JACC: HEART FAILURE VOL. ■, NO. ■, 2024

Genetic Landscape of Patients With Dilated Cardiomyopathy and a Systemic Immune-Mediated Disease

Sophie L.V.M. Stroeks, MD,[a,b,c,g] Michiel T.H.M. Henkens, MD, PhD,[a,d,e] Fernando Dominguez, MD, PhD,[c,j,k] Marco Merlo, MD,[c,f] Debby M.E.I. Hellebrekers, PhD,[c,g] Esther Gonzalez-Lopez, MD, PhD,[c,j,k] Matteo dal Ferro, MD,[c,f] Juan Pablo Ochoa, MD, PhD,[c,j,k] Francesco Venturelli, MD,[c,f] Godelieve R.F. Claes, PhD,[c,g] Max F.G.H.M. Venner, MD,[a] Ingrid P.C. Krapels, MD, PhD,[c,g] Els K. Vanhoutte, MD, PhD,[c,g] Pieter van Paassen, MD, PhD,[h] Arthur van den Wijngaard, PhD,[c,e] Maurits A. Sikking, MD,[a] Rick van Leeuwen, BSc,[a] Myrurgia Abdul Hamid, MD,[d] Xiaofei Li, MD, PhD,[d] Han G. Brunner, MD, PhD,[g,i] Gianfranco Sinagra, MD, PhD,[c,f] Pablo Garcia-Pavia, MD, PhD,[c,j,k] Stephane R.B. Heymans, MD, PhD,[a,b] Job A.J. Verdonschot, MD, PhD[a,c,g]

ABSTRACT

BACKGROUND Systemic immune-mediated diseases (SIDs) are a well-known cause of dilated cardiomyopathy (DCM), a cardiac phenotype influenced by genetic predispositions and environmental factors.

OBJECTIVES This study sought to examine if an underlying genetic predisposition is present in patients with DCM and SID.

METHODS Genotyped DCM-SID patients (n = 183) were enrolled at 3 European centers. Genetic variants were compared with healthy control subjects (n = 20,917), DCM patients without SID (n = 560), and individuals with a suspicion of an SID (n = 1,333). Clinical outcomes included all-cause mortality, heart failure hospitalization, and life-threatening arrhythmias.

RESULTS The SID diagnosis preceded the DCM diagnosis by 4.8 months (Q1-Q3: −68.4 to +2.4 months). The prevalence of pathogenic/likely pathogenic (P/LP) variants in DCM patients with an SID from the Maastricht cohort was 17.1%, compared with 1.9% in healthy control subjects ($P < 0.001$). In the Madrid/Trieste cohort, the prevalence was 20.5% ($P < 0.001$). Truncating variants showed the strongest enrichment (10.7% [OR: 24.5] (Maastricht) and 16% [OR: 116.6 (Madrid/Trieste); both $P < 0.001$), with truncating *TTN* (titin) variant (TTNtv) being the most prevalent. Left ventricular ejection fraction at presentation was reduced in TTNtv-SID patients compared with DCM patients with SID without a P/LP ($P = 0.016$). The presence of a P/LP variant in DCM-SID had no impact on clinical outcomes over a median follow-up of 8.4 years (Q1-Q3: 4.9-12.1 years).

CONCLUSIONS One in 6 DCM patients with an SID has an underlying P/LP variant in a DCM-associated gene. This highlights the role of genetic testing in those patients with immune-mediated DCM, and supports the concept that autoimmunity may play a role in unveiling a DCM phenotype in genotype-positive individuals.

From the [a]Cardiovascular Research Institute Maastricht, Department of Cardiology, Maastricht University, Maastricht, the Netherlands; [b]Cardiovascular Sciences, KU Leuven, Leuven, Belgium; [c]European Reference Network for Rare, Low Prevalence and Complex Diseases of the Heart (ERN GUARD-Heart), Amsterdam, the Netherlands; [d]Department of Pathology, Maastricht University Medical Centre, Maastricht, the Netherlands; [e]Netherlands Heart Institute (NLHI), Utrecht, the Netherlands; [f]Cardiovascular Department, Azienda Sanitaria Universitaria Giuliano Isontina, University of Trieste, Italy; [g]Department of Clinical Genetics, Maastricht University Medical Center, Maastricht, the Netherlands; [h]Department of Nephrology and Clinical Immunology, Maastricht University Medical Center, Maastricht, the Netherlands; [i]Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands; [j]Madrid Hospital Universitario Puerta de Hierro Majadahonda, IDIPHISA, CIBERCV, Madrid, Spain; and the [k]Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain.

ISSN 2213-1779 https://doi.org/10.1016/j.jchf.2024.08.011

ABBREVIATIONS AND ACRONYMS

DCM = dilated cardiomyopathy

EMB = endomyocardial biopsy

ICD = implantable cardioverter-defibrillator

LVEF = left ventricular ejection fraction

NTV = nontruncating variant

P/LP = pathogenic or likely pathogenic

SID = systemic immune-mediated disease

TTNtv = truncating *TTN* variant

TV = truncating variant

VF = ventricular fibrillation

VT = ventricular tachycardia

Systemic immune-mediated diseases (SIDs) include both autoimmune and autoinflammatory diseases that may involve all organs and also the heart.[1] Autoinflammatory diseases are a group of conditions characterized by unprovoked inflammation caused by a primary innate immune system dysfunction. Autoimmune diseases are characterized by immune cell responses against self-antigens in genetically susceptible individuals. However, a dichotomous classification does not reflect clinical evidence, and a continuum from purely autoinflammatory to purely autoimmune diseases should be considered.[1,2] Cardiac involvement in SIDs is not uncommon and is associated with adverse outcomes.[3] The association between cardiac involvement and SIDs is further highlighted by a position statement of the European Society of Cardiology (ESC) working group on myocardial and pericardial disease that emphasizes the importance of an appropriate cardiac diagnostic work-up in patients with SIDs.[2]

Dilated cardiomyopathy (DCM) is a cardiac phenotype that can be caused by SIDs, characterized by left ventricular systolic dysfunction and (bi)ventricular dilation.[4] DCM is a heterogeneous disease that is influenced by both environmental and genetic triggers.[5,6] Rare variants in genes that cause familial DCM have also been significantly enriched in patients with different environmental triggers and acquired triggers, such as alcohol,[7] chemotherapy,[8] and peripartum cardiomyopathy.[9] However, a possible underlying genetic basis in patients with SID-associated DCM remains unknown.

We report the first large-scale international genetic evaluation of patients with DCM and SIDs to determine the frequency of genetic variants associated with inherited cardiomyopathies, in particular truncating variants (TVs) in *TTN* (titin), the most frequent genetic cause of DCM (present in 10%-20% of DCM).[10,11] In this study, we sought to examine if an underlying genetic predisposition is present in patients with DCM and an SID.

