JACC: HEART FAILURE VOL. 13, NO. 9, 2025

ORIGINAL RESEARCH

CARDIOMYOPATHIES

Genetic and Phenotypic Characterization of Nexilin (*NEXN*)–Related Cardiomyopathy

Results From a Multicentric Study

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ABSTRACT

BACKGROUND Nexilin (*NEXN*)–related cardiomyopathies (CMPs) are largely unexplored.

OBJECTIVES This study investigated the causative role of *NEXN* in CMPs, examining its phenotypic expression and prognostic profile.

METHODS Twelve referral centers collected phenotypic/genotypic data of patients with *NEXN* variants. Variant rarity was determined according to gnomAD allele frequency in CMPs. Burden enrichment tested rare *NEXN* variants in hypertrophic (HCM) and dilated cardiomyopathy (DCM)/nondilated left ventricular cardiomyopathy (NDLVC) CMPs against gnomAD non-Finnish Europeans (NFE). Outcomes of validated variants were detailed, with prognostic comparisons to Titin (*TTN*)- and Filamin C (*FLNC*)–related CMP cohorts.

RESULTS Involving 60 *NEXN* carriers with rare, protein-altering variants, a significant enrichment of *NEXN*-truncating variants (tvs) was found in the DCM/NDLVC cohort (0.39% vs 0.09% in gnomAD NFE; $P = 0.0001$), whereas no association was observed with HCM. Patients with DCM/NDLVC with *NEXN*tv (n = 17; median age: 45 years [Q1-Q3: 36-55 years], 88% probands, 53% male) showed mild left ventricular dilatation (indexed end-diastolic volume 69 mL [Q1-Q3: 46-87 mL]), mildly reduced left ventricular ejection fraction (44% [Q1-Q3: 31%-53%]), and myocardial fibrosis (64%). NYHA functional class I was common (71%). During a 45-month median follow-up (Q1-Q3: 11-130 months), 53% of patients were implanted with an implantable cardioverter-defibrillator and 25% had malignant ventricular arrhythmias (MVAs). Compared with *TTN*-CMP, *NEXN*-CMP exhibited earlier and more frequent MVAs at higher ejection fractions, and no significant differences were found against *FLNC*-CMP.

CONCLUSIONS *NEXN*tvs were significantly associated with DCM/NDLVC, characterized by mild cardiac abnormalities, infrequent heart failure, common fibrosis, and arrhythmias. This largest *NEXN* variant carrier cohort to date contributes to defining the causative role of this rare genotype and its associated phenotype. (JACC Heart Fail. 2025;13:102529)

ISSN 2213-1779 https://doi.org/10.1016/j.jchf.2025.102529

ABBREVIATIONS AND ACRONYMS

CMP = cardiomyopathy

DCM = dilated cardiomyopathy

HCM = hypertrophic cardiomyopathy

ICD = implantable cardioverter-defibrillator

LVEDD = left ventricular end-diastolic diameter

LVEDV = left ventricular end-diastolic volume

LVEF = left ventricular ejection fraction

MVA = malignant ventricular arrhythmia

NDLVC = nondilated left ventricular cardiomyopathy

P/LP = pathogenic/likely pathogenic

SCD = sudden cardiac death

tv = truncating variant

In monogenic cardiomyopathies (CMPs), causative variants in different genes (such as *TTN* [Titin], *LMNA* [Lamin A], *DSP* [Desmoplakin], *FLNC* [Filamin C]) can lead to different outcomes.[1-6] Nexilin (full name: Nexilin F-actin binding protein), the 675-amino-acid protein encoded by the *NEXN* gene, is a key component of the sarcoplasmic reticulum-sarcolemma junctional complex in cardiomyocytes.[7] These subcellular structures closely surround Z disks in adult cells.[8] Down-regulation of Nexilin disrupts calcium transients and T-tubule formation in cardiomyocytes, leading to severe dilated cardiomyopathy (DCM) in nonhuman animal models. Consistent experimental evidence has shown abnormal cardiac development and DCM associated with knockout models, homozygous or heterozygous deletion, or homozygous rare variants of *NEXN*.[9-11] In humans, conversely, clinical and genetic features of adult *NEXN*-related CMPs are still poorly characterized,[4,8,12-16] especially regarding heart failure and arrhythmia risk.

At present, the Clingen Consortium classifies *NEXN* as having moderate evidence for causing DCM. Despite the highest degree of experimental evidence, the genetic evidence is lower because of few case-control studies and absence of segregation data.[15-18] Regarding HCM, the association is even more limited.[18,19]

A recent publication on a population survey of *NEXN* variants in 3 French centers described possible association with highly heterogeneous phenotypes (DCM, hypertrophic cardiomyopathy [HCM], and idiopathic ventricular fibrillation[16]), but no genetic evidence nor segregation data were provided.

To date, a comprehensive study with accurate clinical description built on robust genetic evidence (genotype-first approach) is still missing, limiting the clinical actionability of *NEXN* variants. Therefore, in the present international study, we collected genetic and clinical details of patients harboring variants in *NEXN* and affected by any type of structural CMP. We sought first to provide more evidence on the putative mendelian role of *NEXN* in CMPs, and second to better clarify its phenotypic expression and prognostic profile.

METHODS

INTERNATIONAL NEXN REGISTRY. Twelve cardiomyopathy and inherited cardiac disease centers across Europe, Australia, and North America participated in a longitudinal retrospective cohort study. The records of patients affected by any kind of

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

Manuscript received November 20, 2024; revised manuscript received April 29, 2025, accepted May 6, 2025.

structural cardiomyopathy carrying variants in *NEXN* predicted to be pathogenic (P), likely pathogenic (LP), or of uncertain significance according to American College of Medical Genetics and Genomics (ACMG) guidelines[19] were collected by each participating center and anonymized to create an international *NEXN* CMP registry. Only patients with available records were included in the registry. Data collection occurred from April 2021 to July 2022. The study complies with the Declaration of Helsinki and was approved by the Ethics Committee of Trieste University Hospital (43_2009 Em. 02 dd. 22/06/2022) and by the Institutional Review Boards of each participating center.

