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ORIGINAL ARTICLE

Implications of Genetic Testing in Dilated Cardiomyopathy

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BACKGROUND: Genetic analysis is a first-tier test in dilated cardiomyopathy (DCM). Electrical phenotypes are common in genetic DCM, but their exact contribution to the clinical course and outcome is unknown. We determined the prevalence of pathogenic gene variants in a large unselected DCM population and determined the role of electrical phenotypes in association with outcome.

METHODS: This study included 689 patients with DCM from the Maastricht Cardiomyopathy Registry, undergoing genetic evaluation using a 48 cardiomyopathy-associated gene-panel, echocardiography, endomyocardial biopsies, and Holter monitoring. Upon detection of a pathogenic variant in a patient with DCM, familial segregation was performed. Outcome was defined as cardiovascular death, heart transplantation, heart failure hospitalization, and/or occurrence of life-threatening arrhythmias.

RESULTS: A (likely) pathogenic gene variant was found in 19% of patients, varying from 36% in familial to 13% in nonfamilial DCM. Family segregation analysis showed familial disease in 46% of patients with DCM who were initially deemed nonfamilial by history. Overall, 18% of patients with a nongenetic risk factor had a pathogenic gene variant. Almost all pathogenic gene variants occurred in just 12 genes previously shown to have robust disease association with DCM. Genetic DCM was independently associated with electrical phenotypes such as atrial fibrillation, nonsustained ventricular tachycardia, and atrioventricular block and inversely correlated with the presence of a left bundle branch block ($P<0.01$). After a median follow-up of 4 years, event-free survival was reduced in genetic versus patients with nongenetic DCM ($P=0.01$). This effect on outcome was mediated by the associated electrical phenotypes of genetic DCM ($P<0.001$).

CONCLUSIONS: One in 5 patients with an established nongenetic risk factor or a nonfamilial disease still carries a pathogenic gene variant. Genetic DCM is characterized by a profile of electrical phenotypes (atrial fibrillation, nonsustained ventricular tachycardia, and atrioventricular block), which carries increased risk for adverse outcomes. Based on these findings, we envisage a broader role for genetic testing in DCM.

Key Words: arrhythmia ■ cardiomyopathy, dilated ■ heart failure ■ phenotype ■ prevalence

Dilated cardiomyopathy (DCM) is often inherited, and >60 genes have been associated with it in various studies.[1] Testing by gene panels is generally accepted in patients with familial DCM, in the absence of an environmental cause.[2,3] In patients with familial DCM, the genetic yield can be as high as 55%.[4] Focusing genetic testing on familial cases only is likely too restrictive. In fact, the yield of genetic testing in nonfamilial DCM varies from 11% to 26% in different studies.[5,6] Also, genetic and nongenetic risk factors are not mutually exclusive, and the extent to which these factors interact in DCM pathogenesis is not fully known. There is accumulating evidence for a more interactive model in which genetic variants increase susceptibility for nongenetic factors to trigger the phenotype.[7,8] For example, genetic variants are prevalent in patients with DCM with specific triggers such as

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Nonstandard Abbreviations and Acronyms

AF	atrial fibrillation
AVB	atrioventricular block
DCM	dilated cardiomyopathy
HCM	hypertrophic cardiomyopathy
LBBB	left bundle branch block
LTA	life-threatening arrhythmia
NSVT	non-sustained ventricular tachycardia
TTNtv	truncating titin variant
VUS	variant of unknown significance

excess alcohol consumption, myocarditis, or cardiotoxic chemotherapy.[9–11] To accommodate such interactions between genetic and nongenetic risk factors, the World Heart Federation developed a new classification scheme, called MOGE(S), which quantifies disease modifiers in relation to the genetic background.[12] Electrical phenotypes are electrical disturbances that could be either cause or consequence of DCM. Atrial fibrillation (AF) and nonsustained ventricular tachycardia (NSVT) are examples of electrical phenotypes, which are often associated with specific gene variants.[13,14] Despite the association of these electrical phenotypes with both genetic variants and life-threatening arrhythmias and their clinical implications, these phenotypes are not considered in recent classification and definitions of cardiomyopathies.[2,8,12] It remains to be determined whether the electrical phenotypes mediate the clinical course of (genetic) DCM.

This study aimed (1) to determine the genetic yield in a large, well-phenotyped DCM cohort (both familial and nonfamilial; acquired and idiopathic) using a panel of 48 genes and (2) to determine the electrical phenotype landscape in genetic and nongenetic DCM at the time of diagnosis, and their subsequent role in the clinical course of DCM.

METHODS

Please see the Data Supplement for complete methods. All detailed information about gene variants and corresponding classification, definitions, and outcome are provided in the Data Supplement. The study was performed according to the declaration of Helsinki and was approved by the institutional Medical Ethics Committee. All patients gave written informed consent. The data that support the findings of this study are available from the corresponding author on reasonable request.

RESULTS

Genetics of DCM

Comprehensive genetic testing allowed us to categorize 689 patients with DCM in 3 groups: 129 carried a pathogenic gene variant (29%), 111 were familial but without an evident genetic cause (16%), and 449 remained negative after careful family history and testing of the 48-gene panel (65%; Table 1; Figure I in the Data Supplement). Truncating *TTN* variants (TTNtv) were most prevalent (n=67/689; 9.7%), followed by *LMNA* (n=21/689; 3%), truncating *FLNC* variants (FLNCtv; n=3/172; 1.7%), and *MYH7* (n=78/689; 1%; Figure 1; Tables II and III in the Data Supplement). The overwhelming majority of the 132 detected pathogenic gene variants (89%; n=118) occurred in 12 genes that have previously been shown to have robust evidence for DCM disease association.[15] We found pathogenic gene variants in *RBM20* (n=5) and *FLNC* (n=3), both 2 genes that were not evaluated by previous mentioned study but carry convincing evidence for DCM pathogenicity. In addition, variants in *TTR* (n=2), *EMD* (n=1), *MYL2* (n=1, homozygous), and *SCN5A* (n=1) were present in a small number of patients and their pathogenicity was supported by additional clinical and/or functional evidence (Table III in the Data Supplement).

As expected, pathogenic variants were more frequent in familial than in nonfamilial DCM (36% versus 13%, respectively; $P<0.001$). The number of variants of unknown significant (VUSs) was comparable between familial and nonfamilial patients with DCM (38% versus 32%; $P=0.16$; Tables II and IV in the Data Supplement). Although our series is relatively large, no pathogenic variant was detected in 65% (31/48) of the genes on our diagnostic panel. Fifty-four percent (26/48) of the genes on the panel yielded only VUSs (Figure 1).