METHODS

COHORTS. The study comprises 5 cohorts (**Figure 1**). In cohort 1 (SID and DCM), 143 patients with DCM and an SID were recruited from the Maastrich Cardiomyopathy Registry (NCT04976348) at the MUMC (Maastricht University Medical Center) that prospectively includes individuals with heart failure-like symptoms or who underwent cardiac or genetic testing.[12] All patients were cardiologically evaluated, and the autoimmune or autoinflammatory disease was classified according to the position statement of the ESC Working Group on Myocardial and Pericardial Disease.[2] The DCM diagnosis was defined according to the World Health Organization criteria and the latest ESC proposal.[13,14] Patients enrolled in the current study presented with a left ventricular ejection fraction (LVEF) <50%, without the presence of occlusion of a major coronary artery branch according to coronary angiography, pericardial disease, congenital heart disease, or acute myocarditis. All DCM patients were index patients. Patients received guideline-directed medical therapy titrated to the maximal tolerated dose and appropriate device therapy (implantable cardioverter-defibrillator [ICD] and cardiac resynchronization therapy-defibrillator implantation) according to the latest ESC guidelines.[14]

Cohort 2 (SID and DCM validation cohort) consisted of 21 and 24 patients with DCM and an SID derived from the Heart Muscle Disease Registry of Trieste Registry and from the Hospital Puerta de Hierro's Inherited Cardiac Diseases Unit in Madrid, respectively. Cohort 2 was used as a validation cohort to confirm the findings from cohort 1 to ensure the reliability and generalizability of the findings. Cohort 2 had similar inclusion and exclusion criteria as described for cohort 1. The 2 patient groups combined served as 1 validation cohort and are visualized separately in **Figure 1**.

Cohort 3 (healthy control subjects) consisted of 20,917 healthy individuals who underwent exome sequencing as part of a trio analysis for their children with intellectual disabilities at the MUMC and the Radboud University Medical Center in Nijmegen, the Netherlands. Exome sequencing data are available for

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 June 7, 2024; revised manuscript received August 5, 2024, accepted August 20, 2024.

anonymous use as a control cohort without the availability of any clinical information, including echocardiography data.

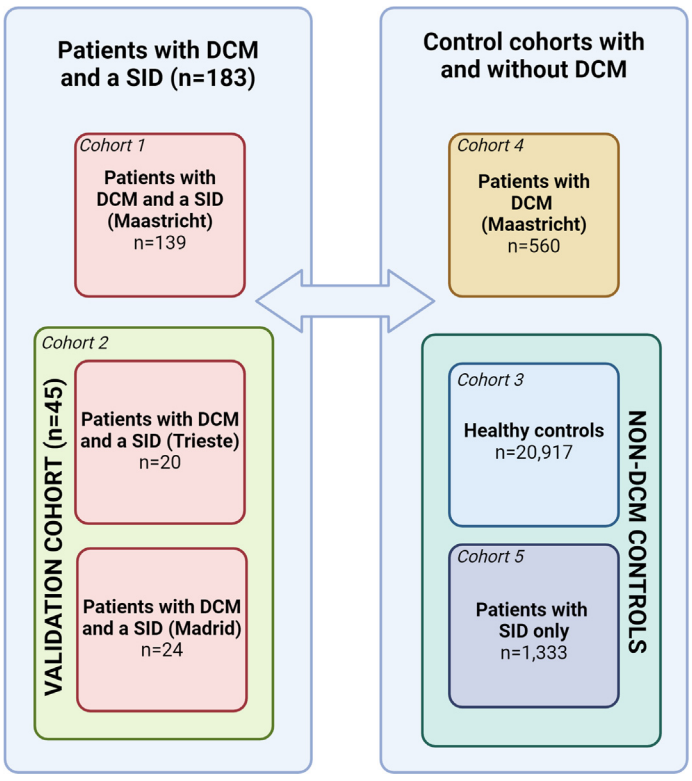
Cohort 4 (DCM only) consisted of 560 patients with DCM and no history of an SID, recruited from the Maastricht Cardiomyopathy Registry with similar inclusion and exclusion criteria as described for cohort 1. All DCM patients were index patients.

Cohort 5 (SID only) consisted of 1,333 patients who were suspected of having an SID and who were referred for exome sequencing to unveil any immunogenic etiology. Exome sequencing data of this cohort is available for anonymous use as a control cohort without the availability of any clinical information, including echocardiography data.

All participants in cohorts 1 (DCM and SID) and 4 (DCM only) provided written informed consent or were included if they died before informed consent was signed and did not object to the use of their medical data (opt-out approach), as approved by the Medical Ethical Committee of MUMC for inclusion in the Maastricht Cardiomyopathy Registry. Cohort 2 (DCM and SID validation cohort) provided written informed consent as approved by the local institutional review boards in Trieste and Madrid. Cohorts 3 (healthy control subjects) and 5 (SID only) gave informed consent that their exome sequencing can be used anonymously as a reference cohort. The study was performed according to the Declaration of Helsinki.

DISEASE CLASSIFICATION OF SIDs. SIDs encompass both autoinflammatory diseases and autoimmune diseases. An overview of the classification of different SIDs is presented in Supplemental Table 1. Autoinflammatory diseases constitute a growing family of conditions characterized by recurrent episodes of inflammation that occur spontaneously, without high autoantibody titers or the involvement of autoreactive T lymphocytes. This pattern reflects a primary dysfunction in the innate immune system. Examples of autoinflammatory SIDs include inflammatory bowel diseases, sarcoidosis, and gout. Autoimmune diseases are characterized by dysregulated responses of B cells, T cells, and dendritic cells. These responses lead to a loss of tolerance to self-antigens, resulting in predominantly cell-mediated or autoantibody-mediated immune responses. Examples of autoimmune SIDs include rheumatic arthritis, Sjögren syndrome, and systemic lupus erythematosus. Mixed SIDs refer to disorders where both autoimmune and autoinflammatory mechanisms contribute to the disease pathogenesis. These conditions exhibit features of both types of immune dysregulation, involving aberrant responses of both the innate and adaptive immune systems. Mixed SIDs include ankylosing spondylitis and reactive arthritis.

FIGURE 1 Overview of the Cohorts Included in This Study



The genetic results of the primary DCM and SID cohort from Maastricht (cohort 1; n = 139) was compared with 20,917 healthy individuals (cohort 3), 560 DCM-only patients (cohort 4), and 1,333 SID-only patients (cohort 5). A further 44 DCM and SID patients from Madrid and Trieste (cohort 2) were compared with the same 3 control cohorts. DCM = dilated cardiomyopathy; SID = systemic immune-mediated disease.