Based on the phenotype assessed by the referring center, patients were broadly categorized as HCM or DCM/nondilated left ventricular cardiomyopathy (NDLVC).[6] This dichotomous approach was chosen based on recent evidence on phenotypic heterogeneity of nonhypertrophic cardiomyopathies,[1,20] in order to avoid any possible bias in burden enrichment testing.

GENETIC TESTING AND VARIANT CURATION. Genetic testing was performed at participating centers or at accredited genetic laboratories by means of next-generation sequencing following site-specific protocols, with different multigene panels including *NEXN*. Genetic data were retrospectively collected for probands and available family members, and any additional variant detected in other cardiomyopathy-related genes was reported.

All *NEXN* variants were re-analyzed regardless of their ACMG classification: only protein-altering variants were considered, regardless of their zygosity, and classified as truncating (nonsense, frameshift, and essential splice site variants) or nontruncating (missense variants and in-frame indels).

These were further filtered according to their specific allele frequency in gnomAD. The cutoff to define variants as rare was set according to previous studies on genetic cardiomyopathies (0.000084 for DCM/NDLVC and 0.00004 for HCM).[21,22]

Any patient carrying pathogenic variants in other cardiomyopathy-related genes was excluded from clinical analysis (regardless of the type of *NEXN* variant carried).

BURDEN TESTING. Burden testing,[17,23] on probands alone, was performed in a subgroup of centers as detailed in the Supplemental Methods. Enriched *NEXN* variants were considered likely causative. Only patients harboring these variants, collected from all participating centers, were considered to be affected by *NEXN* CMP.

PHENOTYPIC CHARACTERIZATION. Each participating center collected demographic, family history, clinical history, and treatment data for each patient at baseline evaluation (defined as the first medical evaluation performed upon disease presentation) and at the last available evaluation prior to the instant of data collection (July 2022). Details of collected data and methods are presented in the Supplemental Methods.

STUDY OUTCOMES. The natural history of the disease was studied according to 2 separate outcomes: 1) a primary outcome of all-cause mortality, heart transplantation, and left ventricular assist device implantation; and 2) a secondary outcome of sudden cardiac death (SCD) and malignant ventricular arrhythmias (MVA), defined as sustained ventricular tachycardia, ventricular fibrillation, and ventricular arrhythmias interrupted by resuscitation or appropriate implantable cardioverter-defibrillator device (ICD) therapy.

Two previously published cohorts of P/LP *TTN* truncating variant (*TTN*tv) carriers and of *FLNC*tv carriers were used for comparison as models of classic DCM (*TTN*tv) and DCM/NDLVC with a pronounced arrhythmogenic profile (*FLNC*tv).[1,2]

STATISTICAL ANALYSIS. Variables were expressed as mean ± SD or median (Q1-Q3) for continuous variables, or n (%) for categoric variables, as appropriate. Categoric variables were compared between groups by means of the parametric chi-square test or nonparametric Fisher test. Comparisons on continuous variables were made by means of the nonparametric Kruskal-Wallis test, as variables did not meet normal distribution. Burden comparisons to test for enrichment were performed using 1-sided Fisher test.

For each variant class with a significant burden excess, the corresponding etiologic fraction was computed, a derivative of OR, representing the prior probability that a rare variant is the cause of disease in a dominant model of inheritance, given that it is found in a patient.

Cumulative incidence curves for the primary and secondary endpoints were estimated considering left truncation on the age scale.

The *P* values reported were estimated by means of univariable Cox models in the "start-stop" counting format, to consider both left truncation and the cause-specific outcome in the case of the secondary endpoint. The R library "survival" was used. A value of $P < 0.05$ was considered to be statistically

TABLE 1 Burden Testing of *NEXN* Variants

Type of Variant	Phenotype of Case Cohort	Number of Variants in Case Cohort	Case Burden, %	Variants in Control Cohort, n	Control Burden, %	*P* Value (1-Sided Fisher Test)	OR (95% CI)	Etiologic Fraction (95% CI)
Truncating	DCM/NDLVC	12	0.39	53	0.09	**2.73E-05**	**4.2 (2.3-7.8)**	**0.76 (0.6-0.88)**
	HCM	2	0.11			0.49	–	–
Nontruncating	DCM/NDLVC	19	0.62	349	0.62	0.53	–	–
	HCM	8	0.46			0.84	–	–

Results of the burden comparison for rare truncating and nontruncating variants in *NEXN* between DCM/NDLVC patients (n = 3,053) and non-Finnish Europeans in gnomAD (exomes; n = 56,264) and between HCM patients (n = 1,740) and non-Finnish Europeans in gnomAD (exomes, n = 56,264), divided by protein truncating and nontruncating variants. **Bold** indicates significantly enriched variants.

DCM = dilated cardiomyopathy; HCM = hypertrophic cardiomyopathy; NDLVC = nondilated left ventricular cardiomyopathy.

significant. IBM-SPSS software (version 29) was used to for the statistical analyses.

RESULTS

CHARACTERIZATION OF *NEXN* VARIANTS AND PATIENT SELECTION. Data from 88 patients were initially collected. These patients harbored 65 different *NEXN* variants, 16 truncating and 48 nontruncating (40 missense and 8 short in-frame deletions) in heterozygosity, and 1 in-frame deletion in homozygosity (Supplemental Table 1).

