a gene with only moderate evidence of involvement in DCM.[9] Mazzarotto et al[10] found that patients with DCM were significantly enriched for aggregated protein-altering variants (truncating+nontruncating) in *NEXN*.

Moreover, *NEXN* mutations have been described in other subtypes of cardiomyopathy. Wang et al's[11] segregation and in vitro data indicated that p.(Gln131Glu) and p.(Arg279Cys) variants were responsible for hypertrophic cardiomyopathy (HCM) phenotypes. Two case reports described *NEXN* variants in patients with left ventricular noncompaction (LVNC).[12,13] To date, only one case report on sudden cardiac death (SCD) in a person with a *NEXN* variant has been published.[14]

The prevalence, phenotypes, and prognosis associated with *NEXN* variants have not been systematically evaluated in large cohorts of patients with cardiomyopathies or in the context of SCD, sudden infant death syndrome (SIDS), and idiopathic ventricular fibrillation (IVF).

The objectives of the present study were to evaluate the prevalence of *NEXN*-related cardiomyopathies or SCD/SIDS/IVF and to describe the phenotypes and prognoses in individuals carrying a putative pathogenic variant in *NEXN*. These individuals were identified by assessing a cohort of 9516 index patients with cardiomyopathy or SCD/SIDS/IVF and who had been referred for screening with next-generation sequencing (including sequencing of the *NEXN* gene).

METHODS

The full methods are available as Supplemental Material. All patients or (for children) authorized family members gave their written informed consent to genetic testing and use of genetic data for research purposes. The study protocol complied with the ethical tenets of the 1975 Declaration of Helsinki and its subsequent amendments. In line with the French legislation on studies of routine clinical practice, the study protocol was approved by the clinical research unit (at Amiens-Picardie University Hospital) with competency for studies not requiring approval by an institutional review board. Furthermore, the study database was anonymized and registered with the French National Data Protection Commission (*Commission nationale de l'informatique et des libertés* [Paris, France]; reference: PI2022_843_0059). The data that support the findings of this study are available from the corresponding author on reasonable request.

RESULTS

Study Population

Overall, 9516 probands (6274 DCM/LVNC, 2153 HCM, and 1089 SCD/SIDS/IVF) were referred for genetic testing with a custom panel. After careful review, 41 patients were found to carry a *NEXN* putative pathogenic variant as defined in the method section.

Ten patients carried a pathogenic/likely pathogenic (LP) variant in another cardiomyopathy-associated gene (ie, double heterozygous) and were, therefore, excluded from the analysis. Hence, 31 probands were included. We estimated the prevalence of *NEXN* putative pathogenic genetic variants to be 0.33% (CI, 0.17–0.50) among individuals with DCM/LVNC, 0.14% (CI, 0.03–0.25) among individuals with HCM, and 0.46% (CI, 0.26–0.66) among individuals with SCD/SIDS/IVF.

Genetic Characterization

Twenty-nine unrelated probands carried a single heterozygous *NEXN* variant. One patient carried a homozygous splice-site variant (c.865-1G>A), and another was a compound heterozygote for 2 *NEXN* stop variants p.(Arg391*) and p.(Arg392*); these 2 patients will be described separately.

In all, we identified 28 *NEXN* putative pathogenic variants (Table S1; Figure 1): 15 missense variants, 6 nonsense variants, 4 frameshift variants, and 3 variants affecting a consensus splice-site (2 of which potentially led to in-frame exon skipping). The mRNA analysis confirmed the in-frame exon skipping for the variant c.-52-2A>G; this gave a shorter mRNA, corresponding to exon skipping and an in-frame deletion of 9 amino acids. Ten of the 15 missense variants were located in the actin-binding domain and 4 in the immunoglobulin domain. All missense variants were classified as variant of uncertain significance (VUS), while the remaining variants were classified as likely pathogenic.