Genetic Yield in DCM With Nongenetic Risk Factors

Thirty-three percent (228/689) of the patients had at least one nongenetic risk factor that could have contributed to the phenotype (Figure 2). In these patients with DCM with a nongenetic risk factor, a pathogenic gene variant was found in 18% (41/228) (Figure 3A). This is comparable to patients without such a nongenetic risk factor (19%; 88/461; $P=0.73$). Pathogenic gene variants were detected in all subgroups of the cohort, whether familial or nonfamilial, and in the presence or absence of established nongenetic risk factors. The prevalence of pathogenic gene variants was similar for the various nongenetic risk factors, ranging from 15% to 19% (Figure 3B). Patients with diabetes and hypertension (both 17%) equally carried pathogenic gene variants.

Taken together, we find that as many as 1 in 5 of the patients with DCM with a nongenetic risk factor or comorbidity carry a pathogenic gene variant and that the finding of a nongenetic risk factor does not preclude a genetic predisposition.

Table 1. Baseline Characteristics and Clinical Features of 689 Patients With DCM

	Genetic (n=129)	Familial unknown-genetic (n=111)	Nongenetic (n=449)	Total (n=689)	*P* Value
Demographics					
Age at diagnosis, y	52±12 (22–80)	51±13 (18–78)	54±13 (18–90)	53±13 (18–90)	0.042*
Male	90/129 (70%)	65/111 (59%)	284/449 (63%)	439/689 (64%)	NS
Hypertension	45/129 (35%)	43/111 (39%)	175/449 (39%)	263/689 (38%)	NS
Diabetes	13/129 (10%)	14/111 (13%)	49/449 (11%)	76/689 (11%)	NS
Body mass index	26.8±4 (16–38)	26.9±5 (18–48)	26.7±5 (16–44)	26.7±5 (16–48)	NS
Autoimmune disease	7/129 (7%)	11/111 (10%)	40/449 (9%)	60/689 (9%)	NS
Toxic trigger (chemotherapy/drug or alcohol abuse)	12/129 (9%)	6/111 (5%)	47/449 (11%)	65/689 (9%)	NS
Presentation					
Family history of DCM	63/129 (49%)	111/111 (100%)	0/449 (0%)	174/689 (25%)	<0.001*†‡
NYHA class III or IV	46/129 (36%)	36/111 (32%)	109/449 (24%)	191/689 (28%)	0.01†
Out of hospital cardiac arrest	11/129 (9%)	7/111 (6%)	29/449 (7%)	47/689 (7%)	NS
Initial ECG/Holter					
Atrial fibrillation	47/129 (36%)	23/111 (21%)	97/449 (22%)	167/689 (24%)	0.001†/0.008‡
AV block	28/129 (22%)	11/111 (10%)	45/449 (10%)	84/689 (12%)	<0.001†/0.014‡
First/second/third degree AV block	24/2/2	10/1/0	34/5/6	68/8/8	
Left bundle branch block	27/129 (21%)	32/111 (29%)	131/449 (29%)	190/689 (28%)	NS
Nonsustained ventricular tachycardia	62/129 (48%)	29/111 (26%)	116/449 (26%)	207/689 (30%)	<0.001†‡
Premature ventricular complexes >20%	3/129 (2%)	8/111 (7%)	39/449 (9%)	50/689 (7%)	0.014†
Initial echocardiography					
Left ventricular ejection fraction	30±11 (10–49)	32±10 (13–49)	32±11 (8–49)	31±11 (8–49)	NS
LVEF <35%	88/129 (68%)	70/111 (63%)	261/449 (58%)	419/689 (61%)	0.039†
Indexed left ventricular end-diastolic diameter	30±4 (18–47)	32±5 (21–53)	31±5 (20–51)	31±5 (18–53)	NS
Endomyocardial biopsy					
Cardiac inflammation	22/129 (17%)	21/111 (19%)	85/449 (19%)	128/689 (19%)	NS
Significant viral load present	8/129 (6%)	5/111 (5%)	64/449 (14%)	77/689 (11%)	0.015†/0.005*
Medication					
β-Blocker	104/129 (81%)	93/111 (84%)	390/449 (87%)	587/689 (85%)	NS
ACE-inhibitors/ARB	110/129 (85%)	92/111 (83%)	400/449 (89%)	602/689 (87%)	NS
Mineralocorticoid receptor antagonist	73/129 (57%)	55/111 (50%)	210/449 (47%)	338/689 (49%)	NS
Diuretics	67/129 (52%)	58/111 (52%)	214/449 (48%)	339/689 (49%)	NS

Values are depicted as percentages or mean±SD (minimum–maximum). Significance <0.05 using Student *t* test or χ^2-test/Fisher Exact test where appropriate. ACE indicates angiotensin-converting enzyme; AV, atrioventricular; DCM, dilated cardiomyopathy; LVEF, left ventricular ejection fraction; NS, not significant; and NYHA, New York Heart Association.

*Familial unknown-genetic vs nongenetic.

†Genetic vs nongenetic.

‡Genetic vs familial unknown-genetic.

Prevalence of Pathogenic Variants in Apparent Nonfamilial DCM

A pathogenic gene variant was found in 13% (n=66) of patients with sporadic DCM, that is, those without a familial history of DCM (Table II in the Data Supplement). Data on family segregation were available in 35 of these patients (53%). In 2 families, the pathogenic variant in the index patient was proven to be de novo, as both parents tested negative for the variant. In 14 families, at least one relative carried the pathogenic variant and had cardiac abnormalities after cardiac examination. In 2 additional families in which only cardiac examination was performed, a relative with cardiac abnormalities was detected. Overall, 16 out of 35 (46%) of the initially nonfamilial DCM pedigrees turned out to be familial after segregation (Table II in the Data Supplement). Figure III in the Data Supplement shows all initially nonfamilial pedigrees in which segregation analysis could be performed.