Thirteen of these variants (n = 25) were excluded due to an allele frequency greater than the prespecified cutoff, and another 3 because they were predicted not to affect splice site or protein sequence. Of the remaining 49 rare variants (n = 60), 36 (12 truncating, 22 nontruncating, 1 homozygous) were carried by patients with DCM/NDLVC (n = 46) and 13 (3 truncating, 10 nontruncating) by patients with HCM (n = 14).

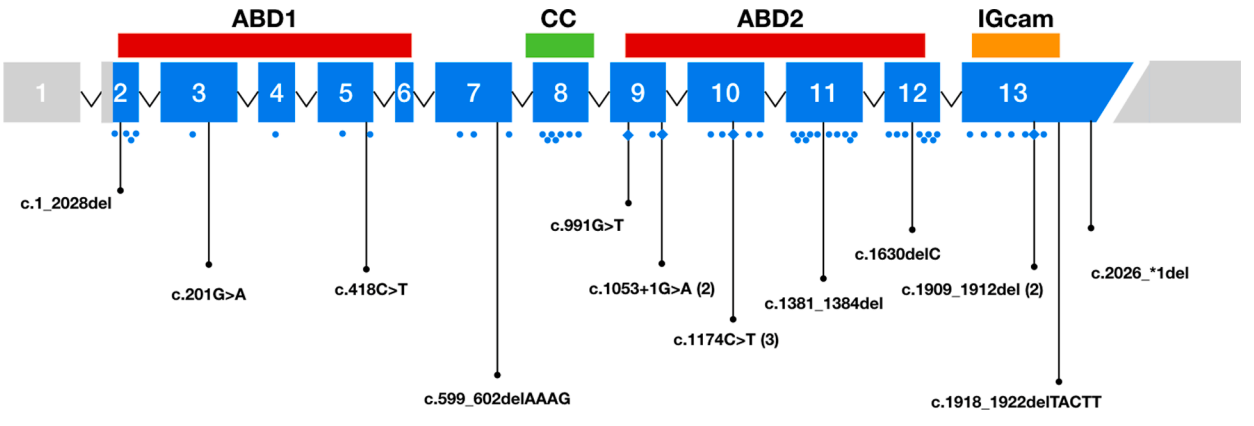
BURDEN TESTING OF RARE *NEXN* VARIANTS. Given the phenotypic and allelic heterogeneity of the population, burden testing was performed to validate the association of each class of *NEXN* rare variants with the reported CMP phenotype.

In the DCM/NDLVC cohort (n = 3,053), tvs were significatively enriched compared with the reference control population of gnomAD non-Finnish Europeans (NFE), with a burden of 0.39% in cases vs 0.09% in controls ($P < 0.0001$) (**Table 1**, Supplemental Tables 2 to 4).

The OR of *NEXN*tv for DCM/NDLVC was 4.2 (95% CI: 2.3-7.8), and the etiologic fraction was 0.76 (95% CI: 0.6-0.88).

When addressing each *NEXN*tv separately, those (n = 5) absent in gnomAD NFE had an OR of

FIGURE 1 Truncating Variant (tv) Locations Across *NEXN* Gene



Annotations on transcript NM_144573.3. Rare isolated *NEXN*tvs in the dilated cardiomyopathy/nondilated left ventricular cardiomyopathy cohort. Numbers in brackets refer to the number of patients carrying the variant. Translated exons are depicted as blue boxes; functional regions are indicated by the bars above. Blue dots represent *NEXN*tvs reported in gnomAD NFE.

TABLE 2 Baseline Characteristics of *NEXN*tv Carriers (N = 17)

Age, y	45 (39-55)
Missing	6 (1)
Male	53 (9)
Missing	0
European ancestry	76 (13)
Missing	0
Probands	88 (15)
Missing	0
Family history of cardiomyopathy	47 (8)
Missing	0
Family history of sudden cardiac death	35 (6)
Missing	0
Hypertension at baseline	6 (1)
Missing	0
Phenotype	
Missing	0
DCM	59 (10)
NDLVC/others	41 (7)
Dyspnea/palpitation/chest pain/syncope/asymptomatic, %	23/23/17/17/17
NYHA functional class III or IV	6 (1)
Missing	0
NYHA functional class I	71 (12)
Sinus rhythm	88 (15)
Missing	0
Atrial fibrillation	12 (2)
Left bundle branch block	6 (1)
Missing	6 (1)
Negative T waves	43 (6)
Missing	6 (1)
Excluded (bundle branch block)	12 (2)
PVCs/24 h	515 (43-5,071)
Missing	29 (5)
PVCs >1,000 in 24 h	42 (5)
Nonsustained ventricular tachycardia	42 (5)
Missing	29 (5)
Echocardiography (probands only)	
LVEF, %	42 (32-51)
LVEF ≤35%	28 (4)
LVEDD, mm	58 (51-63)
Missing	12 (2)
LVEDVi, mL/m^2	75 (52-87)
LV hypertrophy	0
RV dysfunction	28 (4)
Missing	6 (1)
Mitral regurgitation ≥ moderate	0
Missing	6 (1)
CMR LGE	50 (7)
Missing	18 (3)
Fibrosis on EMB	30 (5)
Missing	70 (12)
Fibrosis overall (EMB or LGE)	64 (9)
Missing	18 (3)
Beta-blocker	53 (9)
Missing	0
ACEI/ARB/ARNI	23 (4)
Missing	0
Diuretic therapy	23 (4)
Missing	0

Continued on the next page

Values are median (Q1-Q3) or n% (n).

