ESC European Society of Cardiology

European Journal of Heart Failure (2023) **25**, 1256–1266
doi:10.1002/ejhf.2886

RESEARCH ARTICLE

Clinical characteristics of heart failure with reduced ejection fraction patients with rare pathogenic variants in dilated cardiomyopathy-associated genes: A subgroup analysis of the PARADIGM-HF trial

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Received 17 February 2023; revised 18 April 2023; accepted 8 May 2023; online publish-ahead-of-print 1 June 2023

Aims To evaluate the prevalence of pathogenic variants in genes associated with dilated cardiomyopathy (DCM) in a clinical trial population with heart failure and reduced ejection fraction (HFrEF) and describe the baseline characteristics by variant carrier status.

Methods and results This was a *post hoc* analysis of the Phase 3 PARADIGM-HF trial. Forty-four genes, divided into three tiers, based on definitive, moderate or limited evidence of association with DCM, were assessed for rare predicted loss-of-function (pLoF) variants, which were prioritized using ClinVar annotations, measures of gene transcriptional output and evolutionary constraint, and pLoF confidence predictions. Prevalence was reported for pLoF variant carriers based on DCM-associated gene tiers. Clinical features were compared between carriers and non-carriers. Of the 1412 HFrEF participants with whole-exome sequence data, 68 (4.8%) had at least one pLoF variant in the 8 tier-1 genes (definitive/strong association with DCM), with *Titin* being most commonly affected. The prevalence increased to 7.5% when considering all 44 genes. Among patients with idiopathic aetiology, 10.0% (23/229) had tier-1 variants only and 12.6% (29/229) had tier-1, -2 or -3 variants. Compared to non-carriers, tier-1 carriers were younger (4 years; adjusted p-value [p_{adj}] $= 4 \times 10^{-3}$), leaner (27.8 kg/m^2 vs. 29.4 kg/m^2; $p_{adj} = 3.2 \times 10^{-3}$), had lower ejection fraction (27.3% vs. 29.8%; $p_{adj} = 5.8 \times 10^{-3}$), and less likely to have ischaemic aetiology (37.3% vs. 67.4%; $p_{adj} = 4 \times 10^{-4}$).

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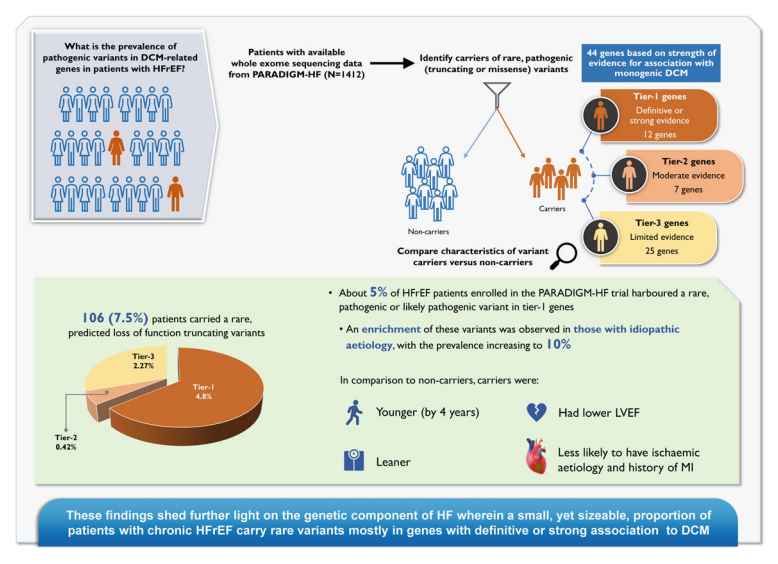
Conclusion Deleterious pLoF variants in genes with definitive/strong association with DCM were identified in ~5% of HFrEF patients from a PARADIGM-HF trial subset, who were younger, had lower ejection fraction and were less likely to have had an ischaemic aetiology.