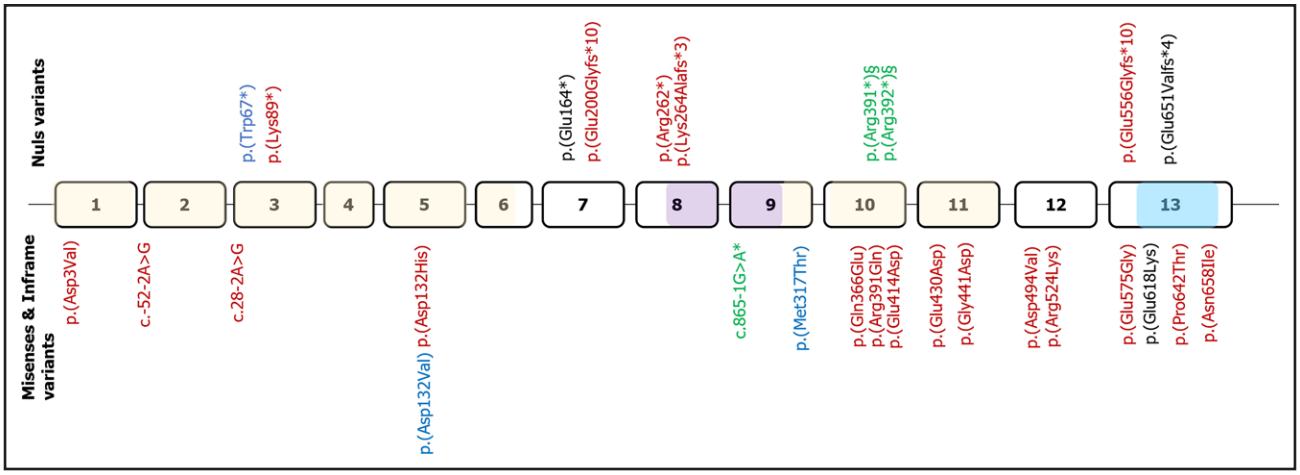

Figure 1. Schematic representation of the *NEXN* gene with the 28 variants of the study.
Exons are shown in black. The colored regions correspond to nexilin's domains (the actin-binding domain in yellow, the coiled coil domain in purple, and the immunoglobulin domain in blue). Null variants are shown above the gene, and missense and in-frame variants are shown under the gene. Variants carried by individuals with dilated cardiomyopathy (DCM)/left ventricle non-compaction (LVNC): red; HCM: blue; sudden infant death syndrome (SIDS): green; idiopathic ventricular fibrillation (IVF): black. The symbol § after the variant's name indicates that it was identified in association with another *NEXN* variant.

Clinical Characteristics of the NEXN Variant Carriers at Diagnosis

Of the 29 patients (16 males; 55.2%), 21 presented with DCM, 3 presented with HCM, 3 presented with IVF, and 2 presented with SIDS (Table 1). Median (interquartile range) age at diagnosis was 32.0 (26.0–49.0) years. Table 2 displays the baseline characteristics of the 29 index cases.

In the DCM group, one patient was diagnosed after inaugural VF, one was diagnosed after a stroke (left ventricular thrombus), one was diagnosed with cardiogenic shock, and 5 were diagnosed fortuitously. The other patients were diagnosed with symptoms of heart failure (dyspnea or acute heart failure) or palpitations. Four patients in the DCM group also met the magnetic resonance imaging (MRI) criteria for LVNC. Nine (42.9%) of the patients with DCM presented a complete left bundle branch block (Figure S1). None reported clinical symptoms of skeletal muscle involvement. The median (interquartile range) left ventricular ejection fraction (LVEF) at diagnosis was 37.5% (26.25–50.00), and the median left ventricle end diastolic diameter at diagnosis was 61.0 mm (58.25–63.75). Only one patient presented with RV impairment at diagnosis. All the adult patients with DCM had normal coronary artery evaluation results. MRI data were available for 15 patients and highlighted late gadolinium enhancement in only 4 of these.

The 3 patients with HCM had predominant asymmetrical septal hypertrophy, and one displayed a significant obstruction. Cardiac MRI showed late gadolinium enhancement in 2 of these 3 patients.

Three patients presented with aborted cardiac arrest due to IVF at the ages of 23, 45, and 32, respectively. The ECG, transthoracic echocardiography, and MRI results were normal. We also found *NEXN* variants in 2 cases of SIDS, which occurred at the ages of 6 months and 2 years, respectively; no signs of cardiomyopathy were observed in their autopsies.

In addition to the 29 probands carrying a single heterozygous *NEXN* variant, 2 other probands were homozygous or heteroallelic for *NEXN* variants; both presented with severe neonatal DCM. One infant, diagnosed with DCM at the age of 9 months, was found to carry a homozygous splicing variant (c.865-1G>A; No. 30.1). In 2023 (at 5 years of age), the infant had a LVEF of 23%. The other case (No. 31.1) was a fetus who presented intrauterine fetal demise. Autopsy revealed severe DCM, and a genetic analysis subsequently identified 2 heteroallelic stop variants in *NEXN* p.(Arg391*) and p.(Arg392*).

The patients' characteristics at diagnosis are described in detail in Table S2.

Familial Analysis

There was a family history of cardiomyopathy in 5 cases and of sudden death in 7 cases (Table S2). Clinical data on relatives was available for 2 families only.

Case No. 31.1 had inherited the p.(Arg392*) variant from the mother and the p.(Arg391*) variant from the father. The mother (No. 31.3) was diagnosed with DCM at the age of 30, during the familial screening. Her LVEF of 45% on diagnosis improved to 55% with treatment. The father (No. 31.2) did not wish to be evaluated.