ACEI = angiotensin-converting enzyme inhibitor; ACM = arrhythmogenic cardiomyopathy; ARB = angiotensin receptor blocker; ARNI = angiotensin receptor neprilysin inhibitor; CMR = cardiac magnetic resonance; DCM = dilated cardiomyopathy; EMB = endomyocardial biopsy; LVEDVi = indexed left ventricular end-diastolic volume; LGE = late gadolinium enhancement; LV = left ventricular; LVEF = left ventricular ejection fraction; LVEDD = left ventricular end-diastolic diameter; *NEXN*tv = Nexilin truncating variant; PVC = premature ventricular contraction; RV = right ventricle.

55 (95% CI: 2.3-1,358). Notably, enrichment and OR were greater among subcohorts with: 1) high-normal to mildly increased median left ventricular end-diastolic diameter (LVEDD); and 2) higher prevalence (>30%) of ICD recipients (Supplemental Tables 5 and 6).

Conversely, nontruncating *NEXN* variants showed the same prevalence in cases and controls (0.62%; $P = 0.53$). Based on these tests, all patients carrying nontruncating *NEXN* variants with a DCM/NDLVC phenotype were excluded from further analysis, because these variants were considered to be likely noncausative.

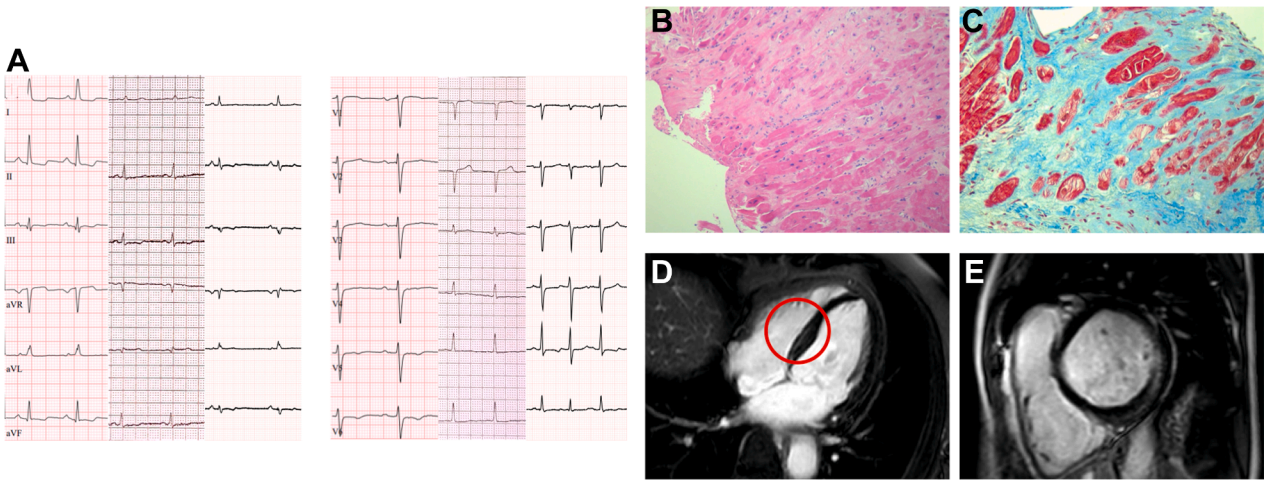
The same analysis was performed in the HCM study case cohort, where 2 *NEXN*tvs (0.11%) and 8 nontruncating rare variants (0.46%) were found, with both burdens similar to those observed in gnomAD NFE (**Table 1**, Supplemental Tables 2 to 6). Accordingly, HCM patients carrying rare *NEXN* variants were excluded from further analysis (Supplemental Table 7).

STUDY POPULATION. After multiple filtering as described above (Supplemental Figure 1), 18 patients from our initial registry, with DCM/NDLVC, were found to be carriers of a likely causative *NEXN* variant. One of them was also carrier of a pathogenic variant in another validated DCM gene (*FLNC*tv) and therefore excluded.

The final study population included 17 patients (16 with *NEXN*tv and 1 with *NEXN* homozygous in-frame deletion c.1949_1951del). *NEXN*tv locations across the *NEXN* gene are shown in **Figure 1**. No region harbored variants more frequently than others; 75% of *NEXN*tvs were absent from gnomAD NFE.

Fifteen patients were probands (88%). Forty-seven percent of patients reported documented family history of CMP, mostly DCM. Family history of SCD was present in 35% of patients. Detailed familial pedigrees with clinical assessment of relatives were available from 4 probands, showing dominant

FIGURE 2 Representative Features of *NEXN*tv Carriers



(A) Electrocardiography recordings of 3 different *NEXN*tv carriers. (B, C) Histologic staining from endomyocardial biopsies, showing extensive fibrosis in both (B) hematoxylin-eosin and (C) Azan-Mallory staining. (D, E) Late gadolinium enhancement short-axis sequences of 2 different patients, with nonischemic patterns.

inheritance pattern with incomplete penetrance. In 3 of these families, all carrying rare *NEXN*tvs absent from gnomAD NFE, co-segregation was confirmed (Supplemental Figure 2).

Baseline clinical characteristics are detailed in **Table 2** and Supplemental Table 8.

Median age of disease onset was 46 years (Q1-Q3: 35-55 years), with only 3 patients affected before the age of 18 years. Nine patients were male (53%), and 13 were of European ancestry (76%).

The reported phenotypes at presentation were DCM in 59% (n = 10) and NDLVC (n = 4) or other phenotypes (n = 3) in 41%. Two patients of the "other phenotypes" group were initially labeled as affected by long QT syndrome. However, after clinical reassessment, electrocardiography (ECG) showed normal QT interval, inferolateral T-wave abnormalities, and low QRS complex voltages, leading to an NDLVC diagnosis (n = 6). The third patient had atrial arrhythmias (atrial fibrillation and 2nd-degree atrioventricular block) at a young age (16 years), family history of DCM, and mildly dilated left ventricle (LV), leading to a DCM diagnosis (n = 11). Predominant right ventricular involvement was not reported in any patient.