Medical records of all DCM patients were checked manually and by text mining of medical records (with the use of CTcue software). Patients were divided into groups of autoimmune, autoinflammatory, or mixed disease patterns based on the specific underlying disease as stated by the position statement of the ESC Working Group on Myocardial and Pericardial Disease (Supplemental Table 1).[2] SIDs were diagnosed through an extensive diagnostic work-up by the rheumatologist or immunologist at our tertiary referral center. This could include physical examination, the analysis of immune disease specific biomarkers (antinuclear antibodies, rheumatoid factor, and immunoglobulin levels), positron emission tomography-computed tomography or other imaging technique as appropriate, and obtaining a biopsy of

an organ suspected to be involved, eg, cardiac, renal, or skin biopsies. An overview of the diagnosed SIDs per patient is presented in Supplemental Table 2.

To exclude the presence of an SID, all included DCM patients had blood collection as part of the diagnostic work-up for DCM. These tests included assessments for soluble interleukin-2, neopterin, antinuclear antibodies, and antineutrophil cytoplasmic antibodies. Patients which were suspected of having an SID after this baseline screening were forwarded to the immunologist or rheumatologist.

ENDOMYOCARDIAL BIOPSY. Endomyocardial biopsies (EMBs) were taken from the right ventricular septum via the internal jugular vein with the use of a transcatheter bioptome (Cordis). Biopsies were collected as part of routine diagnostics for DCM to identify other triggers related to DCM, such as inflammation, storage diseases, and metabolic disorders. Two specimens were used for immunohistologic analysis on 4-μm-thick tissue sections from formalin-fixed paraffin-embedded EMBs as part of routine clinical care. Increased cardiac inflammation was defined as ≥14 CD45 cells/mm^2, including up to 4 CD68-infiltrating cells/mm^2 according to the latest ESC position statement.[15] We excluded patients with a significant viral load in their EMB from this study.

GENETIC TESTING AND VARIANT CLASSIFICATION. Participating individuals from cohorts 1 (DCM and SID), 2 (DCM and SID validation cohort), and 4 (DCM only) received genetic testing with the use of targeted next-generation sequencing panels. Although the next-generation sequencing panels could differ in the number of genes tested in specific cohorts, all panels included at least the robust DCM-associated genes as curated by the ClinGen consortium.[16] A family history of cardiac-related disease and sudden cardiac death was obtained from pedigree analysis. Familial inheritance was defined as recommended by the ESC: 1) 2 or more individuals (first- or second-degree relatives) have DCM-fulfilling diagnostic criteria for definite disease; or 2) the presence of an index patient fulfilling diagnostic criteria for DCM and a first-degree relative with autopsy-proven DCM and sudden death at <50 years of age.[17]

All variants were classified according to American College of Medical Genetics and Genomics guidelines.[18,19] Only patients with a likely pathogenic or pathogenic variant (class 4 or 5) in 1 or more robustly DCM-associated genes (*TTN*, *DSP*, *MYH7*, *LMNA*, *BAG3*, *TNNT2*, *TNNC1*, *PLN*, *ACTC1*, *NEXN*, *TPM1*, *VCL*, *FLNC*, *RBM20*, and *SCN5A*) were scored as genotype positive.[20] Variants of unknown significance in these genes were scored as genotype negative. An overview per patient of the pathogenic or likely pathogenic (P/LP) variants in the DCM cohorts is presented in Supplemental Table 3.

Participating individuals from cohorts 3 (healthy control subjects) and 5 (SID only) underwent whole-exome sequencing. The exome sequencing data was retrieved anonymously and analyzed for the presence of rare variants in robust DCM-associated genes, defined as an overall minor allele frequency <0.001. In all cohorts, all found variants were sorted on the basis of amino acid change and divided into 2 groups; all DCM nontruncating variants (DCM-ntv), and all DCM TVs (DCM-tv; nonsense, frameshift, or essential splice-site variants).

BASELINE EVALUATION OF DCM PATIENT COHORTS. Baseline evaluation was defined as the first outpatient clinic visit of a DCM patient in the medical center that enrolled the patient. All participants received routine clinical evaluation with medical history, including symptoms, genetic testing, electrocardiography, blood collection, and echocardiography. Echocardiographic measurements were performed in the standard parasternal, apical, and subxiphoidal views at baseline. Echocardiography observers were blinded to clinical and genetic parameters.

CLINICAL FOLLOW-UP AND OUTCOMES. Follow-up data on mortality, heart failure hospitalization, and ventricular tachycardia/fibrillation (VT/VF) were collected from patients' medical records, municipal population registries, and telephone contact with general practitioners. VT/VF was defined as nonfatal ventricular fibrillation (with or without ICD shock), hemodynamic unstable sustained VT, or sustained VT with appropriate ICD shock. The combined endpoint was the combination of all-cause mortality, heart failure hospitalization, and VT/VF.

STATISTICAL ANALYSIS. Groups were compared by means of Student's *t*-test or 1-way analysis of variance. Noncontiguous categoric variables were expressed as n (%) and compared with the use of Fisher test as appropriate. Although variants in many genes were tested, we performed a prespecified focused analysis on protein-altering variants in 15 robust DCM-associated genes, as specified earlier.[20] We prespecified 6 comparisons of association tests: cohort 1 (Maastricht DCM-SID patients) vs cohorts 3 (healthy control subjects), 4 (DCM patients), and 5 (SID-only patients), and cohort 2 (Madrid/Trieste

TABLE 1 Baseline Demographics of the Maastricht (Cohort 1) and Trieste and Madrid (Cohort 2) Groups of Patients With DCM and a SID Compared With a DCM Cohort Without SID

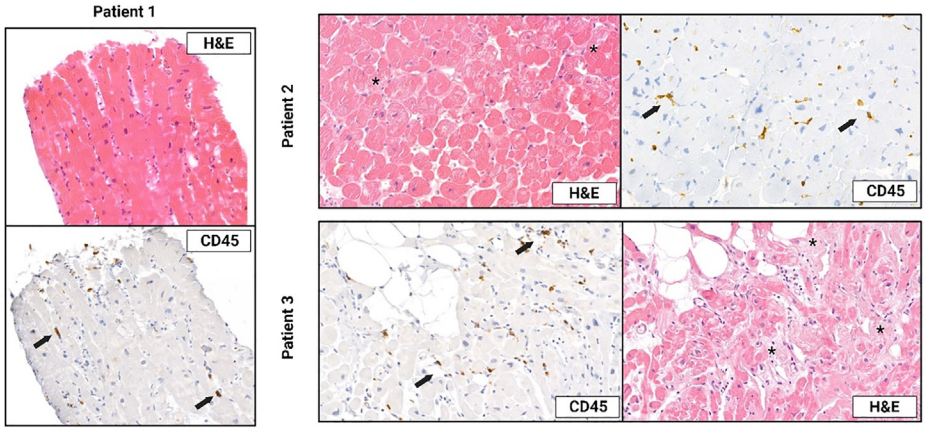
		Index Cohort (Cohort 1)	Validation Cohort (Cohort 2)	
	DCM Without SID (n = 560)	DCM-SID Maastricht (n = 139)	DCM-SID Trieste (n = 20)	DCM-SID Madrid (n = 24)
Age at presentation, y	57 (50-65)	58 (49-65)	57 (37-62)	55 (47-62)
Men	361 (64)	91 (65)	10 (50)	20 (83)
LVEF, %	25 (19-36)	38 (27-47)	33 (25-39)	30 (20-36)
Classification of SID[2]				
Autoimmune disease	–	51 (36)	13 (65)	8 (33)
Mixed pattern disease	–	32 (23)	1 (4)	1 (4)
Autoinflammatory disease	–	43 (31)	5 (25)	15 (63)
Both autoimmune and autoinflammatory disease	–	8 (6)	0 (0)	0 (0)
Both autoimmune disease and mixed pattern	–	3 (2)	1 (4)	0 (0)
Both autoinflammatory disease and mixed pattern	–	1 (1)	0 (0)	0 (0)