Genetic DCM Is Associated With Electrical Phenotypes and Adverse Events

After a median of 4-year follow-up, 20% (143/689) of the cohort had experienced at least one serious adverse event. Adverse events occurred more frequently in those

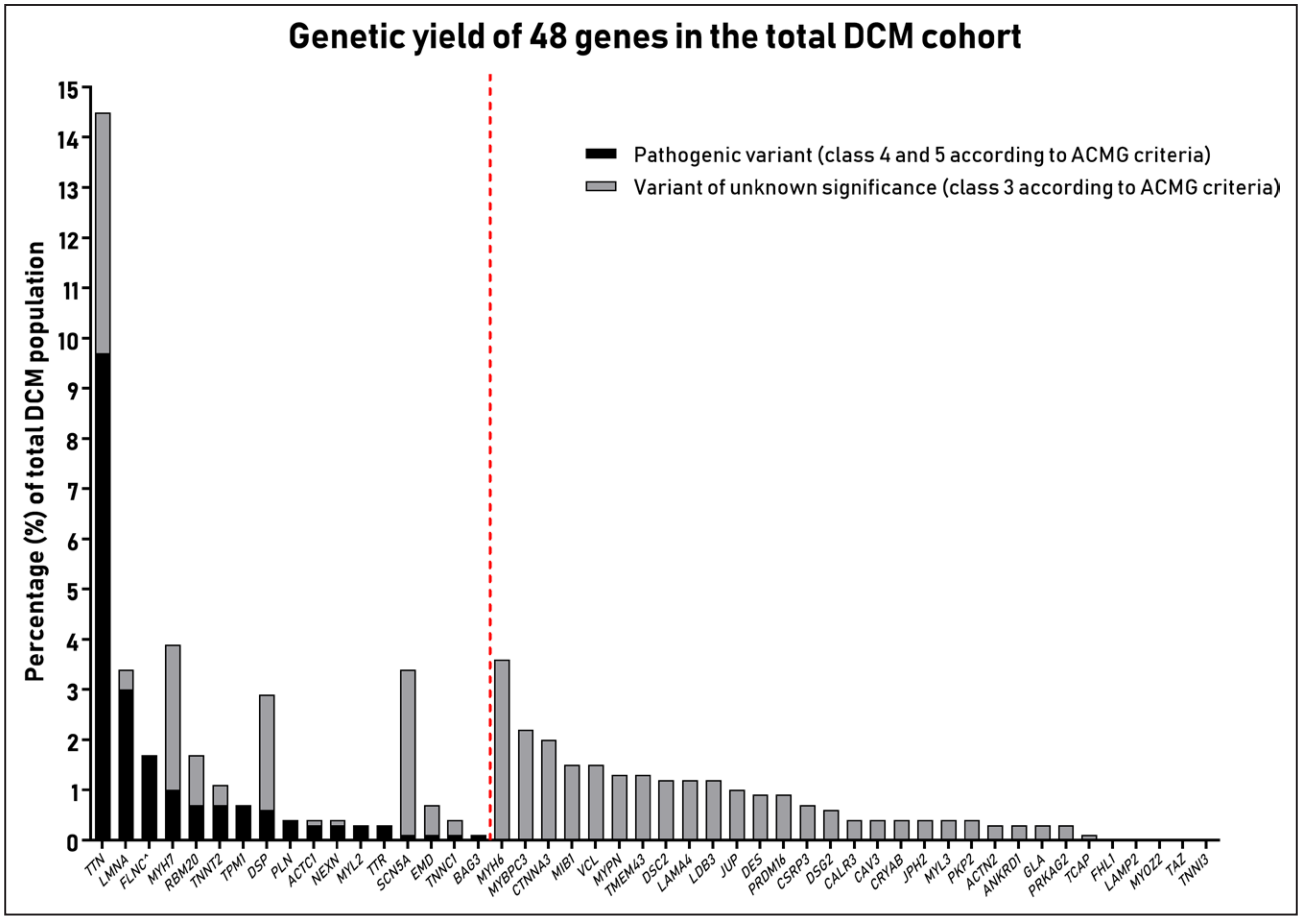

Figure 1. Genetic yield of the 48 cardiomyopathy-associated gene panel in 689 patients with dilated cardiomyopathy (DCM). Genes are ordered according to prevalence of pathogenic variants. ^ *FLNC* was only sequenced in 172 patients with DCM. The prevalence is calculated within this subgroup. ACMG indicates American College of Medical Genetics.

with genetic than those with nongenetic DCM (31% versus 18%; hazard ratio [HR], 1.6 [1.1–2.4]; log-rank P=0.01; Figure 4A; Table 2). Having a pathogenic variant and an additional nongenetic risk factor does not change outcome (log-rank P=0.28; Figure IV in the Data Supplement). The adverse outcome of patients with genetic DCM was mainly driven by life-threatening arrhythmias (LTAs; HR, 2.2 [1.3–3.7], P=0.002, Table 2).

Electrical phenotypes are frequently observed in patients with DCM. In our cohort, AF, NSVT, and an atrioventricular block (AVB) were independently associated with genetic DCM, while frequent premature ventricular contractions and left bundle branch block (LBBB) were associated with nongenetic DCM (Table 3; Figure 5). Familial disease and electrical phenotypes are the only distinguishing factor between genetic and nongenetic DCM (Table 3). Electrical phenotypes were also associated with an adverse outcome in DCM (Table 4; Figure 4). This was also mainly driven by the strong association between electrical phenotypes and LTA (HR, 5.0 [2.2–11.6]; P<0.001; Table V in the Data Supplement). We performed a causal mediation analysis to quantify the contribution of electrical phenotypes to the prognosis of genetic DCM. Electrical phenotypes mediate the effect between genetic DCM and an adverse outcome (average causal mediation effect, P<0.001), but there was no average direct effect (P=0.1; Figure 6; Figure II in the Data Supplement). This shows that the adverse outcome of genetic DCM is partly because of the strong association with electrical phenotypes.

Given the strong association between the presence of NSVT and worse outcome (HR, 2.72 [1.95–3.78]; P<0.001; Table 4), we distinguished between specific adverse events. Baseline NSVT represents a strong predictor for an LTA (HR=6.1) and is also a marker of overall poor outcome (Table VI in the Data Supplement). NVST is prevalent in genetic DCM and occurred in 62 (48%) patients in this group (Table 1; Figure 5). In contrast, LBBB and frequent premature ventricular contractions are more common in nongenetic DCM, and these phenotypes are not associated with adverse events (Table 3). These data underline the prognostic relevance of the electrical phenotypes that are frequent in genetic DCM.