Palpitations (23%) and dyspnea (23%) were the most prevalent symptoms, followed by syncope (17%) and chest pain (17%). Three subjects were asymptomatic and diagnosed through ECG/echocardiography abnormalities found by family screening (n = 2) or incidental ECG finding (n = 1).

Heart failure-related symptoms at presentation were uncommon (71% of patients were in NYHA functional class 1). Most patients were in sinus rhythm (88%), and left bundle branch block (LBBB) was rare (6%). ECG repolarization abnormalities were frequent (43%), with no dominant location of T-wave inversion.

At first Holter ECG, 42% of patients had frequent ventricular ectopic beats (ie, >1,000/24 h). Median left ventricular ejection fraction (LVEF) was 42% (Q1-Q3: 32%-51%), and median indexed left ventricular end-diastolic volume (LVEDV) was 75 mL/m^2 (Q1-Q3: 52-87 mL/m^2). Up to 60% of patients had normal indexed LVEDV, especially if female (Supplemental Figure 3).

Cardiac magnetic resonance data were available for 14 patients, with the scan being obtained 1 month after diagnosis (Q1-Q3: 0-5 months); 50% had late gadolinium enhancement. The pattern of fibrosis was nonischemic, with no predominant localization and heterogeneous distribution. When accounting also for those patients in which endomyocardial biopsy was performed (n = 5), up to 64% (n = 9) showed significant ventricular fibrosis at either methodology (**Figure 2**).

FOLLOW-UP AND OUTCOMES. During a median follow-up of 45 months (Q1-Q3: 11-130 months), heart failure-related symptoms remained uncommon, with only 1 patient (as indicated in **Table 2**) in NYHA functional class >II. Median LVEDV modestly increased to 80 mL/m^2 (Q1-Q3: 52-86 mL/m^2), and LV systolic dysfunction persisted as mild to moderate. Twenty-five percent presented atrial fibrillation.

TABLE 3 Follow-Up Characteristics of *NEXN*tv Carriers

Follow-up duration, mo	45 (11-130)
Missing	0
Atrial fibrillation	25 (4)
Missing	6 (1)
NYHA functional class III or IV	8 (1)
Missing	23 (4)
Echocardiography	
LVEF, %	45 (36-54)
Missing	18 (3)
LVEF ≤35%	21 (3)
LVEDD, mm	55 (48-62)
Missing	29 (5)
LVEDVi, mL/m^2	80 (52-86)
LV hypertrophy	0
RV dysfunction	33 (4)
Missing	29 (5)
Mitral regurgitation ≥ moderate	8 (1)
Missing	29 (5)
Beta-blocker	76 (13)
Missing	0
ACEI/ARB/ARNI	53 (9)
Missing	0
Diuretic therapy	41 (7)
Missing	0
All cause death	6 (1)
Missing	6 (1)
MVA	25 (4)
Missing	6 (1)
SCD	0
Heart transplantation	0
Missing	6 (1)
VAD	0
Missing	6 (1)
ICD placement	53 (9)
Missing	0
ICD intervention	19 (3)
Missing	6 (1)

Values are median (Q1-Q3) or n% (n).

ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; ARNI = angiotensin receptor-neprilysin inhibitor; ICD = implantable cardioverter-defibrillator; LVEDD = left ventricular end-diastolic diameter; LVEDVi = indexed left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; MVA = malignant ventricular arrhythmia; RV = right ventricle; SCD = sudden cardiac death; VAD = ventricular assist device.

Nine patients (53%) underwent ICD placement for both primary (n = 7) and secondary prevention (n = 2). Primary prevention indications were persistent LVEF dysfunction (ie, LVEF <35% despite 6 months or more of optimal medical therapy) and risk factors such as unexplained syncope, extensive late gadolinium enhancement, or family history of SCD. One patient died of noncardiac causes. No heart failure-related events (ie, death, heart transplantation, or left ventricular assist device implantation) were reported. Four patients experienced SCD/MVA: 1 patient had aborted SCD from documented ventricular fibrillation (VF), 1 patient had a resuscitated cardiac arrest and underwent ICD placement, which correctly interrupted another VF a few months later, and 2 patients had appropriate ICD therapies because of ventricular tachycardia/VF at 13 and 126 months of follow-up (**Table 3**). These 4 MVA events occurred with heterogeneous LVEFs, in 2 cases with LVEF >35%.

To further prove *NEXN*tv disease specificity, patients were compared with the 23 excluded subjects carrying rare isolated nontruncating *NEXN* variants (Supplemental Table 9). Carriers of rare *NEXN* missense variants had more dilated LVs (median indexed LVEDV 88 mL/m^2 [Q1-Q3: 72-106 mL/m^2]) and tended to be in higher NYHA functional classes and to be hypertensive. They presented significantly more LBBB and moderate or severe mitral regurgitation on baseline echocardiography. When comparing outcomes, carriers of *NEXN*tv variants had SCD/MVAs more frequently than their nontruncating counterparts (25% vs 6%; $P = 0.056$).

COMPARISON WITH *TTN*-CMP AND *FLNC*-CMP. *NEXN*tv carriers were compared with 95 patients with *TTN*tv-CMP and 37 patients with *FLNC*tv-CMP. *NEXN*tv carriers showed significantly smaller LVEDD and higher LVEF at enrollment compared with *TTNtv* carriers. Compared with *FLNC*tv carriers, these values were similar, though with a trend toward smaller LVEDD and higher LVEF in *NEXN*tv carriers.