Values are median (Q1-Q3) or n (%).
DCM = dilated cardiomyopathy; LVEF = left ventricular ejection fraction; SID = systemic immune-mediated disease.

DCM-SID patients) vs subsequently cohorts 3, 4, and 5.

The Kaplan-Meier method was used to compute the survival curves (comparison between groups by log-rank test) using a combined patient group from all DCM-SID patients from Maastricht, Madrid, and Trieste combined (cohorts 1 and 2). Univariable Cox proportional hazards regression analysis was performed to assess the association of genetic status with event-free survival, followed by multivariable Cox proportional hazards regression analysis when appropriate. HRs were adjusted for age, sex, and baseline LVEF. Time-to-event analysis was performed for the combined endpoint, including all-

FIGURE 2 Detailed (Clinical) Description of Examples of Patients With DCM and a SID With an Identified Pathogenic or Likely Pathogenic Variant



	Demographics			Genotype and immunology			Phenotype						
	Sex	Age	SID	Gene	Variant	Troponin-T	NT-proBNP	LVEF (%)	CMR LGE	EMB fibrosis	EMB CD3+	EMB CD45+	EMB CD68+
P1	M	53	Sjögren/SLE	*TTN*	*p.Asp15574**	26	*2609*	20	No focal fibrosis	*Minimal*	4.9/mm²	9.2/mm²	6.1/mm²
P2	M	44	Gout	*LMNA*	*p.Glu161Lys*	46	*1647*	41	Basal, midseptal, and inferolateral	*Interstitial*	17.7/mm²	28.8/mm²	2.2/mm²
P3	M	26	Psoriasis	*BAG3*	*p.Gln304**	18	*92*	15	No focal fibrosis	*Focal patches*	8.3/mm²	12.6/mm²	8.6/mm²

Histopathology images are presented for the patients, showing hematoxylin-eosin (H&E) and pan-leukocyte (CD45) stainings. Arrows indicate places of cell infiltration; asterisks depict areas of cardiac fibrosis. CMR = cardiac magnetic resonance; EMB = endomyocardial biopsy; LVEF = left ventricular ejection fraction; LGE = late gadolinium enhancement; NT-proBNP = N-terminal pro-B-type natriuretic peptide; other abbreviations as in Figure 1.

TABLE 2 ORs of the Excess of Rare Genetic Variation in Cases With DCM With or Without a SID Compared With a Cohort of Healthy Individuals

	Healthy Control (Cohort 3) (n = 20,917)		DCM-SID Maastricht (Cohort 1) (n = 139)				DCM-SID Madrid/Trieste (Cohort 2) (n = 44)			
	n	%	n	%	OR (95% CI)	*P* Value	n	%	OR (95% CI)	*P* Value
DCM-ntv	297	1.4	7	5	3.7 (1.7-7.5)	0.004	2	4.5	3.3 (0.8-12.0)	0.13
DCM-tv	102	0.2	15	10.7	24.5 (13.9-43.3)	<0.001	7	16	116.6 (59.9-215.9)	<0.001
TTNtv	64	0.3	11	7.9	27.8 (14.6-53.6)	<0.001	5	11.4	41.8 (17.3-103)	<0.001
Total number of variants	399	1.9	24	17.1	9.6 (6.0-15.2)	<0.001	9	20.5	13.2 (6.4-26.9)	<0.001

Fisher test 2-sided level of significance = 0.05; with Bonferroni correction for 4 tests = 0.0125.
ntv = nontruncating variant; TTNtv = truncating TTN variant; tv = truncating variant; other abbreviations as in Table 1.

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cause mortality, heart failure hospitalization, and life-threatening arrhythmias. The proportional hazards assumption was tested using Schoenfeld residuals, with plots and a global test to assess overall validity. The goodness-of-fit of the model was evaluated by means of Cox-Snell residuals, ensuring that the assumptions underlying the Cox proportional hazards model were adequately verified. For the LVEF analysis, a linear regression model was used to compare the median LVEF between patients with SID and those in the reference group. The prespecified main analysis assessed the significance of an interaction term between TTNtv and SID in a multivariable model predicting LVEF to determine whether TTNtv and SID in combination have any additional effect beyond the effects of TTNtv and SID separately. Assumptions of linearity, homoscedasticity, and normality of residuals were verified with the use of diagnostic plots, including residual vs fitted values in Q-Q plots. Statistical significance was accepted at the 95% CI ($P \leq 0.05$). Statistical analysis was performed with the use of SPSS 23.0 (IBM Corp) and Prism GraphPad 9.0.2.

RESULTS

COHORT REVIEW. In the Maastricht DCM-SID cohort (cohort 1), the median age at presentation was 58 years (Q1-Q3: 49-65 years) and 64% were men (Table 1). The median LVEF was 38% (Q1-Q3: 25%-47%). All patients were evaluated by an immunologist and classified according to the latest ESC position statement: 51 had an autoimmune disease (36%), 43 had an autoinflammatory disease (31%), and 32 had a mixed disease pattern (23%). Eight patients had both an autoimmune disease and an autoinflammatory disease (6%), 3 patients had both an autoimmune disease and a mixed pattern disease (2%), and 1 patient had both an autoinflammatory disease and a mixed pattern disease (1%).

In the DCM-SID validation cohorts of Madrid and Trieste (cohort 2), the median age at presentation was 56 years (Q1-Q3: 47-62 years), 67% were men, and the median LVEF was 30% (Q1-Q3: 22%-40%). Twenty-one had an autoimmune disease (48%), 20 had an autoinflammatory disease (45%), and 2 had a mixed disease pattern (5%).

ASSOCIATION OF SID AND DCM. Of the DCM-SID patients from the Maastricht cohort (cohort 1), 75% had an SID diagnosis before the diagnosis of DCM and 26% were diagnosed with an SID during the diagnostic work-up for DCM. The median time between DCM diagnosis and SID diagnosis/event was −0.39 years (Q1-Q3: −5.7 to +0.2 years), showing that the immune event was generally 4 to 5 months before the diagnosis of DCM.