The genetic DCM group constitutes of a heterogeneous mixture of variants. The subgroup of patients with either a TTNtv or *LMNA* variant were large enough to

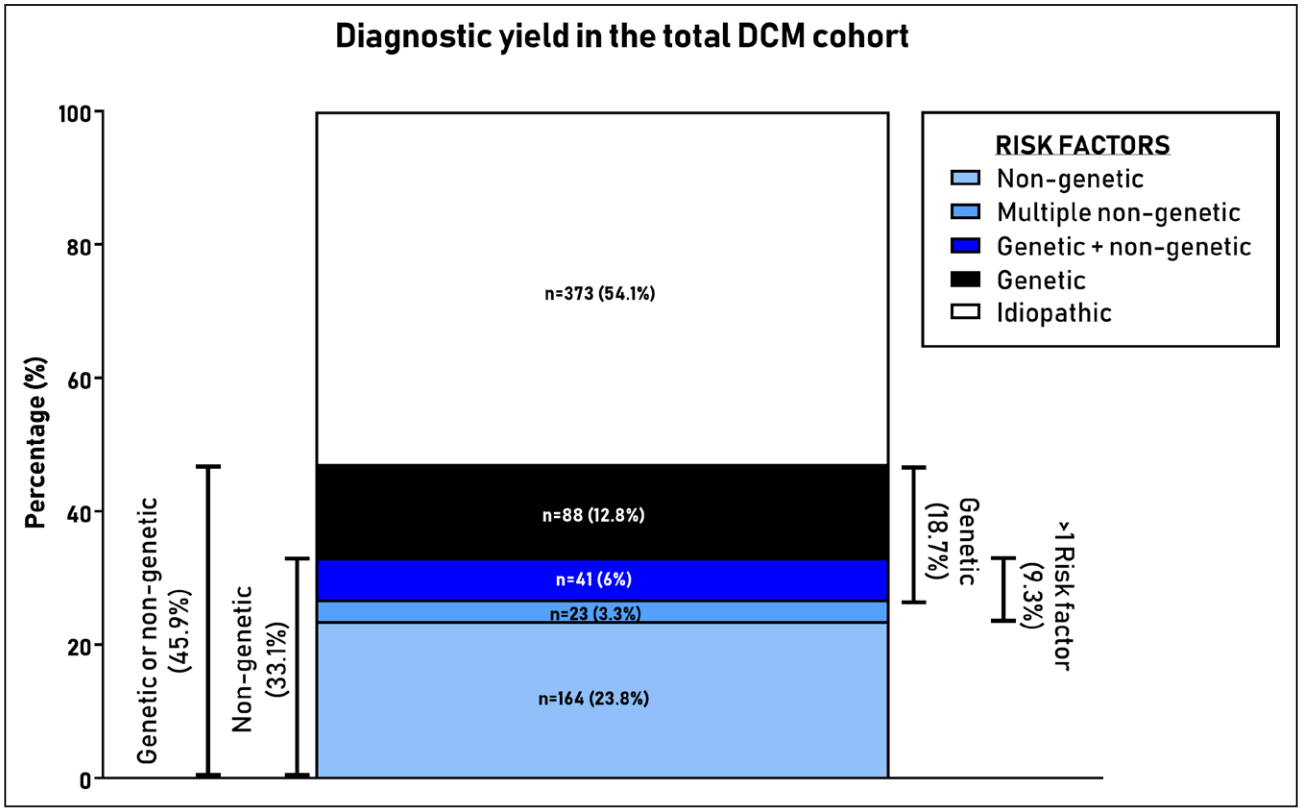

Figure 2. Diagnostic yield in 689 patients with dilated cardiomyopathy (DCM) after complete diagnostic work-up.

investigate separately. The TTNtv patients had an electrical signature, which closely resembles that of the total genetic DCM cohort: a strong association with AF, NSVT, and AVB and a significant underrepresentation of LBBB (Figure 5). In contrast, LBBB is common in patients with a *LMNA* variant, in addition to AF, NSVT, and AVB.

Finally, we performed the analysis with the exclusion of *LMNA* variants as they might drive the worse prognosis because of their known malignant arrhythmic profile. In general, the results remained the same, showing AF, NSVT, and AVB associated with genetic DCM (Table VII in the Data Supplement). The adverse outcome was worse in genetic DCM compared with nongenetic DCM (Figure V in the Data Supplement), which was mainly driven by LTA (Table VIII in the Data Supplement). This worse outcome was strongly mediated by the association with electrical phenotypes (average direct effect P=0.14; average causal mediation effect, $P<0.001$; Figure VI in the Data Supplement), implying that the adverse outcome of non-*LMNA* genetic DCM relies strongly on the association with electrical phenotypes.

DISCUSSION

The overall genetic yield in unselected DCM is 19% using strict criteria to classify genetic variants. This study shows that patients with apparently nonfamilial DCM still carry a pathogenic gene variant in 13%, and that after family segregation a clinically affected relative carrying the variant was found in 46% of what first appeared to be nonfamilial pedigrees. Furthermore, we find a pathogenic gene variant in 18% of the patients with DCM with a nongenetic risk factor or comorbidity. Clearly, neither the absence of a family history nor the presence of a nongenetic risk factor excludes the chance of finding genetic DCM. Importantly, genetic DCM carries an increased risk of adverse events, which is partly mediated by electrical phenotypes such as NSVT, AF, and AVB (Figure 6). Based on these results, we propose that genetic testing is clinically relevant and warranted across a wide range of clinical situations.

Diagnostic Yield in DCM

We detected at least one genetic or nongenetic risk factor in 46% of patients after a complete diagnostic work-up. This diagnostic yield is comparable to that in previous reports on large DCM populations studied before large-scale genetic testing became available.[16] A recent study in 100 patients with DCM identified a cause in 86% of patients after endomyocardial biopsy and cardiac magnetic resonance imaging but without genetic testing.[17] Inflammation-associated disease was reported in 49% of the patients with DCM in that study. Such inflammation-associated disease was defined by suspected or previous myocarditis on magnetic resonance imaging without confirmation by endomyocardial biopsy. Such a scenario would