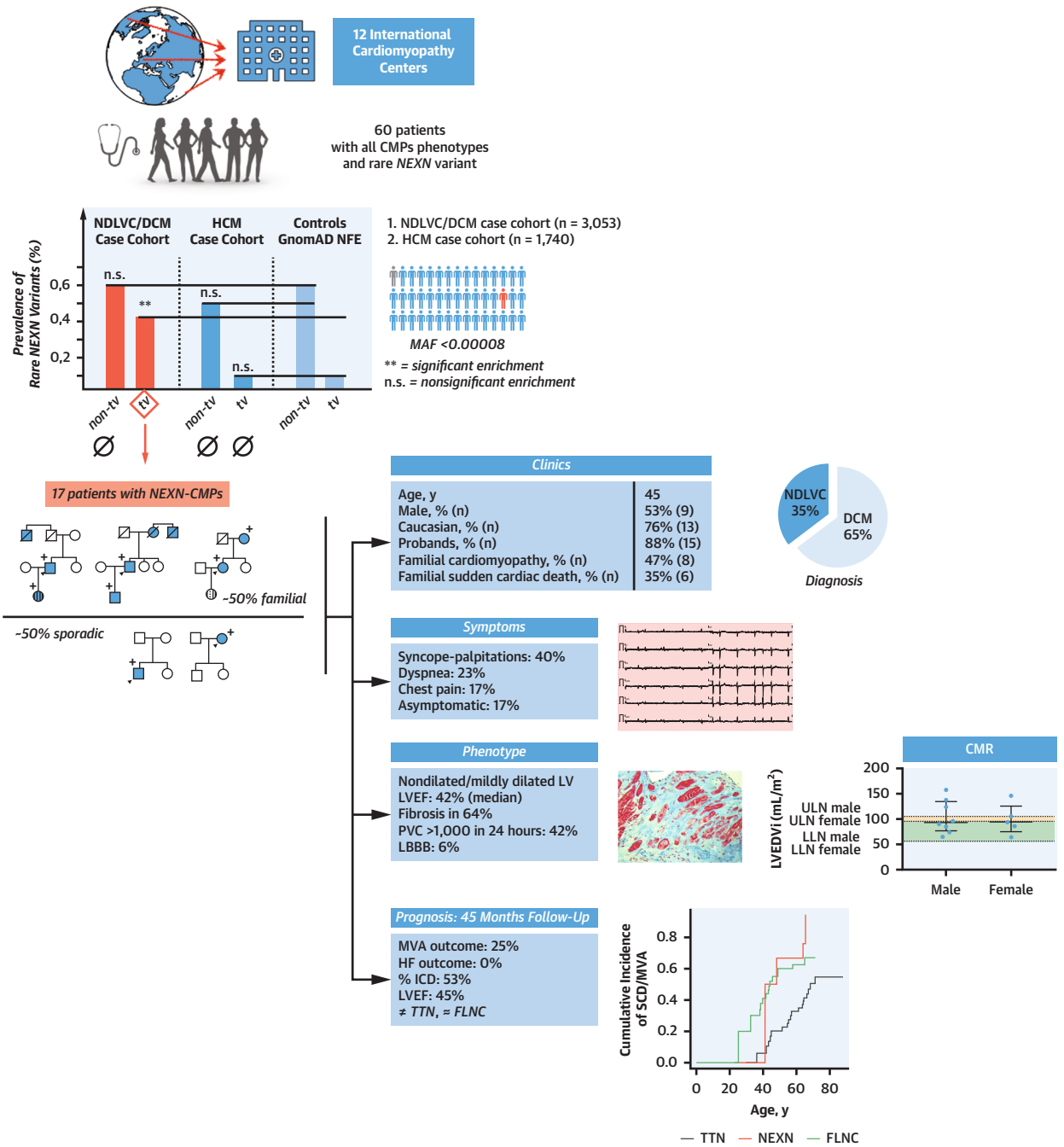
*NEXN*tv carriers showed a tendency toward a more even distribution between sexes (Supplemental Table 10). When comparing outcomes, cumulative incidence function curves for the secondary outcome stratified by decades of age showed similar risks between *NEXN*tv and *FLNC*tv carriers ($P = 0.69$) (Supplemental Figures 4 and 5).

DISCUSSION

This study is the first to provide a comprehensive cardiac evaluation of a well characterized international population of *NEXN* variants carriers through a genotype-first approach. The following main findings are reported: 1) *NEXN*-related CMP is a rare condition; 2) only *NEXN*tv showed potential mendelian association with a distinct DCM/NDLVC cardiac phenotype; and 3) *NEXN*tv-CMP may present an arrhythmogenic profile in young adult age (**Central Illustration**). These results could have a significant impact in clinical management of these patients.

GENETIC EVIDENCE. Despite a wide international collaborative effort, only 60 carriers of rare and isolated *NEXN* variants were collected. After multiple

CENTRAL ILLUSTRATION Study Workflow and Results



Perotto M, et al. JACC Heart Fail. 2025;13(9):102529.

The study began with burden enrichment testing, comparing the study case cohort with gnomAD non-Finnish Europeans (NFE), where only *NEXN*tvs were enriched, in the NDLVC/DCM subgroup. Family pedigrees are depicted: probands indicated with arrowheads, affected individuals with gray, positive genetic testing for *NEXN*tv with plus signs, and intermediate phenotypes with slashes. The right side of the figure lists all characteristics of the study population in terms of clinics, symptoms, phenotype, and outcomes. CMP = cardiomyopathy; CMR = cardiac magnetic resonance; DCM = dilated cardiomyopathy; *FLNC* = Filamin C; HCM = hypertrophic cardiomyopathy; HF = heart failure; ICD = implantable cardioverter-defibrillator; LBBB = left bundle branch block; LLN = lower limit of normal; LV = left ventricular; LVEDVi = indexed left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; MAF = minor allele frequency; MVA = major ventricular arrhythmias; NDLVC = nondilated left ventricular cardiomyopathy; *NEXN* = Nexilin; PVC = premature ventricular contractions; SCD = sudden cardiac death; *TTN* = Titin; tv = truncating variant; ULN = upper limit of normal.

filtering, we described the clinical features of 17 patients likely affected by *NEXN*-CMP. With a final estimated prevalence of 0.40% among our case cohort, *NEXN*-CMP is a rare clinical entity.