In the subgroup of DCM-SID patients from the Maastricht cohort (cohort 1) for whom an EMB was obtained as part of routine clinical care (n = 467), those with an SID more often had significant cardiac inflammation (23/112 [21%] vs 42/355 [12%]; $P = 0.02$) and often had patches of fibrosis (Figure 2). In contrast to the findings in the cardiac tissue, there were only minor to no findings on cardiac magnetic resonance.

GENETIC CONTRIBUTION TO SID-ASSOCIATED DCM. To investigate the potential genetic contribution of cardiomyopathy genes in patients with DCM and an SID, we examined cases for the presence of P/LP variants in robust genes associated with DCM. We identified 23 distinct variants involving 6 different genes in DCM-SID patients from the Maastricht cohort (cohort 1). The prevalence of variants in the DCM-SID group was significantly higher than in the healthy control population (17.1% vs 1.9%; OR: 9.6 [95% CI:

TABLE 2 Continued

DCM Without SID (Cohort 4) (n = 560)				SID Without DCM (Cohort 5) (n = 1,333)			
n	%	OR (95% CI)	*P* Value	n	%	OR (95% CI)	*P* Value
29	5.2	3.8 (2.6-5.7)	<0.001	0	0	0.0 (0.0-0.2)	<0.001
64	11.4	26.5 (19.1-36.6)	<0.001	0	0	0.0 (0.0-0.5)	0.003
52	9.3	33.4 (23.0-48.6)	<0.001	0	0	0.0 (0.0-0.9)	0.03
93	16.6	10.3 (8.1-13.1)	<0.001	0	0	0.0 (0.0-0.1)	<0.001

6.0-15.2]; $P < 0.001$) (Table 2). In the Madrid/Trieste DCM and SID validation cohort (cohort 2), we identified 9 distinct variants involving 4 different genes (22.2%). In line with the Maastricht DCM-SID cohort, there was a significant excess of P/LP variants compared with the control population (20.5% vs 1.9%; OR: 13.2 [95% CI: 6.4-26.9]; $P < 0.001$). Interestingly, in the cohort of SID-only patients (cohort 5), no rare TVs or NTVs were identified in any of the 15 DCM-associated genes (0% vs 1.9%; OR: 0.0 [95% CI: 0.0-0.1]; $P < 0.001$).

Among patients with immune-mediated SID, 14% were genotype positive, compared with 22% of those with inflammatory-mediated SID and 18% with mixed-pattern SID. The most common immune-mediated SIDs were rheumatoid arthritis, antibody-associated vasculitis, and systemic lupus, with 8%, 11%, and 30% of patients, respectively, having a P/LP in a DCM-associated gene. The most common inflammatory-mediated SIDs were gout, inflammatory bowel disease, rheumatic polymyalgia, and sarcoidosis, with 22%, 11%, 25%, and 25% of patients, respectively, having a P/LP in a DCM-associated gene. Psoriasis was the most common mixed-pattern SID, with 19% of patients having a P/LP in a DCM-associated gene. An overview of the prevalence per SID in DCM patients with and without a P/LP is presented in Supplemental Table 4. In addition, Supplemental Table 5 presents the prevalence of SIDs in men and women with DCM and a P/LP.

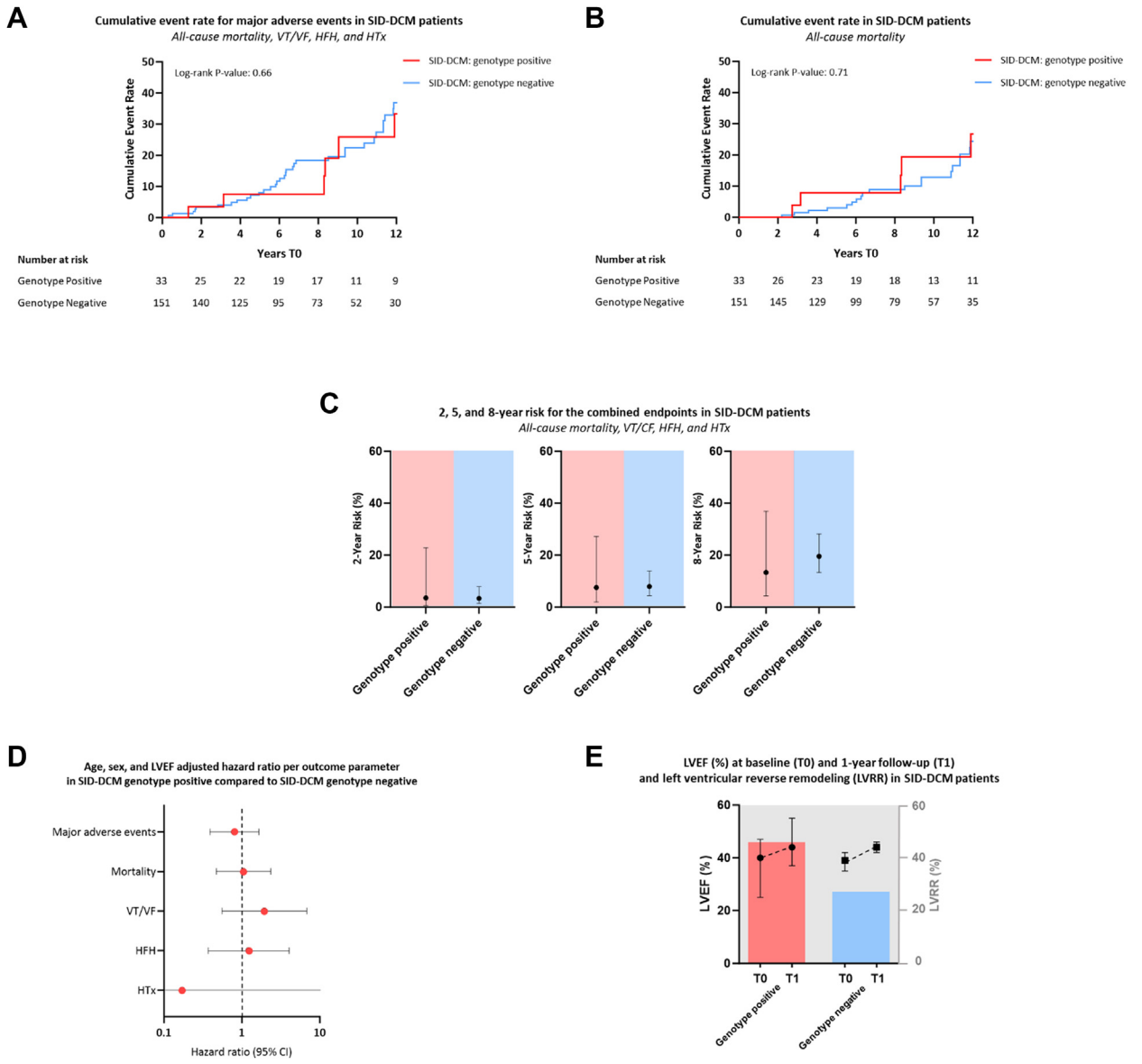
A family history of DCM was identified in 17% of DCM-SID patients. Of the genotype-positive DCM-SID patients, 35% had a familial history of DCM, compared with 27% in the DCM only group ($P = 0.60$) The notable presence of familial DCM in DCM-SID patients underscores the importance of considering genetic testing and family screening in this subgroup, even after an etiology has been identified.