Graphical Abstract



Subgroup analysis of the PARADIGM-HF trial in heart failure with reduced ejection fraction patients with rare pathogenic variants in dilated cardiomyopathy-associated genes. DCM, dilated cardiomyopathy; HFrEF, heart failure with reduced ejection fraction; LVEF, left ventricular ejection fraction; MI, myocardial infarction.

Keywords HFrEF • Dilated cardiomyopathy • Rare truncating pLoF variants • Prevalence • Clinical characteristics • Titin

Introduction

Heart failure (HF) affects more than 64 million people worldwide and is a major global health and socioeconomic burden.[1] Despite remarkable advances in our understanding of HF pathophysiology and therapeutic strategies, a gap remains in our knowledge of the molecular mechanisms of HF pathogenesis.[2]

Heart failure has a substantial degree of heritability (26–34%).[3] However, the genetic component of HF is heterogeneous and complex, mirroring the diverse aetiology, pathophysiology, and clinical course of HF.[4] Although recent studies have identified both frequent[5–8] and rare genetic variations[5,6,9] associated with HF, often coinciding with the same genetic loci, the prevalence and extent to which the genetic component contributes to HF pathogenesis remain to be elucidated.

Cardiomyopathies are a heterogeneous group of diseases of the myocardium, which include dilated, hypertrophic, restrictive and arrhythmogenic cardiomyopathies, and are a frequent cause of HF.[10] Classically, dilated cardiomyopathy (DCM) is characterized by enlargement and dilatation of one or both ventricles along with impaired contractility due to primary disease of the heart muscle, which in some cases has a genetic cause.[11] DCM with no other apparent systemic involvement but including genetic causes has been commonly classified as idiopathic,[12,13] with a suggested prevalence of 1 in 250 individuals.[12]

Variants in >250 genes across different gene ontologies encoding proteins involved in diverse aspects of myocardial cell structure and functions have been implicated in the pathogenesis of DCM, with variable penetrance and clinical manifestations,[4,7,14–16] a testimony to its complex genetic architecture.[4,17] Pathogenic (or likely pathogenic) variants are observed in 13–25% of unselected patients with sporadic DCM and up to 40% in those with familial DCM.[16,18] However, the prevalence and contribution of DCM-related gene variants in the pathogenesis of HF with reduced ejection fraction (HFrEF) as a broader category of both ischaemic and non-ischaemic aetiology are not completely understood.

The objective of this *post hoc* analysis was to evaluate the prevalence of rare pathogenic and likely pathogenic variants in

44 DCM-associated genes (proposed by the Clinical Genome Resource [ClinGen] DCM Gene Curation Expert Panel)[14] among a subset of patients with HFrEF (irrespective of the underlying ischaemic or non-ischaemic clinical classification) from the PARADIGM-HF, a Phase 3 clinical trial,[19] in whom whole exome sequencing (WES) and clinical data are available ($n = 1412$). Furthermore, we describe the demographic and clinical characteristics of the carriers versus non-carriers in this well-phenotyped cohort.

Methods

Study design and participants

This *post hoc* analysis included 1412 participants with WES data in the PARADIGM-HF trial conducted between December 2009 and March 2014. The design and primary results of PARADIGM-HF have been reported previously.[19] Briefly, PARADIGM-HF was a randomized, double-blind clinical trial comparing the efficacy of sacubitril/valsartan with enalapril in patients with chronic HFrEF, as reported by the investigators based on clinical antecedents. The trial was conducted in accordance with the Declaration of Helsinki and approved by an ethics committee at each study centre and all patients included in this analysis provided written informed consent for genetics.

Genetic data availability

Germline single nucleotide polymorphisms (SNPs) were obtained by WES of DNA samples available from 1412 patients (details in online supplementary *Appendix S1*).