Rare *NEXN*tvs emerged as the unique class of variants significantly enriched. Nontruncating variants, conversely, did not. The work by Mazzarotto et al[15] on enrichment of DCM-causing genes showed that in a "pure" nonarrhythmic DCM cohort composed of patients with severely dilated LVs and free of devices, *NEXN*tv variants were not enriched. We found that *NEXN*tvs were enriched especially in cohorts of patients with mildly or nondilated LVs and frequently treated with devices. Because the selection of the appropriate phenotype is crucial in case-control studies, these differences between cohorts at enrollment could explain both results on burden testing (Mazzarotto et al[15] and the present study), which are complementary. Consistently, *NEXN*tv-CMP shows minor LV dilatation compared with *TTN*tv, *FLNC*tv, and missense *NEXN* variant carriers.

However, the strongest and most independent support of the association of *NEXN*tv with nondilated LV is provided by the recently published work on the same topic by Hermida et al.[18] In reanalysis of their data, the only subgroup of patients in which *NEXN*tvs are enriched (compared with gnomAD NFE) is the SCD/idiopathic ventricular fibrillation (IVF) group (*NEXN*tv prevalence in SCD/IVF patients 4/1,089 [0.36%]; $P = 0.02$ vs gnomAD NFE).[18] Both the phenotype and the genetic enrichment are remarkably similar to our results. In Hermida et al's study,[18] *NEXN*tvs are not enriched in DCM (8/6,274 [0.12%]; $P = 0.4$ vs gnomAD NFE) or in HCM (1/2153 [0.04%]; $P = 0.53$ vs gnomAD NFE). Hermida et al,[18] however, did not perform case-control genetic analyses and included missense variants, describing an association with DCM and LBBB.

The population in the present study was largely composed of probands. This fact may be due to incomplete penetrance and is coherent with calculated values of etiologic fraction. Accordingly, although burden testing supports mendelian actionability, *NEXN* could also be hypothesized as a "near-mendelian" gene,[24] with a limited penetrance and a complex etiology for CMPs, such that the interaction with environment may be necessary for *NEXN*tv to be fully penetrant.

Indeed, the overall reported OR of *NEXN*tv in DCM/NDLVC CMP is low, similar to those of *LMNA* missense variants and *MYH7* (Myosin heavy chain 7) missense variants (for DCM).[17,18] This may have several explanations. First may be potential pitfalls of burden testing in *NEXN*-CMP due to phenotype selection, where the values of OR and etiologic fraction could be diluted by the presence of a share of severely dilated LVs in the case cohort, while on the other side, some affected carriers could be undiagnosed (or misdiagnosed with channelopathies as in 2 cases in the present registry) and thus not included. This hypothesis is supported by analyses in separate subcohorts (Supplemental Table 2). Second, *NEXN*tv might not be all equally mendelian, with heterogenous penetrance. In our population, the rarest tvs, with allele count of 0 in the control cohort, showed much higher OR and segregation in families, whereas the OR was lower for less rare tvs (allele count >0 to <8) and segregation not demonstrated. This hypothesis is supported by recent evidence on the complex spectrum of heritability in CMPs.[25] Of note, the consistent evidence of severe fetal and infantile DCM associated with biallelic *NEXN* variants[12,26] aligns with our findings on adult *NEXN*tv CMP, suggesting a continuum of penetrance that ranges from recessive to dominant or semidominant, depending on zygosity, rarity, and type of *NEXN* variants.

In the present registry, self-reported family history of CMP or SCD was recorded for one-half of the cases, and the 3 families in which *NEXN*tv co-segregation was documented showed silent CMPs in asymptomatic subjects, thus strengthening the recommendation for variant co-segregation studies.

In the context of HCM, we and Hermida et al[18] independently described rare sporadic cases of HCM associated with *NEXN*tv, in absence of evidence from case-control studies. However, our studies lack sufficient evidence to support this hypothesis. *NEXN* has not been associated with HCM nor hypertrophic LV traits in previous genome-wide association studies.[27]

CLINICAL DESCRIPTION. Most *NEXN*tv carriers had LV diastolic volumes in normal ranges. One of the 2 patients with dilated volumes was affected by LBBB (unique case), possibly increasing LV dilatation. Median LV function was only moderately impaired. This is consistent with the absence or mildness of heart failure symptoms at enrollment.

Considering the evolution of the disease on the age axis, LV dilatation in *NEXN*tv-CMP seems to develop later in life than *TTN*tv-CMP. However, a relevant proportion of patients had myocardial fibrosis since disease onset. In keeping with all this, ventricular arrhythmias represented the most common clinical feature of this CMP.

After 4 years of follow-up, median LVEF did not significantly decrease, with a minority of patients

developing heart failure. One-half of the patients received ICDs. As in other arrhythmogenic genotypes such as *FLNC*, SCD and MVAs were not consistently associated with severe LV dysfunction.[2] Interestingly, in our cohort, all SCD/MVA episodes occurred in female patients.