In family No. 2 (Figure S2), the *NEXN* variant (c.-52-2G>A) was found in a 30-year-old woman who presented with severe DCM, conductive disorders, and atrial and ventricular arrhythmias. She received a

Table 1. Summarized Baseline and Follow-Up Data

	Variant	Phenotype	Sex	Age at diagnosis (y, unless otherwise stated)	Bundle branch block	LVEF (TTE; at diagnosis)	Length of follow-up, y	LVEF change	Event during follow-up	CEID
1	p.(Asp3Val)	DCM	M	30	No	50%	14	↔	0	0
2.1	c.-52-2A>G	DCM	F	30	cRBBB, LPFB	50%	6	↓	Heart transplantation	ICD
2.3	c.-52-2A>G	No cardiomyopathy	M	79	LAFB	60%	4	…	AV block	Pacemaker
3	c.28-2A>G	DCM	M	44	cLBBB	15%	9	↑	Acute heart failure	ICD
4	p.(Asp132His)	DCM	M	38	cLBBB	25%	4	↑	0	0
5	p.(Glu200Glyfs*10)	DCM	F	4 mo	No	Severe LV dysfunction	31	↑	0	0
6	p.(Arg262*)	DCM	F	44	No	45%	10	↔	0	0
7	p.(Arg391Gln)	DCM	F	49	cLBBB	30%	4	↑	0	ICD with CRT
8	p.(Arg392*)	DCM	M	63	cLBBB	30%	3	↑		Pacemaker
9	p.(Glu414Asp)	DCM	F	22	No	35%	21	↑	0	0
10	p.(Glu430Asp)	DCM	M	49	iLBBB	40%	14	↔	0	0
11	p.(Gly441Asp)	DCM	M	48	cLBBB	25%	1	↑	0	0
12	p.(Asp494Val)	DCM	F	9	cLBBB	20%	0	…	Cardiogenic shock; death	…
13	p.(Arg524Lys)	DCM	M	34	cLBBB	50%	6	↔	Appropriate shock	ICD
14	p.(Glu556Glyfs*10)	DCM	F	31	cLBBB	20%	11	↑	0	ICD with CRT
15	p.(Glu575Gly)	DCM	M	24	No	40%	6	↑	Appropriate shock	ICD
16	p.(Pro642Thr)	DCM	M	32	cLBBB	50%	4	↑	0	0
17	p.(Asn658Ile)	DCM	M	64	cLBBB	50%	9	↔	0	0
18	p.(Lys89*)	DCM with LVNC	M	73	cLBBB	30%	5	↑	0	0
19	p.(Lys264Alafs*3)	DCM with LVNC	F	29	No	30%	7	↑	0	ICD
20	p.(Gln366Glu)	DCM with LVNC	F	29	No	55%	0	↔	0	0
21	p.(Gln366Glu)	DCM with LVNC	M	28	iLBBB	45%	1	↑	0	0
22	p.(Trp67*)	HCM	F	71	No	65%	6	…	0	Pacemaker
23	p.(Asp132Val)	HCM	F	62	No	70%	14	…	0	0
24	p.(Met317Thr)	HCM	M	74	No	75%	4	…	0	0
25	p.(Glu164*)	IVF	M	23	No	60%	2	…	Appropriate shock	ICD
26	p.(Glu618Lys)	IVF	F	45	No	60%	3	…	0	ICD
27	p.(Glu651Valfs*4)	IVF	M	32	No	60%	0	…	0	ICD
28	c.865-1G>A	SIDS	M	6 mo	…	…	2	…	…	…
29	p.(Arg391*)	SIDS	F	2	…	…	12	…	…	…
30.1	c.865-1G>A (homozygous)	DCM	F	3 mo	No	15%	2	↑	0	0
31.1	p.(Arg391*)+p.(392*)	IUFD (DCM)	F	…	…	…	…	…	…	…
31.3	p.(Arg392*)	DCM	F	37	No	45%	6	↑	0	0

AV indicates atrioventricular; CEID, cardiac electronic implantable device; cLBBB, complete left bundle branch block; cRBBB, complete right bundle branch block; CRT, cardiac resynchronization therapy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter defibrillator; iLBBB, incomplete left bundle branch block; IUFD, intrauterine fetal demise; IVF, idiopathic ventricular fibrillation; LAFB, left anterior fascicle block; LPFB, left posterior fascicle block; LV, left ventricle; LVEF, left ventricle ejection fraction; LVNC, left ventricle noncompaction; SIDS, sudden infant death syndrome; and TTE, transthoracic cardiac echocardiogram.

heart transplant at the age of 35. Her *NEXN* variant had been inherited from the father (No. 2.3), who had normal transthoracic echocardiography data. However, he had been treated for years with a combination of a beta blocker and an angiotensin II receptor blocker for hypertension and coronary disease; hence, this treatment might have prevented the development of DCM. He received a pacemaker for 2:1 atrioventricular block. The 49-year-old sister (No. 2.2) who did not carry the variant had a normal transthoracic echocardiography and MRI. One brother died from sudden death, and another brother received a heart transplant for severe DCM and died after an infectious complication linked to his immunosuppressive treatment. Unfortunately, DNA from the brothers was not available for genetic analysis.

Clinical Course

Follow-up data were available for the 27 patients alive after diagnosis (Tables 1 and 3). The median (interquartile range) length of follow-up was 6.0 (3.0–10.0) years.