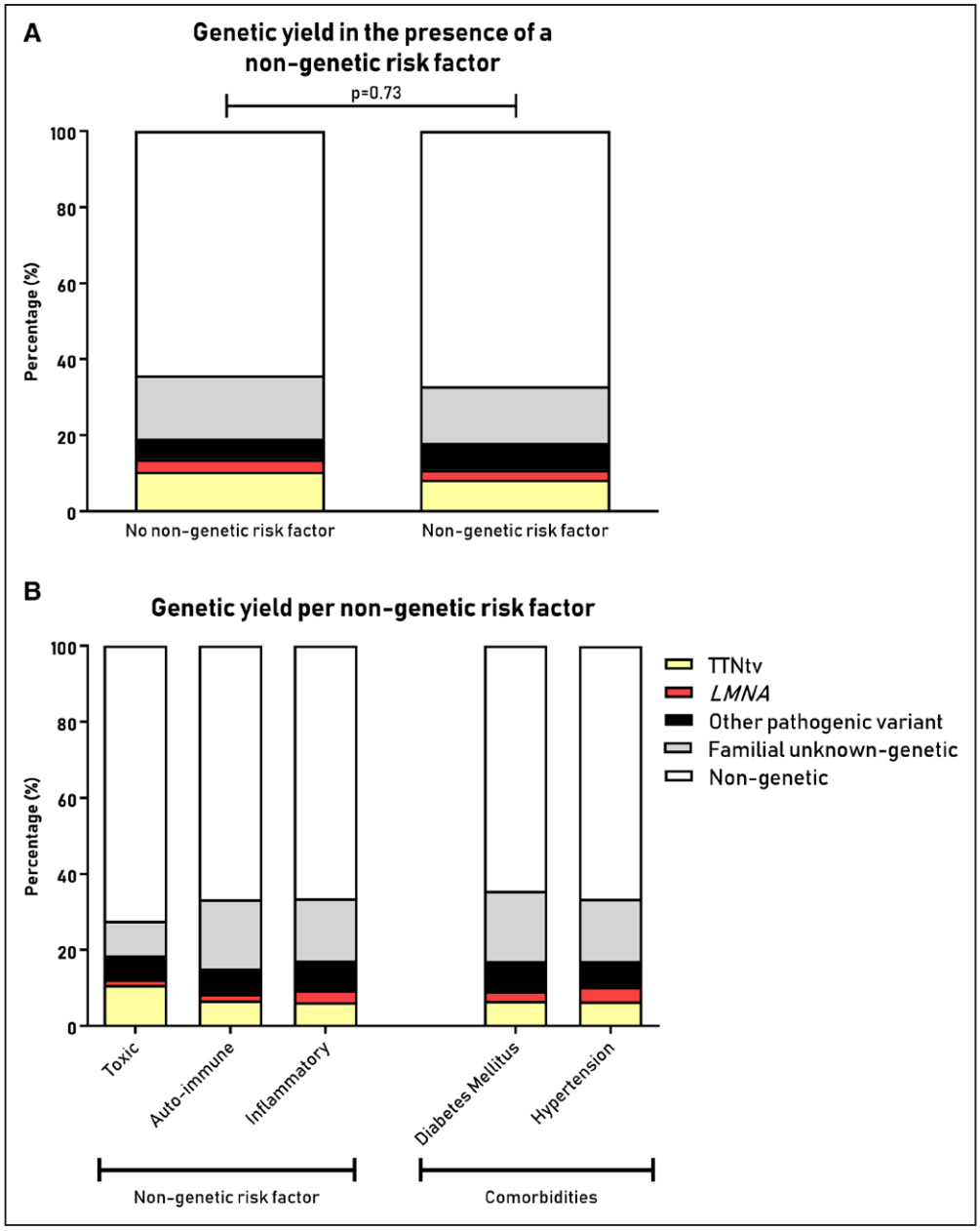

Figure 3. Genetic yield in patients with dilated cardiomyopathy (DCM).
Results are divided in patients with and without a nongenetic risk factor (**A**) and divided per subgroup of nongenetic risk factors and/or comorbidities (**B**). *P* is calculated using χ^2-test comparing the prevalence of pathogenic gene variants between groups. TTNtv indicates truncating titin variant.

not fit our diagnostic criteria for DCM. There is a continued need for standardized criteria for diagnosis and the definition of pertinent risk factors, to better understand DCM pathogenesis, as previously proposed by the World Health Federation.[12] We decided to treat electrical disturbances as separate phenotypes, rather than as independent risk factors. Our data do not allow us to determine whether these electrical disturbances are cause or consequence of DCM.

Genetic Yield in Nonfamilial and Acquired DCM

Genetic diagnostics is widely accepted in familial DCM, especially when there are no other specific triggers.[2] We find that limiting DNA testing to familial DCM may not be the best strategy, as pathogenic variants were present in 13% of patients with nonfamilial DCM in our cohort. Conversely, of those who were tested positive for a pathogenic DCM gene variant in our study, almost half lacked a positive family history at baseline. Family history provided by patients is frequently incomplete, and some of these relatives will develop overt DCM at a later age.[18] Accurate family segregation revealed a pathogenic gene variant and clinically relevant DCM in a relative in 46% of the nonfamilial DCM pedigrees. There may be diagnostic as well as prognostic value to search for the genetic substrate in all patients with DCM and subsequently in other family members.