GENE-BASED BURDEN IN PATIENTS WITH DCM AND SID. Overall, TVs in DCM-associated genes showed the strongest enrichment, in both the Maastricht DCM-SID (cohort 1; 10.7% vs 0.2%; OR: 24.5 [95% CI: 13.9-43.3]) and the Madrid/Trieste DCM-SID cohort (cohort 2; 16% vs 0.2%; OR: 116.6 [95% CI: 59.9-215.9]) compared with the healthy control cohort (cohort 3; both $P < 0.001$) (Table 2). In the Maastricht cohort (cohort 1), 11 TTNtvs were identified in 11 unrelated DCM-SID patients (7.9% prevalence), compared with 64 in the healthy control cohort (0.3% prevalence; OR: 27.8 [95% CI: 14.6-53.6]; $P < 0.001$), and 5 TTNtvs in 5 unrelated DCM-SID patients (11.4% prevalence) in the Madrid/Trieste DCM-SID cohort (OR: 41.8 [95% CI: 17.3-103]; $P < 0.001$). In the Maastricht DCM and SID cohort, other detected TVs were also enriched in DCM-SID patients in aggregate (*BAG3*, *FLNC*, and *RBM20*; $P < 0.001$).

Nontruncating variants (NTVs) also were significantly enriched in the DCM-SID cohort compared with the healthy control cohort (in the Maastricht DCM-SID cohort: 5.0% vs 1.4%; OR: 3.7 [95% CI: 1.7-7.5]; $P = 0.004$; but not in the Madrid/Trieste DCM-SID cohort) (Table 2). The genes with the highest prevalence of NTVs were *MYH7*, *LMNA*, and *TNNT2*. In total, there were, respectively, 4, 4, and 3 variants in 11 unrelated patients in the 2 DCM-SID cohorts (prevalences of 2.1%, 2.1%, and 1.6%), compared with 36, 25, and 5 variants in the healthy control cohort (prevalences of 0.2%, 0.02%, and 0.1%; all $P < 0.001$). Other genes carrying NTVs were limited to *TPM1* and *RBM20*.

CLINICAL OUTCOMES. Over a median follow-up of 8.4 years (Q1-Q3: 4.9-12.1 years), there were 56 major adverse events (30%) in the combined DCM-SID cohorts (cohorts 1 and 2), of which 41 were mortality. Seventeen patients were hospitalized with heart failure, 12 patients experienced a VT/VF, and 2 patients underwent heart transplantation. Overall, the outcome for DCM-SID patients with or without a P/LP variant was similar (log rank $P = 0.66$ for major adverse events; log rank $P = 0.71$ for all-cause mortality) (Figures 3A to 3C). No differences were found in

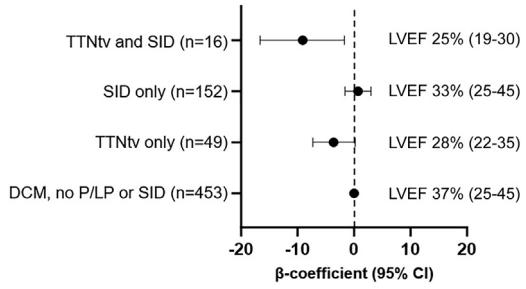
FIGURE 3 Clinical Outcomes of Patients With DCM and a SID Stratified by the Presence of a P/LP Variant



(A) Cumulative event rate for major adverse events during follow-up in the combined SID-DCM cohort (cohorts 1 and 2), stratified by the presence of a P/LP variant. Major adverse events include all-cause mortality, VT/VF, HFH, and HTx. Median duration of follow-up was 8.6 years (Q1-Q3: 5.3-11.8 years) in Maastricht DCM-SID patients and 5.9 years (Q1-Q3: 2.1-14.6 years) in Madrid/Trieste SID-DCM patients. (B) Cumulative event rate for all-cause mortality during follow-up in the combined SID-DCM cohort (cohorts 1 and 2), stratified by the presence of a P/LP variant. (C) 2-, 5-, and 8-year risk for major adverse events in the combined SID-DCM cohort (cohorts 1 and 2) of DCM-SID patients, stratified by the presence of a P/LP variant. (D) Age, sex, and baseline LVEF–adjusted HR for major adverse events in the combined SID-DCM cohort (cohorts 1 and 2), stratified by the presence of a P/LP variant. (E) LVEF at baseline (T0) and 1-year follow-up (T1) in SID-DCM patients and the percentage of patients that showed left ventricular reverse remodeling (LVRR) at 1-year follow-up in SID-DCM patients, stratified by the presence of a P/LP variant. HFH = heart failure hospitalization; HTx = heart transplantation; LVEF = left ventricular ejection fraction; P/LP = pathogenic or likely pathogenic; VF = ventricular fibrillation; VT = ventricular tachycardia; other abbreviations as in Figure 1.

the age, sex, and baseline LVEF adjusted HRs for major adverse events, mortality, VT/VF, heart failure hospitalization, and heart transplantation between DCM-SID patients with and without a P/LP variant (Figure 3D). In addition, there was no significant differences in left ventricular reverse remodeling between DCM-SID patients with and without a P/LP variant (Figure 3E).

FIGURE 4 Effect of TTNtv and SIDs on LVEF



Regression coefficients and 95% CIs from the linear regression model evaluating the effects of TTNtv and SIDs on baseline LVEF. TTNtv = truncating TTN variant; other abbreviations as in Figures 1 and 3.