Ten of the 27 patients (37.0%) received an implantable cardiac defibrillator. Seven patients had DCM: 6 patients (including 2 with cardiac resynchronization therapy) received an implantable cardiac defibrillator as primary prevention, and one received an implantable cardiac defibrillator as secondary prevention. The 3 other patients were diagnosed with IVF and received an implantable cardiac defibrillator as secondary prevention.

In patients with DCM, the response to drug therapy was good: LVEF increased in 13 patients (61.9%), including the 2 with cardiac resynchronization therapy (Figure 2), with a median (interquartile range) LVEF of 48.5% (40.0–55.0) at last follow-up (P=0.023 versus LVEF at diagnosis). Twenty of the 21 patients with DCM (95.2%) were being treated with beta-blockers at last follow-up and 19 (90.5%) were taking an angiotensin-converting enzyme inhibitor, an angiotensin II receptor blocker, or an angiotensin receptor neprilysin inhibitor.

Three of the *NEXN* carriers experienced severe arrhythmic events after diagnosis: 2 patients with DCM (one with a history of aborted cardiac arrest at the time of DCM diagnosis) and one patient with IVF received appropriate shocks for ventricular fibrillation. Two patients (No. 10 and No. 13) underwent (unsuccessful) ablation: one for frequent premature ventricular contractions, and the other for sustained ventricular tachycardia. Only 4 patients developed supraventricular tachycardia: atrial tachycardia in one case, and atrial fibrillation in the others.

We reported 2 severe hemodynamic events. One patient (No. 2.1) developed refractory cardiogenic shock after ablation for atrial tachycardia and required a heart transplant at the age of 35. Another proband (No. 12) died from heart failure at 9 years of age, 1 week after having been diagnosed with DCM in the context of cardiogenic shock. During the follow-up period, one patient with DCM had to be admitted to hospital for acute heart failure.

The patients' age distribution at the time of the first major cardiac event (regardless of whether the latter occurred at the time of diagnosis or during the follow-up) is shown in Figure 3.

Detailed follow-up data for each patient are given in Table S3.

Phenotype Genotype Correlations

Details of the phenotypes associated with null, missense, and in-frame splicing variants are given in Table 1.

Table 2 compares patients with missense variants, classified as VUS, to those with remaining variants, all classified as likely pathogenic. No differences were observed in phenotypes, age at diagnosis, or gender. The severity of patients with DCM was not statistically different, although there was a tendency for a lower LVEF in patients with an LP variant. Table 3 shows no significant difference in outcomes between VUS and LP variant carriers, particularly regarding response to drug therapy.

Among patients with a missense variant, we did not observe a relationship between the phenotype and the variant's location within the gene; variants in the same protein domain were associated with different phenotypes. Two missense variants, p.(Asp132His) and p.(Asp132Val), carried by one patient with DCM and one with HCM, were located in a hotspot in exon 5.

DCM was the most frequent phenotype (n=21). Six cases carried a null variant, 13 carried a missense variant, and 2 carried an in-frame exon-skipping variant. Considering the 2 cases of SIDS, one was heterozygous for the p.(Arg391*) variant, and the other was heterozygous for the c.865-1G>A splice variant. These variants were also found, in the homozygous or heteroallelic state, in the 2 cases of antenatal/perinatal DCM (No. 30.1 and no. 31.1).

DISCUSSION

The present work constitutes the first systematic review of rare *NEXN* variants, based on an analysis of 9516 probands with cardiomyopathy or SCD/SIDS/IVF. We identified 31 index patients carrying putative pathogenic variants in the *NEXN* gene. Most of the variants were associated with a DCM phenotype.

NEXN-Linked DCM

DCM was the most frequently observed phenotype (in 21 patients). The largest previous series of cases of *NEXN*-mediated DCM (published in 2009) included only 9 individuals: one carried the p.(Pro611Thr) variant, 2 carried