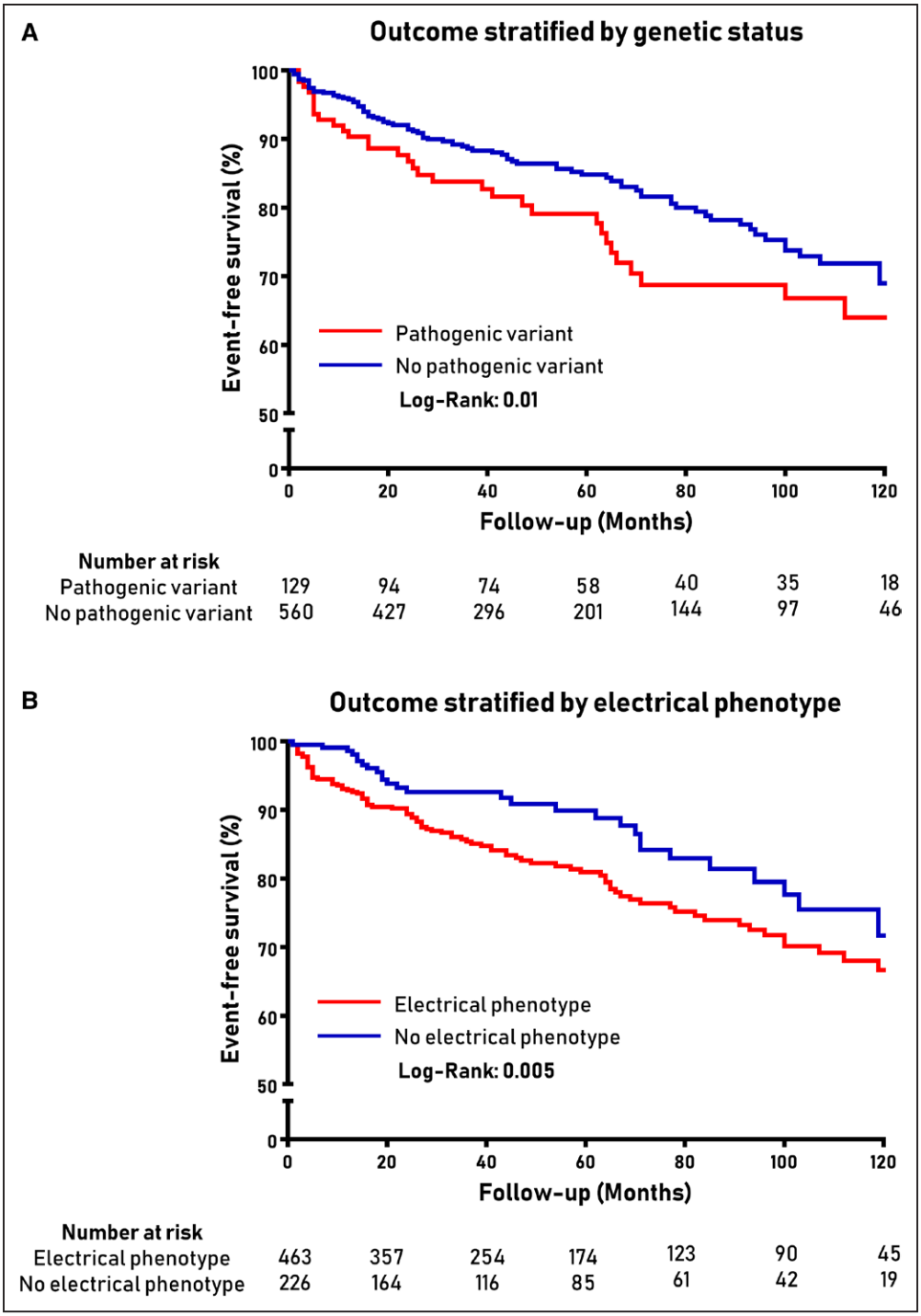

Figure 4. Survival curves show freedom from composite end point (cardiac death or transplantation, heart failure hospitalization or life-threatening arrhythmia).
Results are stratified on genetic status (**A**), and stratified on the presence of an electrical phenotype (**B**). Event-free survival is measured from time of diagnosis. Curves are compared using the log-rank test.

Recent reports of an increased burden of genetic variants in patients with DCM exposed to chemotherapy or alcohol suggest that the clinical impact of a pathogenic gene variant may be amplified by exposure to such nongenetic risk factors.[7,8,11,19] In one study, patients with DCM with a TTNtv who consumed excess alcohol had a more severe clinical presentation compared with DCM with neither TTNtv nor excess alcohol consumption.[11] We detected pathogenic gene variants in an average of 18% of patients with DCM across a range of nongenetic risk factors. Showing that the finding of a nongenetic risk factor does not exclude a genetic predisposition. Also,

Table 2. Association of Genetic Status With Adverse Outcomes on Cox Proportional Hazards Analysis

	Pathogenic variant (n=129)	No pathogenic variant (n=560)	Hazard ratio (95% CI)	*P* Value
Death	9 (7%)	37 (7%)	…	NS
Heart transplantation	7 (5%)	6 (1%)	5.1 (1.4–19.2)	0.015
Heart failure hospitalization	22 (17%)	59 (11%)	…	NS
Life-threatening arrhythmia	23 (18%)	46 (8%)	2.2 (1.3–3.7)	0.002
Combined end point	40 (31%)	103 (18%)	1.6 (1.1–2.4)	0.011
Combined end point without life-threatening arrhythmia	27 (21%)	83 (15%)	…	NS

Significance <0.05 using Cox regression analysis. NS indicates not significant.

there was no detectable effect of having an additional nongenetic risk factor on prognosis in our cohort (Figure IV in the Data Supplement).

Composition of Diagnostic Gene Panels for DCM

Genetic testing aims to test the smallest number of DCM genes that maximizes the diagnostic yield, while simultaneously keeping the number of VUSs as low as possible. A VUS complicates the clinical translation of genetics to the patient and family, as the clinical consequences are unknown. The number of genes reported as disease-causing for DCM is constantly increasing, but many such genes currently lack reliable and robust evidence.[15,20]

In our cohort, a genetic variant was present in 19% of patients with DCM, which is slightly lower compared with most published studies using high-throughput genetic screening.[4,5,21,22] One important difference is that familial DCM was less frequent in our cohort (25%) compared with ≈50% in most published studies. Our study was a consecutive series of patients, not selected for familial occurrence. We used a diagnostic panel including 47/48 genes in contrast to gene panels including up to 149 genes used for genetic screening in previous studies.[5,21] Another important difference is the criteria used for interpreting pathogenicity of variants. We used the strict American College of Medical Genetics guidelines to systematically classify variants and incorporated evidence per gene as suggested by Mazzarotto et al.[15] Previous studies sometimes used less strict and uniform criteria. For example, a previous study reported 2 or more gene variants in up to 38% of patients with DCM. This number greatly exceeds previous studies.[22] One possible explanation for this discrepancy is the inclusion of *TTN* missense variants, the majority of which are now considered to be likely benign.[23]

It has been argued that only 12 genes have sufficiently robust evidence for DCM-related pathogenicity, and that the inclusion of more genes to a DCM screening panel is questionable.[15,20] Classifying variants in further non-established DCM genes should be done with caution. In our study evaluating only the proposed established DCM genes would have detected the overwhelming majority of pathogenic gene variants (89%; 118/132). Additional pathogenic variants were detected in *RBM20* and *FLNC* that were not reviewed by Mazzarotto et al,[15] in patients with multisystemic disease (*EMD* and *TTR*), an arrhythmogenic form of DCM (*SCN5A*), or in a rare homozygous form (*MYL2*). These genes collectively added 2% to the genetic yield, of which 1.2% attributed to *FLNC* and *RBM20*. This confirms that pathogenic gene variants mostly reside in the core DCM genes: *TTN, LMNA, MYH7, TNNT2, TPM1, DSP, VCL, BAG3, TNNC1, ACTC1, NEXN, PLN*, with the addition of *RBM20* and *FLNC*. Focusing genetic testing to solely these 14 genes would substantially reduce VUSs with 70%. These VUSs were detected in these nonestablished DCM genes with questionable diagnostic utility in a clinical setting. We found 3 pathogenic hypertrophic

Table 3. Uni- and Multivariable Model for Predicting the Likelihood of a Pathogenic Variant in Patients With Dilated Cardiomyopathy

Multivariable model for predicting a pathogenic variant						
	Univariable			Multivariable		
Variable	OR	95% CI	*P* Value	OR	95% CI	*P* Value
Familial disease	3.86	2.6–5.8	<0.001	3.72	2.4–5.7	<0.001
Atrial fibrillation	2.1	1.4–3.2	<0.001	1.68	1.1–2.6	0.024
Nonsustained VT	2.65	1.8–3.9	<0.001	2.7	1.8–4.2	<0.001
AV block	2.5	1.5–4.1	<0.001	2.5	1.4–4.4	0.002
Left bundle branch block	0.65	0.4–1.0	0.06	0.51	0.3–0.9	0.01
>20% PVCs	0.26	0.1–0.8	0.03	0.19	0.1–0.6	0.008
NYHA≥III	1.59	1.1–2.4	0.03	…	…	…

AV indicates atrioventricular; LVEDD, left ventricular end-diastolic diameter; NYHA, New York Heart Association; OR, odds ratio; PVC, premature ventricular contraction; and VT, ventricular tachycardia.