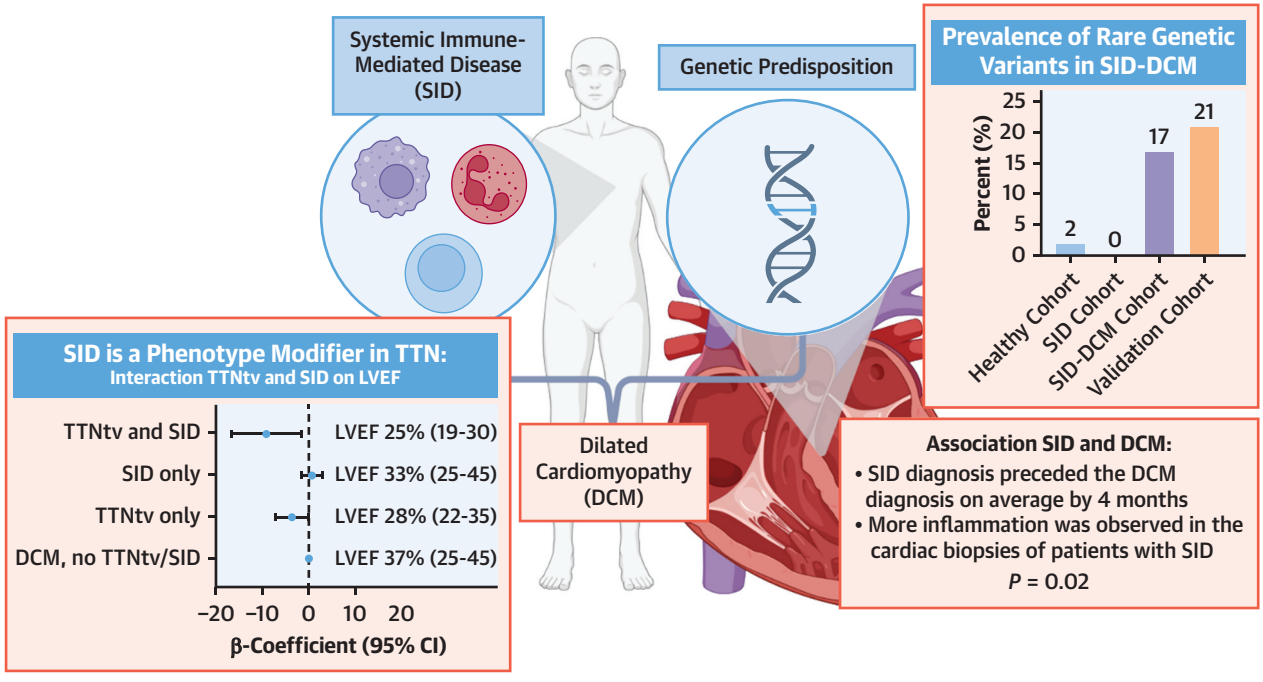
SID AS A PHENOTYPIC MODIFIER IN DCM. Because TTNtv was the most frequent type of DCM-causing genetic variants found in DCM-SID, we investigated the interaction of TTNtv with cardiac phenotype in DCM-SID from the Maastricht, Madrid, and Trieste cohorts combined (cohorts 1 and 2) (Figure 4). The median LVEF at baseline was 28% in DCM patients with a TTNtv only, which was not significantly lower than those DCM patients without a TTNtv (P = 0.06). In the multivariable analysis, only patients with both TTNtv and an SID (n = 16) had a statistically significantly lower LVEF ($P_{TTNtv \times SID}$ = 0.016; β = −9.1% [95% CI: −16.6% to −1.7%]), showing a biological interaction between TTNtv and SID on the absolute baseline LVEF reduction.

DISCUSSION

One in 6 patients with an SID and a DCM have a P/LP variant in a cardiomyopathy-associated gene, which is significantly higher than in healthy control subjects and patients with a suspected immune disorder without myocardial involvement (Central Illustration). Therefore, testing for P/LP variants in DCM patients with an SID is crucial, as is ensuring adequate treatment and follow-up for both the DCM and the SID.

SID AS A SECOND HIT IN GENETICALLY PREDISPOSED HEARTS. DCM is increasingly acknowledged as a final phenotype caused by genetic and environmental

CENTRAL ILLUSTRATION Genetic Landscape of Patients With DCM and a SID



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DCM = dilated cardiomyopathy; LVEF = left ventricular ejection fraction; SID = systemic immune-mediated disease; TTNtv = truncating *TTN* variant.

disease triggers.[21] Previous landmark studies have shown that specific environmental factors are present in combination with genetic variants within DCM populations of chemotherapy,[8] alcohol,[7] pregnancy,[9] and myocarditis.[22] The present study is the first showing the prevalence of genetic variants in an etiology-characterized cohort of patients with DCM and an SID. One in 6 DCM-SID patients had a genetic predisposition to DCM, which is significantly higher than in control cohorts. It is common that genetic testing is not performed in patients with an SID who develop DCM, because a nonischemic etiology has already been identified. Our study highlights that genetic testing is still important, because the genetic yield is similar to patients with DCM associated with other etiologies. The identification of a P/LP gene variant is crucial not only for the individual patient (eg, treatment implications for specific genotypes [*LMNA*, *FLNC*, *DSP*]), but also for the family, because the identification of a P/LP gene variant will allow cascade screening to identify patients at risk for developing DCM.

IMMUNE DISEASES AND DCM. The cardiovascular system and particularly the myocardium are often critical targets in SIDs, even in asymptomatic patients.[2] For this reason, the working group on myocardial and pericardial diseases of the ESC provided a first orientation for an appropriate multidisciplinary diagnostic work-up in SID-related myocardial disease. The present study is the largest to date demonstrating a potential genetic predisposition for myocardial involvement in DCM patients with an underlying SID. We also identified an interaction between TTNtv and SID in the context of baseline LVEF: Patients with a TTNtv and SID have a decreased LVEF at presentation compared with those with only a TTNtv. These 2 lines of evidence both support a model whereby an immune disease interacts with the cardiac genotype, contributing to myocardial involvement that can affect the disease onset and severity. The same interaction was previously shown in those TTNtv carriers with excess alcohol consumption[7] and for TTNtv with chemotherapy,[8] thereby providing important evidence for gene-environment interactions in DCM.

Immune-mediated diseases are a well described etiology for DCM through different pathomechanisms that differ among SIDs.[2,4,14,23] They most often lead to myocarditis followed by left ventricular dysfunction.[24,25] We also noted more chronic cardiac inflammation in the cohort of DCM-SID patients compared with DCM-only patients, which could imply myocardial involvement in chronic systemic immune-mediated inflammation. Recently, the genetic architecture of myocarditis was shown to have a major overlap with inherited cardiomyopathies,[22] further strengthening the genetic predisposition for DCM in those with immune-related involvement. Further (preclinical) studies should determine if the underlying genetic defect renders the heart more susceptible to myocardial involvement in SID or if it prevents the heart from remodeling after an inflammatory trigger. Interestingly, the presence of antiheart autoantibodies is an independent predictor of DCM development in healthy relatives of DCM patients.[26] These autoantibodies were also found in probands with arrhythmogenic right ventricular cardiomyopathy and their affected relatives. This emphasizes an important role of autoimmunity in genetic forms of DCM.[27]

In the present study, we have shown a predominance of men with DCM and SID even though many SIDs are more predominant in women. Several factors could contribute to this observation. First, it is possible that women are underrepresented in DCM screening because of differing clinical approaches or health-seeking behaviors between genders.[28] Second, we and others observed that for almost all DCM-associated genes the disease penetrance is higher in men than in women who carry a P/LP variant.[11,29,30] Our findings indicate that women, despite having a higher prevalence of SIDs, may have greater protection from DCM development, highlighting the need for further research into these protective factors.