Table 2. Baseline Characteristics

		All patients, N=29	Probands with VUS variants, N=16	Probands with LP variants, N=13	*P* value
Type of variant					
Missense		16 (55.2)	16 (100)	0 (0)	<0.001
Frameshift		4 (13.8)	0 (0)	4 (30.8)	
Nonsense		6 (20.7)	0 (0)	6 (46.2)	
Splice		3 (10.3)	0 (0)	3 (23.1)	
Phenotype					
DCM		21 (72.4)	13 (81.3)	8 (61.5)	0.22
HCM		3 (10.3)	2 (12.5)	1 (7.7)	
IVF/SIDS		5 (17.2)	1 (6.3)	4 (30.8)	
Demographic variables					
Age at diagnosis, y		32.0 (26.0–49.0)	36.0 (28.25–49.0)	31.0 (12.5–53.5)	0.45
Male sex		16 (55.2)	10 (62.5)	6 (46.2)	0.47
Family history					
DCM		5 (17.2)	3 (18.8)	2 (15.4)	1
SCD <50 y old		7 (24.1)	5 (31.3)	2 (15.4)	0.41
Mode of diagnosis					
Fortuitous		7 (24.1)	6 (37.5)	1 (7.7)	0.09
Symptomatic		22 (75.9)	10 (62.5)	12 (92.3)	
Details of symptoms in symptomatic patients					
Palpitation		3 (10.3)	3 (18.8)	0 (0)	
Dyspnea		9 (31.0)	4 (25.0)	5 (38.5)	
Acute heart failure		2 (6.9)	0 (0)	2 (15.4)	
Severe arrhythmic or hemodynamic event		7 (24.1)	3 (18.8)	4 (30.8)	
Stroke		1 (3.4)	0 (0)	1 (7.7)	
Clinical status					
NYHA	NYHA I	13 (48.1)	11 (68.8)	2 (18.2)	0.005
	NYHA II	5 (18.5)	0 (0)	5 (45.5)	
	NYHA III/IV	9 (33.3)	5 (31.3)	4 (36.4)	
ECG (n=27)					
First degree AV block		2 (7.4)	0 (0)	2 (18.2)	0.16
QRS morphology	Thin	14 (51.9)	8 (50.0)	6 (54.4)	0.42
	cRBBB	1 (3.7)	0 (0)	1 (9.1)	
	iLBBB or cLBBB	12 (41.4)	8 (50.0)	4 (36.4)	
Abnormal TWI		3 (11.1)	2 (12.5)	1 (9.1)	1
Low QRS voltage limb leads		2 (7.4)	1 (6.3)	1 (9.1)	1
Echocardiography (for DCM patients, n=21)					
LVEF (%; n=20)		37.5 (26.25–50.0)	40.0 (27.5–50.0)	30.0 (20.0–45.0)	0.18
LVEF <50% (n=21)		20 (95.2)	12 (92.3)	8 (100)	1
LVEDD, mm (n=16)		61.0 (58.25–63.75)	61.0 (58.0–63.25)	61.5 (59.0–67.0)	0.49
LV dilatation (n=18)		16 (88.9)	9 (81.8)	7 (100)	0.50
RV dysfunction (n=21)		1 (4.8)	1 (7.7)	0 (0)	1
Cardiac magnetic resonance imaging (for DCM patients, n=15)					
LVEF (%) (n=15)		40.0 (31.0–46.0)	42.5 (27.25–46.75)	32.0 (31.0-)	0.45
LVEF <50% (n=15)		14 (93.3)	11 (91.7)	3 (100)	1
LV dilatation (n=12)		9 (75.0)	7 (70.0)	2 (100)	1
LV noncompaction (n=15)		4 (26.7)	2 (16.7)	2 (66.7)	0.15
LGE (n=15)		4 (26.7)	2 (16.7)	2 (66.7)	0.15
RV dysfunction (n=15)		0 (0)	0 (0)	0 (0)	

AV indicates atrioventricular; cLBBB, complete left bundle branch block; cRBBB, complete right bundle branch block; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; iLBBB, incomplete left bundle branch block; IVF, idiopathic ventricular fibrillation; LGE, late gadolinium enhancement; LP, likely pathogenic; LV, left ventricle; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; RV, right ventricle; SCD, sudden cardiac death; SIDS, sudden infant death syndrome; TWI, T wave inversion; and VUS, variant of uncertain significance.

the p.(Tyr652Cys) variant, and 6 carried the p.(Gly650del) variant (Table S4).[2] The 9 patients originated from 2 distinct DCM cohorts (containing 90 and 910 individuals, respectively), and none had pathogenic variants in the *MYH7, TNNT2, MYBPC3, ACTC1, TPM1, LMNA, SGCD, LDB3,* and *DES* genes. However, the study was performed before the advent of next-generation sequencing, and frequently DCM-associated genes (eg, *TTN*, *RBM20*, and *BAG3*) were not analyzed. Furthermore, the p.(Tyr652Cys) and the p.(Gly650del) variants had a high minor allele frequency, with, respectively, 22 and 42 occurrences in the gnomAD database (Table S4), which suggests that these variants are not pathogenic in the heterozygous state. However, the development of DCM in a mouse model of the p.Gly650del variant[6] indicates that the latter is pathogenic in the homozygous state.

Importantly, homozygous variants (c.865-1G>A) and compound heterozygous variants, p.(Arg392*) and p.(Arg391*), were associated with severe antenatal or perinatal phenotypes in our cohort. Severe DCM phenotypes associated with homozygous *NEXN* variants have been described previously. In the homozygous state, the p.(Arg392*) variant was associated with a severe DCM phenotype.[8] Furthermore, the homozygous p.(Glu528del) deletion has been identified in 2 siblings with severe