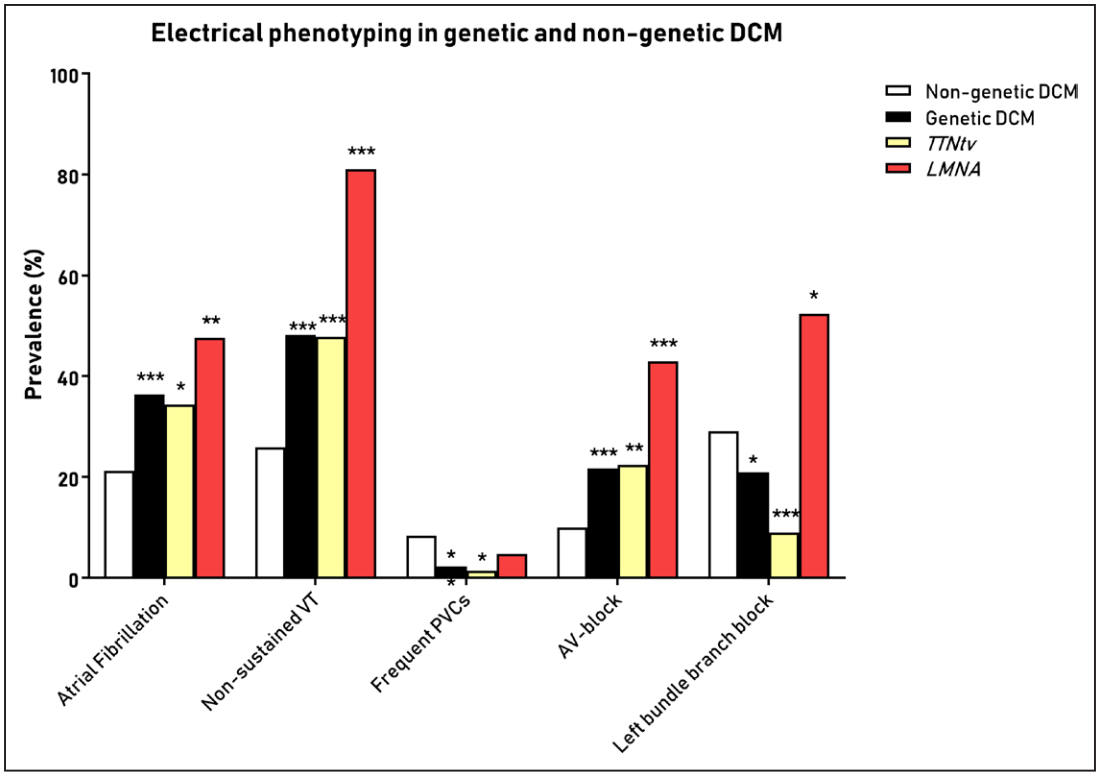

Figure 5. Prevalence of atrial fibrillation, nonsustained ventricular tachycardias (VTs), atrioventricular (AV) block, left bundle branch block, and prevalent premature ventricular contraction (PVC) within genetic and nongenetic dilated cardiomyopathy (DCM).
P is calculated using χ^2-test comparing the prevalence with the nongenetic DCM group. LMNA indicates lamin A/C; TTNtv, truncating titin variant. **P*<0.05; ***P*<0.01; ****P*<0.001.

cardiomyopathy (HCM) founder mutations in *MYBPC3* (Table IV in the Data Supplement), which are difficult to interpret when detected in a patient with DCM. None of these patients appeared to have end-stage HCM. This is a remarkable finding, as these pathogenic variants are solely linked to HCM in the literature. That a gene can be associated with both HCM and DCM is not entirely without precedent. An example of this would be *MYH7*, which is both an established DCM and HCM gene.[15,20]

We note that DCM founder mutations affecting specific genes occur in distinct geographic areas. Thus, the precise composition of a diagnostic gene panel should ideally include those genes that are more commonly involved locally.

Genotype-Phenotype Associations in DCM

The electrical signature of genetic DCM is characterized by AF, NSVT, and AVB in the absence of LBBB and frequent premature ventricular contractions. Previous studies described the arrhythmogenic potential in *LMNA* and *TTN*-associated DCM and related this to a higher degree of cardiac fibrosis.[13,14] It is possible that our risk stratification could be further improved using novel imaging techniques such as cardiac magnetic resonance imaging to detect and quantify this cardiac fibrosis. [24] We showed that the electrical phenotype of genetic DCM may mediate a worse prognosis compared with nongenetic DCM (Figure 6). Genetic DCM was mainly related

Table 4. Electrical Phenotypes in Association to Adverse Outcome in All Patients

	Adverse outcome (n=143)	No adverse outcome (n=546)	Hazard ratio (95% CI)	*P* Value
Electrical phenotype (n=463)	111 (24%)	352 (76%)	1.76 (1.18–2.63)	0.005
No electrical phenotype (n=226)	32 (14%)	194 (86%)	…	…
Atrial fibrillation (n=167)*	48 (29%)	119 (71%)	1.64 (1.16–2.33)	0.005
Nonsustained VT (n=207)*	79 (38%)	128 (62%)	2.72 (1.95–3.78)	<0.001
AV block (n=84)*	24 (29%)	60 (71%)	…	NS
Left bundle branch block (n=190)	34 (18%)	156 (82%)	…	NS
>20% PVCs (n=50)	13 (26%)	37 (74%)	…	NS

AV indicates atrioventricular; DCM, dilated cardiomyopathy; NS, not significant; PVC, premature ventricular contraction; and VT, ventricular tachycardia.
*Electrical phenotypes, which are significantly associated with genetic DCM.