The homozygous carrier of in-frame deletion c.1949_1951del was included in our population because of its homozygosity and previous experimental models and clinical cases that have associated this homozygous genotype with severe DCM.[11,28]

Although we detected no signal for rare missense variants in our registry, their possible role as risk factors for CMP or in special populations (eg, pediatric patients) needs dedicated study to be properly elucidated. Our case carriers of *NEXN* missense variants are likely to represent a group of heterogeneous "gene-elusive" CMPs, with prevalent DCM phenotype and random noncausative association with *NEXN*. In fact, patients with missense variants and DCM more frequently had LBBB (known to be rare in monogenic mendelian CMPs[29]) and a significantly lower rate of arrhythmic events compared with carriers of *NEXN*tv. This was similar in Hermida et al's study.[18]

To provide the clinician with useful prognostic information, we performed an outcome comparison of *NEXN*tv carriers with previously published cohorts of *TTN*tv-CMP and *FLNC*tv-CMP. As expected, patients with *TTN*tv-CMP were characterized by a lower arrhythmic risk, but we did not find any significant difference between *FLNC*tv-CMP and *NEXN*tv-CMP at 4 years.

In summary, we think that the results of the present study comprehensively suggest considering *NEXN*tv-CMP as a new arrhythmogenic entity. Given the low number of events, these trends warrant validation in larger cohorts.

Recent guidelines do support the inclusion of genotype in the risk assessment of CMPs.[30,31] Clinicians could be encouraged to perform a multiparametric assessment in *NEXN*tv carriers when an arrhythmic phenotype is present, regardless of LVEF values.

Furthermore, CMR and repeated ECG Holter monitoring should be indicated in relatives to detect possible silent manifestations of *NEXN*tv-CMP, and further studies are needed to assess their prognostic value.

FROM BENCH TO THE BEDSIDE: CLINICAL IMPLICATION. To date, ClinGen Resource assigns to *NEXN* the highest score in experimental evidence (8 points on a 0-6 scale). Previous studies with knockout models showed a loss-of-function effect directly associated with a marked reduction of Nexilin levels, resulting in severely dilated CMP.[7] Heterozygous tvs lead to relatively less function reduction, because of compensation from the normal allele. Assuming this, the degree of LV dilatation in *NEXN*-CMP could be directly influenced by cellular protein levels.

There is no experimental evidence yet available on nonsense-mediated decay (NMD). However, NMD prediction models suggest a high likelihood of haploinsufficiency for most of our *NEXN*tvs. In addition, in the absence of dedicated functional studies, we cannot exclude that a subset of nontruncating variants could induce a similar deregulation in Nexilin levels.

STUDY LIMITATIONS. Even though in this study we describe the largest genotype-first study on *NEXN* variants to date, our results are based on a small number of patients. The study is retrospective in nature and thus carries all the limitations of that design.

Most importantly, our enrollment criteria from CMP centers were as wide as possible to ensure that all potentially affected patients were recruited, but we cannot exclude a potential selection bias. In particular, the prevalence of *NEXN* variants in patients with suspected channelopathies may be non-negligible, especially in patients who tested negative in limited gene panels.

Finally, this study was not designed to systematically investigate predicted splice-altering missense variants, which deserve dedicated studies.

CONCLUSIONS

In this study, rare *NEXN*tvs were associated with a distinct CMP phenotype characterized by nondilated, nonhypertrophic, mildly hypokinetic LV with frequent fibrosis and ventricular arrhythmias. Sequencing of the *NEXN* gene should always be included in genetic testing for CMPs and suspected channelopathies, and family screening is recommended for *NEXN*tvs.

ACKNOWLEDGMENTS Prof Rossana Bussani, Associate Professor of Pathological Anatomy, University of Trieste, is gratefully acknowledged for her expert review of endomyocardial biopsies. The authors are grateful to the patients and family members for their participation in these studies.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

This study was supported by the CRTrieste Foundation and Cassa di Risparmio di Gorizia Foundation (to Prof Sinagra), the British Heart Foundation (FS/CRTF/23/24448) Alexander Jansons Myocarditis UK, Rosetrees Trust; and ÚNKP-23-4 New National Excellence Program of the Ministry for Culture and Innovation funded by the National

Research, Development, and Innovation Fund of Hungary. The Centro Nacional de Investigaciones Cardiovasculares (CNIC, Madrid) is supported by the Instituto de Salud Carlos III , the Ministerio de Ciencia e Innovación, and the Pro CNIC Foundation, and is a Severo Ochoa Center of Excellence (grant CEX2020-001041-S funded by MICIN/AEI/10.13039/501100011033). Dr Sepp has received consulting and speaker fees from Boehringer Ingelheim, AstraZeneca, Novartis, Takeda, Bristol Myers Squibb, Bayer, and Merck Sharp & Dohme, outside of the submitted work. Dr Stolfo has received personal fees from Novartis, Merck, GlaxoSmithKline, and Acceleron, outside the submitted work. Dr Merlo has received consulting and unrestricted fees at congresses and congress fees from Novartis and Vifor Pharma. Dr Sinagra has received consulting fees from Novartis, Impulse Dynamics, and Biotronik, and speaker and honoraria from Novartis, Bayer, AstraZeneca, Boston Scientific, Vifor Pharma, Menarini, and Akcea Therapeutics, outside the submitted work. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: *NEXN* should be included in genetic analyses of patients with DCM/NDLVC or channelopathies and of their relatives, because it may be causative of a distinct cardiomyopathy with mild left ventricular dilation and dysfunction, fibrosis, and frequent ventricular arrhythmias in young adults.

TRANSLATIONAL OUTLOOK: More research is needed to clearly define the frequency of *NEXN* cardiomyopathy and to understand its pathogenesis at the cellular level.

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KEY WORDS arrhythmias, cardiac fibrosis, DCM, genetics, inherited cardiac diseases, NDLVC

APPENDIX For an expanded Methods section as well as supplemental figures and tables, please see the online version of this paper.