Table 3. Follow-Up Data

		All patients, N=27	Probands with VUS variants, N=16	Probands with LP variants, N=11	*P* value
Length of follow-up		6.0 (3.0–10.0)	4.0 (1.5–12.75)	6.0 (3.0–10.0)	0.68
Clinical status					
NYHA	NYHA I	15 (55.6)	10 (62.5)	5 (45.5)	0.68
	NYHA II	10 (37.0)	5 (31.3)	5 (45.5)	
	NYHA IV	2 (7.4)	1 (6.3)	1 (9.1)	
ECG (n=25)					
First degree AV block		3 (12.0)	0 (0)	3 (27.3)	0.07
QRS morphology	Thin	12 (48.0)	6 (42.9)	6 (54.5)	0.28
	cRBBB	1 (4.0)	0 (0)	1 (9.1)	
	iLBBB or cLBBB	9 (36.0)	7 (50.0)	2 (18.2)	
	Paced	3 (12.0)	1 (7.1)	2 (18.2)	
Abnormal TWI		4 (16.0)	2 (14.3)	2 (18.2)	1
Low QRS voltage limb leads		3 (12.0)	1 (7.1)	2 (18.2)	0.56
SVT		4 (14.8)	3 (18.8)	1 (9.1)	0.62
Echocardiography (for DCM patients, n=21)					
LVEF (%) (n=20)		48.5 (40.0–55.0)	50.0 (45.5–57.25)	40.0 (32.5–48.75)	0.17
LVEF <50% (n=21)		11 (52.4)	5 (38.5)	6 (75.0)	0.18
LVEF improvement (n=21)		13 (61.9)	7 (53.8)	6 (75.0)	0.40
LV dilatation (n=20)		14 (70.0)	7 (58.3)	7 (87.5)	0.32
RV dysfunction (n=20)		2 (9.5)	1 (7.7)	1 (12.5)	1
CIED					
PM		2 (7.4)	0 (0)	2 (18.2)	0.16
ICD		10 (37.0)	4 (25.0)	6 (54.5)	0.22
Severe arrhythmic event		3 (11.5)	2 (13.3)	1 (9.1)	1
Severe hemodynamic event		1 (3.8)	0 (0)	1 (9.1)	0.42
Treatment (for DCM patients, n=21)					
β-Blocker		20 (95.2)	12 (92.3)	8 (100)	1
ACE inhibitor, ARB, or ARN inhibitor		19 (90.5)	12 (92.3)	7 (87.5)	1
Aldosterone antagonist		8 (38.1)	3 (23.1)	5 (62.5)	0.16

ACE indicates angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; ARN, angiotensin receptor neprilysin; AV, atrio ventricular; CIED, cardiac implantable electronic device; cLBBB, complete left bundle branch block; cRBBB, complete right bundle branch block; DCM, dilated cardiomyopathy; ICD, implantable cardioverter defibrillator; iLBBB, incomplete left bundle branch block; LP, likely pathogenic; LV, left ventricle; LVEF, left ventricle ejection fraction; PM, pacemaker; RV, right ventricle; SVT, supraventricular tachycardia; TWI, T wave inversion; and VUS, variant of uncertain significance.

DCM.[7] Recently, Johansson[15] reported on a fetus who died of severe DCM and who was homozygous for the p.(Ile435Serfs*3) variant. Along with previous reports, our results suggest that homozygous and heteroallelic *NEXN* variants are associated with a severe, early onset DCM phenotype. However, the familial data from family No. 31, with a mild DCM phenotype in the mother, who was heterozygous for the *NEXN* p.(Arg392*) variant, suggest that the association between heterozygous *NEXN* variants, and DCM is based on an autosomal dominant mechanism and results in less severe phenotypes.

As in the present cohort, no clinical signs of skeletal muscle involvement have been reported previously in *NEXN* variant carriers. Right ventricular involvement was rare and only observed in 2 patients with severe DCM (patients No. 12 at diagnosis and No. 2.1 during the follow-up). Interestingly, we observed that the response to drug therapy was usually good (with an increase in LVEF in 65.0% of cases).

Four of the 21 patients with DCM met the MRI criteria for LVNC. The association between *NEXN* variants and LVNC has been described previously. Pardun et al[13] identified the p.(Glu575*) variant in a patient with LVNC, and Yuen et al[12] identified the p.(Cys667Tyr) mutation in a newborn with LVNC and severe LV dysfunction (Table S4). The p.(Glu470Gln) variant previously reported in a patient with LVNC has a high minor allele frequency and is likely to be a polymorphism (Table S4).[13]

Translational Data Supporting the Pathogenicity of Nexilin Loss of Function Variants and the Association with DCM

In 1998, nexilin was first isolated, characterized, and named by Ohtsuka et al[1] as a novel filamentous actin-binding protein. Loss-of-function[2,5] and poison-peptide disease[2,6] mechanisms have been described for *NEXN* variants.