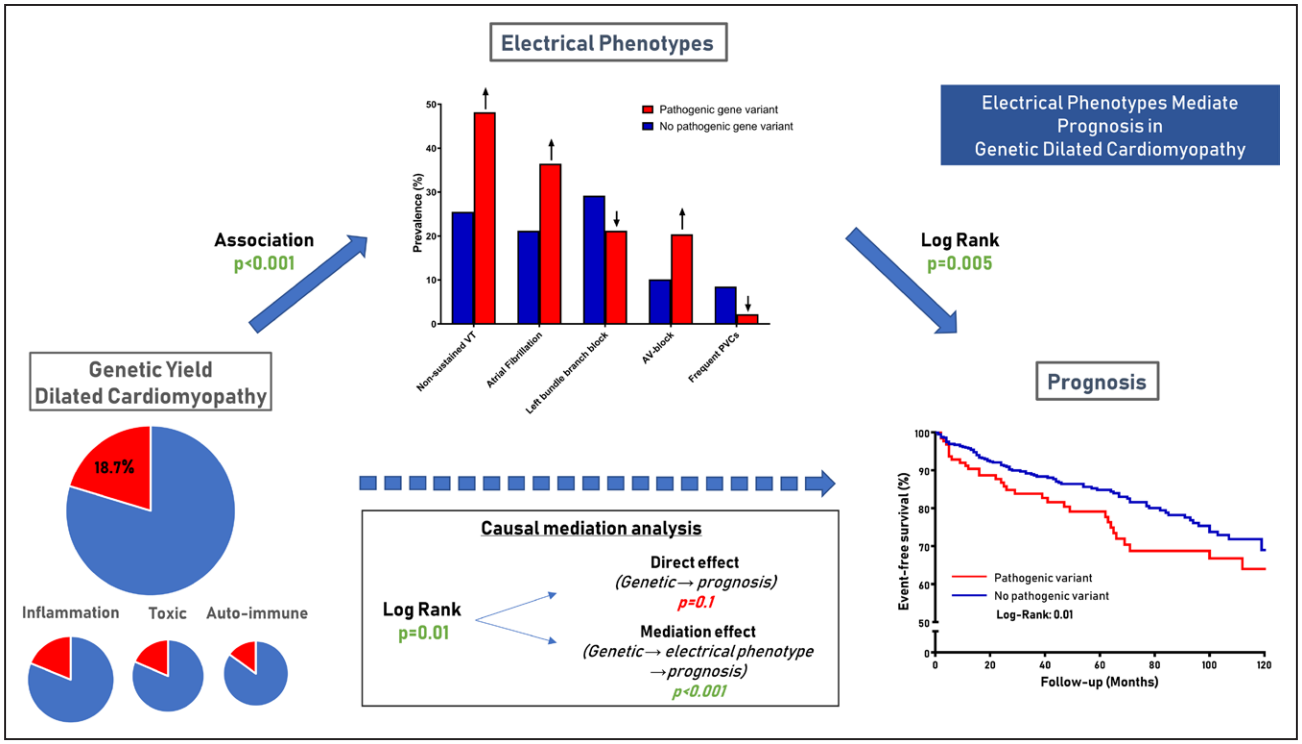

Figure 6. Genetic dilated cardiomyopathy (DCM) has an electrical phenotype characterized by an increased prevalence of nonsustained ventricular tachycardia (VT), atrial fibrillation and atrioventricular (AV) block.
This electrical phenotype mediates the worse prognosis in genetic vs nongenetic DCM. The direct effect is the contribution of genetics to outcome, the mediation effect is the contribution of electrical phenotypes to the association between genetics and outcome. PVC indicates premature ventricular contraction.

to an increased risk of LTA. The relation between LTAs and genetic DCM remained in our study, even after exclusion of *LMNA* variants. This is in contrast to a recent study in which the association between LTAs and genetic DCM failed to reach significance.[25] A subgroup analysis in that study suggested significantly higher risk for LTAs for *LMNA* and desmosomal gene carriers only. Based on our findings, the arrhythmogenic phenotype that predisposes to LTAs would seem to be distributed over a broader range of DCM-associated genes. This may have implications for DCM management. The susceptibility for ventricular arrhythmias in *LMNA* as well as in non-*LMNA* genetic DCM argues for early preventive measures irrespective of left ventricular dysfunction. This should be further investigated in a more extensive study designed to answer this question.

The conduction disorder LBBB is rare in *TTN*-associated DCM but is not uncommon in patients carrying a *LMNA* variant, in line with previous literature.[26] We note that *LMNA* variants are known to affect the septum, which is important for electrical conduction.[27]

Clinical Implications

A key finding of our study is that an established acquired trigger in DCM does not exclude a pathogenic gene variant. Interactions between pathogenic gene variants and nongenetic risk factors may contribute to the incomplete penetrance and variability within families. Overall, our finding of 19% pathogenic variants across a range of nongenetic risk factors (Figure 3) suggests that it may be justifiable to perform genetic diagnostics in all patients with DCM.

There will always be a trade-off between diagnostic sensitivity and specificity in DCM genetic testing. On the one hand, some families who were tested negative in our study may have monogenic causes that are not covered by the 47 gene DCM panel, such as *FLNC* which was only tested in 25% of our cohort. On the other hand, decreasing the number of genes in our diagnostic gene panel to only the genes, which have robust evidence to be DCM-associated (*TTN, LMNA, MYH7, RBM20, TNNT2, TPM1, DSP, VCL, BAG3, TNNC1, ACTC1, NEXN, FLNC,* and *PLN*)[15,20,28] would have detected 95% of all pathogenic gene variants found in this cohort (126/132). Patients with VUSs in the aforementioned established genes (excluding missense variants in *TTN*) showed a similar prognostic trend compared with patient with a classified (likely) pathogenic variant (Figure VII in the Data Supplement). This suggests that at least some of these VUSs are likely pathogenic but currently lack sufficient evidence to be classified as (likely) pathogenic with the American College of Medical Genetics guidelines. The diagnostic and prognostic value of VUSs in

established DCM genes should be analyzed in detail in larger cohorts, as previously performed for HCM.[29,30] In addition, these VUSs could explain some of the familial occurrence in the 111 familial unknown genetic patients with DCM in our cohort.

Study Limitations

This study represents a single-center, retrospective data analysis. Results of this study still need to be confirmed in an external cohort. There is an ongoing debate about the definition of risk factors that contribute to the cardiac phenotype. We tried to be as strict as possible using only previously defined quantifiable nongenetic risk factors, following previous guidelines and position statements. Regardless of whether such factors are truly causative, our main findings underscore the importance of genetic variants in patients with apparent nongenetic risk factors. The exclusion of coronary artery disease and valvular disease does not exclude the possibility that there could be a genetic predisposition in this DCM subgroup. In fact, patient with DCM with nongenetic risk factors still carried pathogenic gene variants in a significant percentage. Our cohort is mainly from white ancestry, and the genetic results should be interpreted and translated within this ancestral context.

CONCLUSIONS

One in 5 patients with an established nongenetic risk factor or nonfamilial disease still carries a pathogenic gene variant. Genetic DCM is characterized by a profile of electrical phenotypes (high prevalence of AF, NSVT, and AVB) and carries increased risk for adverse outcomes. Based on these findings, we envisage a broader role for genetic testing in DCM.

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Disclosures

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