That SID diagnosis precedes DCM and the observed inflammation in the myocardium of DCM patients with SID indicates that SID may contribute to changes in a myocardium already predisposed by a genetic variant. Although this evidence is associative rather than causal, it suggests that while genetic predisposition is a primary driver, SID may trigger adverse myocardial changes affecting phenotypic expression of the genetic variant and disease severity. In this study, we identified illustrative examples where we show an inflammatory component in the heart that contributes to the observed cardiac dysfunction in addition to the identified genetic defect. The exact molecular mechanisms underlying cardiac involvement in SID remain unknown, and this study explored only some of the genetic factors that may influence susceptibility to myocardial involvement and the development of DCM in those patients with an SID.

CLINICAL IMPLICATIONS. Most guidelines recommend genetic testing in DCM patients only when other etiologies have been excluded or if specific clinical clues are present.[31,32] The data presented in this article show that patients with DCM and an SID should still be considered for genetic testing, to identify family members at risk for developing DCM. Although the genetic background of immune diseases is polygenic, it is not uncommon that immune diseases also have a familial inheritance pattern.[33] The early recognition of a P/LP variant in a cardiomyopathy-associated gene can be of additional value for those relatives who also have an immune disease. Although with high variability depending on the affected gene, the finding of a P/LP variant in a cardiomyopathy-associated gene as a secondary finding in an individual without DCM or DCM in the family usually has no large clinical consequences, because the penetrance of such a variant is estimated to be very low.[34] However, if the individual has an SID, more strenuous cardiac follow-up could be considered because they might have a higher chance for myocardial involvement. The impact of guideline-directed medical therapy on survival and adverse outcomes in patients with genetic cardiomyopathy or SID is not well defined, and what may appear as "recovery" in these patients might actually be "remission." It would be suitable to implement an even more rigorous follow-up protocol for individuals with DCM carrying a P/LP variant and SID compared with those who do not have a P/LP variant. Greater and closer collaboration with specialists in rheumatology and immunology can be highly beneficial in providing more comprehensive and effective care for DCM-SID patients. Future studies will have to determine if early recognition of patients with an SID and a high risk of myocardial involvement through genetic testing will benefit from the optimization of immune and DCM therapy.

The presence of a P/LP variant in a patient with DCM and an SID did not influence the clinical outcome of the patient, supporting the contention that genetics play a primary role in these patients. This is in contrast to the general DCM population where patients with a P/LP variant have a worse outcome.[5,35,36] Survival and clinical progression can be significantly influenced by guideline-directed medical therapy, whose impact in an DCM-SID patient cohort remains uncertain. Effective treatment of SID, potentially resulting in remission, could limit its impact on cardiac inflammation over time, explaining the similar long-term outcomes observed in both groups. Future studies should focus on the clinical course and outcome of patients with DCM due to the large variety of SID subtypes and genotypes and study the effects of DCM and SID therapy.

STUDY LIMITATIONS. The recognition and classification of SID in patients with a cardiomyopathy are not fully incorporated into routine diagnostic care, thereby representing a certain selection bias in our population. Second, genetic testing was conducted at different laboratories for the 5 cohorts, which may introduce variability in the results. The different cohorts introduce a potential confounder, which should be considered when interpreting our findings. Third, almost all subjects included in this study are of European descent. Consequently, our findings may not be applicable to the effects of SID and DCM on other racial groups, because of their underrepresentation in our cohorts. Fourth, we do not have any clinical information from the control cohorts that we used, therefore we cannot exclude a potential cardiac phenotype in those "healthy" individuals where a rare variant in a cardiomyopathy-associated gene was detected. However, this is common to many genetic studies, and our control cohort was large enough to minimize the potential effect of individuals with a phenotype among the control subjects. Fifth, our study focused on the prevalence of P/LP variants in DCM-associated genes in well phenotyped patients with DCM and an SID. It is important to note that the lack of detailed data on previous SID-specific immunomodulatory treatments (eg, steroid use) may limit the interpretability of the biopsy results in our subanalysis (eg, patients might have had steroids before cardiac biopsy, leading to absence of cardiac inflammation). In addition, patients in the DCM control cohort, though not found to have pathogenic variants by gene panel testing, may still possess a polygenic background. Finally, we did not have access to cardiac biopsies in all patients with DCM and an SID and could therefore not quantify inflammation in all patients. Although we included only patients with an SID that is associated with myocardial involvement according to the latest position statement of the ESC,[2] not all DCM-SID had significant inflammation in their biopsy, potentially showing lead time bias or sampling bias of the EMB, because some patients may have been under prolonged immunosuppressive treatment.

CONCLUSIONS

One in 6 DCM patients with an SID had an underlying P/LP variant in a DCM-associated gene, supporting the concept that autoimmunity may play a pivotal role in revealing a DCM phenotype in genotype-positive individuals. Long-term and larger cohort studies are needed to determine if genetic testing can help guide the intensity of clinical monitoring in DCM patients with an SID, especially those with persistent inflammation, investigate the response to guideline-directed medical therapy in DCM-SID patients, and predict longer-term event-free survival.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

This work was supported by the Netherlands Cardiovascular Research Initiative, an initiative with support of the Dutch Heart Foundation (Dutch Cardiovascular Alliance Double Doses, 2020-B005). Dr Verdonschot was supported by a Dekker Clinical Scientist grant from the Dutch Heart Foundation. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

ADDRESS FOR CORRESPONDENCE: Dr Job A.J. Verdonschot, Maastricht University Medical Center P. Debyelaan 25, Maastricht, Limburg 0, the Netherlands. E-mail: job.verdonschot@mumc.nl.

PERSPECTIVES

COMPETENCY IN PATIENT CARE AND PROCEDURAL SKILLS: Autoimmunity may play a role in unveiling a DCM phenotype in individuals who are genotype positive. Therefore, patients with DCM and an SID should be considered for genetic testing not only for individual treatment implications but also to identify family members at risk for developing DCM.

COMPETENCY IN MEDICAL KNOWLEDGE: Patients with DCM, an immune disease, and a truncating *TTN* variant presented with a lower LVEF, implying an interaction of factors on cardiac function. Genotype-positive DCM patients with an SID require closer monitoring. Collaborating with rheumatology and immunology experts can enhance care for DCM-SID patients.

TRANSLATIONAL OUTLOOK: Early identification of patients with an SID and pathogenic DCM-associated variant might provide an opportunity to optimize therapy in order to prevent the development of DCM.

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KEY WORDS autoimmunity, dilated cardiomyopathy, genetics, heart failure, systemic immune-mediated disease, titin

APPENDIX For supplemental tables, please see the online version of this paper.