Nexilin knockout in the zebrafish[2] resulted in DCM with destabilized sarcomeric Z-disks and suggested that loss of function is a disease mechanism for this gene. Aherrahrou et al[3] observed that nexilin knockout in the mouse resulted in death soon after birth, DCM and endomyocardial fibroelastosis. However, the Z-disc was not destabilized in this murine model. Following on from these knockout experiments (the results of which emphasized the nexilin importance in cardiac development and function), Spinozzi et al[5] generated a *NEXN* inducible adult cardiomyocyte-specific knockout mouse model, which enabled them to study the role of *NEXN* in mature cardiomyocytes. The researchers confirmed the nexilin's importance for muscle contraction, calcium handling, and maintenance of the T-tubule network. The same group of researchers[4] also found that cardiomyocyte-specific knockout of *NEXN* mice resulted in a severe, progressive DCM (the same phenotype observed in *NEXN* knockout mice), which demonstrated that the disease was a direct consequence of *NEXN* loss in cardiomyocytes. Lastly, the researchers discovered that rather than being a Z-disk protein, nexilin interacts with junctional sarcoplasmic reticulum proteins and is essential for optimal

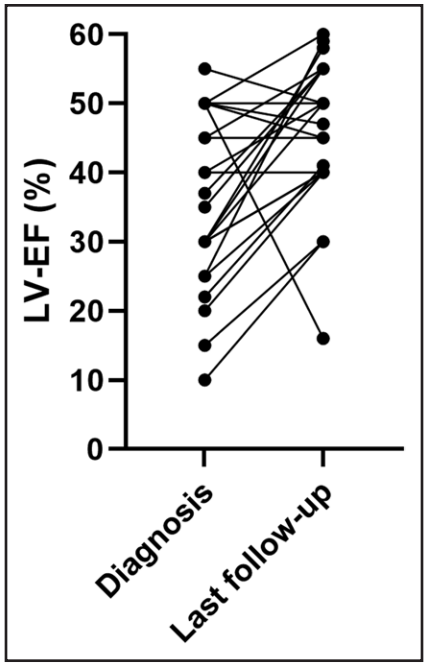

Figure 2. The left ventricular ejection fraction at diagnosis and at last follow-up.
In patients with dilated cardiomyopathy (DCM), the response to drug therapy was good: left ventricle ejection fraction (LVEF) increased in 13 patients (61.9%), including the 4 with cardiac resynchronization therapy, with a median (interquartile range) LVEF of 48.5% (40.0–55.0) at last follow-up (P=0.023 vs LVEF at diagnosis; Wilcoxon's signed-rank test).

calcium transients and the initiation of T-tubule invagination and formation.

Here, we identified heterozygous nonsense *NEXN* variants in patients with DCM; this suggests that the loss of nexilin function in the heterozygous state also leads to DCM (as with homozygous/heteroallelic variants) but gives a less severe phenotype. We also observed loss-of-function variants in cases of HCM or in LVNC; hence, it is possible that this mechanism also leads to other types of cardiomyopathy. Taken as a whole, these data suggest that nexilin haplo-insufficiency is a disease mechanism.

The literature data also suggest that other types of variants (such as in-frame deletions) can lead to DCM via a poison-peptide mechanism. Hassel et al[2] observed disrupted sarcomeric units and detached, blurry Z-disks in cardiac tissue from people carrying the *NEXN* p.(Gly650del) variant—even though the mutant protein was able to bind α-actin, β-actin and α-actinin. In a knock-in mouse model, Liu et al[6] generated a G645del variant (the equivalent of Gly650del in humans). Homozygous G645del mice showed low levels of nexilin expression, progressive DCM, poor T-tubule formation, and disorganization of the transverse-axial tubular system. These results suggested that this nexilin (lacking a single amino acid) acted as a poison peptide. In our work, we identified an in-frame exon skipping variant (c.-52 to 2A>G; family I) in the heterozygous state (leading to the deletion of 9 amino acids) in a patient with severe DCM.

NEXN Might Contribute to the Development of Other Cardiac Phenotypes and Ventricular Arrhythmia

Other cardiac phenotypes (HCM, intrauterine fetal demise, IVF, SIDS) were found in putative *NEXN* variant carriers in our cohort, as in the literature. In contrast, we did not identify *NEXN* variants in patients with arrhythmogenic cardiomyopathy or restrictive cardiomyopathy.

We identified *NEXN* variants, p.(Met317Thr), p.(Asp132Val), and p.(Trp67*), in 3 probands with an HCM phenotype. Wang et al[11] previously reported 2 variants, p.(Gln131Glu) and p.(Arg279Cys), associated with HCM. The proband carrying the p.(Gln131Glu) variant was a 37-year-old female diagnosed with nonobstructive HCM and an interventricular septal thickness of 21 mm. The variant segregated with the HCM phenotype in 2 other affected family members. The *NEXN* p.(Arg279Cys) variant was identified in a 45-year-old male with nonobstructive HCM; however, this variant has a high minor allele frequency (141 occurrences in the gnomAD database) and is more likely to be a benign polymorphism. Functional in vitro experiments[11] have shown that the p.(Gln131Glu) variant decreased NEXN's ability to bind α-actin, suggesting that this variant is associated with a poison peptide mechanism. In our cohort, 3 patients had a phenotype of HCM. One of these patients carried the p.(Asp132Val) variant, located in the actin-binding domain, just next to the p.(Gln131Glu) variant identified by Wang et al. We identified an additional variant, p.(Asp132His), in a patient with DCM, that affects the same amino acid (n°132) in the actin-binding region. This region could thus be considered as a hotspot. However, considering that (1) 2 out of 3 variants were VUS, (2) the prevalence of *NEXN* variants in our population of HCM patients was only 0.14%, and (3) we do not have any segregation data, the link between *NEXN* variants and HCM could not be established.

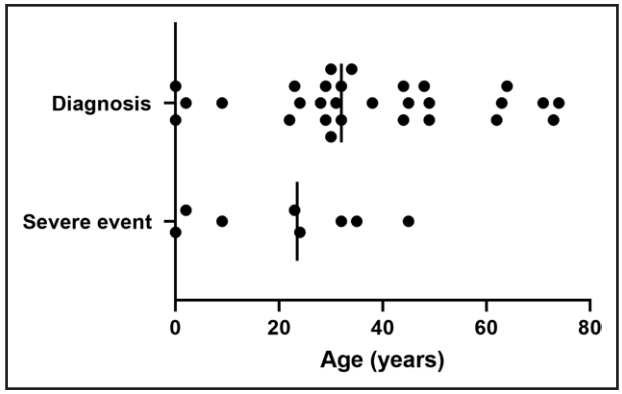

Figure 3. The age at diagnosis and when the first major event occurred.
Median (interquartile range [IQR]) age at diagnosis was 32.0 (26.0–49.0) years. From birth to last follow-up, 8 patients suffered from at least one severe hemodynamic or arrhythmic event, at a median (IQR) age of 23.5 (3.75–34.25) years.

Seven *NEXN* carriers experienced ventricular fibrillation/SCD during their lifetime, with 2 cases in the context of DCM and 5 cases in the absence of overt cardiomyopathy (including 2 cases of SIDS). The 2 cases of SIDS were heterozygous for the c.865-1G>A and p.(Arg391*) variants found in the homozygous/heteroallelic state in the infants with neonatal DCM (No. 30.1 and No. 31.1). The latter cases did not show signs of cardiomyopathy on autopsy, and so it is difficult to say whether the *NEXN* variants were responsible for the sudden death/ventricular fibrillation or were merely incidental findings. We cannot rule out the presence of a second, undetected pathogenic variant responsible for the arrhythmic phenotype. The absence of cardiomyopathy in the cases of SIDS/IVF carrying a heterozygous *NEXN* variant suggests that the disease mechanism differs from that seen in DCM complicated by ventricular arrhythmia/SCD. In the sole published case report on SCD in a patient carrying a *NEXN* variant, the minor allele frequency was suggestive of a VUS.[14] Considering the absence of a rationale for how a *NEXN* variant could lead to VF in the absence of structural heart disease, and given that we have no segregation data, we cannot establish a formal, causal link between *NEXN* variant and IVF/SIDS.

Rationale for Including VUS

Our decision to include VUS was based on several arguments. Since *NEXN* variants are rare, have not been extensively studied, and have not been validated in DCM, few can be classified as P/LP according to the ACMG criteria. Thus, excluding VUS would make it virtually impossible to study the gene. We have linked some of these variants to a specific DCM phenotype, and publication of data on VUS might enable the ACMG's PP5 criterion to be attributed in the future. Publishing these data will also enable other teams to share their observations and thus expand our knowledge of this gene. Some researchers may also be interested in carrying out functional studies, which might enable the PS3 criterion to be attributed to these variants. Hence, the *NEXN* VUS classification might change over time, with designation as P/LP.

Unfortunately, we did not have segregation data; this made it impossible to apply the PP1 criterion. Until now, a causal role of the *NEXN* gene in dilated cardiomyopathies had not been established; indeed, the laboratories in our network did not report these variants to clinicians, and so segregation studies were rare. In the light of our results, we believe that the variants with the highest level of evidence can now be reported to clinicians and assessed in segregation studies.

Limitations

In this cohort we described the clinical course and genetics of DCM/HCM/LVNC associated with *NEXN* variants. However, the lack of informative data on familial segregation prevented us from drawing firm conclusions about a given variant's causal role, penetrance, and expression variability. Lastly, the small number of patients and the few events during the follow-up period prevented us from drawing conclusions about possible specific arrhythmic or hemodynamic risks linked to *NEXN*-mediated cardiomyopathies.

Conclusions

Our data suggest that putative pathogenic *NEXN* variants are mostly associated with DCM (prevalence, 0.33%). Although severe LV dysfunction could be observed when DCM is diagnosed, the LVEF increased with treatment in a significant proportion of patients. The phenotype was sometimes severe (with early onset or severe arrhythmia, or hemodynamic events) but most patients had a favorable outcome. Homozygous and heteroallelic variants were associated with severe early onset phenotypes. HCM and SIDS/IVF phenotypes were also observed, although a causal link with *NEXN* variants cannot be established. The present results should help us to better define the management of *NEXN* variant carriers and refine the counseling given to patients and their families.

ARTICLE INFORMATION

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None.

Supplemental Material

Supplemental Methods
Tables S1–S4
Figures S1–S2
References 16–28

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