Dilated cardiomyopathy-related genes

We examined the presence of rare variants among a panel of 44 genes, categorized into three tiers based on the strength of evidence for association with DCM,[14] as proposed by the ClinGen DCM Gene Curation Expert Panel.[14] The classification utilizes the ClinGen semi-quantitative gene-disease clinical validity classification framework, which sums the scores for published genetic and experimental laboratory evidence. Tier-1 consisted of 12 genes with definitive (*BAG3*, *DES*, *FLNC*, *LMNA*, *MYH7*, *PLN*, *RBM20*, *SCN5A*, *TNNC1*, *TNNT2*, and *TTN*) or strong (*DSP*) evidence for association with monogenic DCM. Tier-2 consisted of seven genes (*ACTC1*, *ACTN2*, *JPH2*, *NEXN*, *TNNI3*, *TPM1*, and *VCL*) that have moderate evidence for association with DCM. Tier-3 comprised 25 genes (*ABCC9*, *ANKRD1*, *CSRP3*, *CTF1*, *DSG2*, *DTNA*, *EYA4*, *GATAD1*, *ILK*, *LAMA4*, *LDB3*, *MYBPC3*, *MYH6*, *MYL2*, *MYPN*, *NEBL*, *NKX2-5*, *OBSCN*, *PLEKHM2*, *PRDM16*, *PSEN2*, *SGCD*, *TBX20*, *TCAP*, and *TNNI3K*) for which limited or disputed evidence is available (*Figure 1*).[14] Seven genes with inconclusive or no evidence[14] were not included in this study.

Rare variant scoring and mutational models to assign carriers and non-carriers

Based on the Ensembl Variant Effect Predictor (VEP) annotations, variants were classified as either predicted truncating loss-of-function (pLoF; 'stop', 'frameshift', 'splicing donor', 'splicing acceptor') or 'missense'.[20] Variants with 'benign' annotations in ClinVar, as well as variants with >1% frequency in any of the major gnomAD[21] populations, were excluded. We therefore defined variants as rare if they occur at ≤1% minor allele frequency (MAF). Rare truncating pLoF variants were assigned a priority score according to the following annotations: ClinVar,[22] Loss of Function Transcript Effect Estimator (LOFTEE),[23] loss of function observed/expected upper-bound fraction (LOEUF) decile – a gene-level measure of constraint against pLoF variants,[23] and a transcript-level annotation metric, the proportion expressed across transcripts (baselevel pext) in left ventricular tissue in Genotype Tissue Expression (GTEx)[24] (details in online supplementary *Appendix S1*). For example, truncating pLoF variants with priority score 1 had unambiguous pathogenic or likely pathogenic ClinVar annotations. Variants with ambiguous or no ClinVar annotations were assigned score 2 if they had high-confidence LOFTEE annotations and affected highly evolutionarily constrained for truncating mutations genes (based on the gnomADs LOEUF deciles) and score 3 if they either had high-confidence LOFTEE annotations and affected non-highly constrained genes or had low confidence LOFTEE annotations and affected highly constrained genes. The truncating variants were also assessed according to the American College of Medical Genetics and Genomics (ACMG) criteria[25] (details in online supplementary *Appendix S1*).

Similarly, the rare missense variants were also assigned priority scores according to five different scoring systems combining various annotations: existing evidence for mutation pathogenicity (ClinVar) and various *in silico* mutation effect predictions: SIFT,[26] PolyPhen-2,[27] EIGEN, metaSVM and metaLR, as well as a constraint metric for missense mutations[23] and pext score[24] (online supplementary *Appendix S1*).

Various sets of rules were used to define the carrier or non-carrier status for the patients, and we refer to each set of rules as a *mutational model*. In such a model, participants with rare variants with specific priority scores and belonging to specific DCM gene tier(s) were considered carriers and the rest were assigned to non-carriers. Separate mutational models were defined for truncating pLoF and missense variants (online supplementary *Table S1*). Based on the amount of evidence supporting a variants' pathogenicity we have established one pre-specified priority mutational model to assign the carrier status for truncating pLoF variants: participants harbouring a truncating pLoF variant with priority score 1, 2, or 3 in tier-1 DCM-related genes only were considered as carriers and the rest of the participants were assigned to non-carriers. Other mutational models permit definition of truncating pLoF variant carriers on tier-2 and/or tier-3 DCM-related genes as well as predicted pathogenic missense variant carriers, as defined in online supplementary *Table S1*.

Statistical analysis

Clinical characteristics of variant carriers versus non-carriers were contrasted via linear regression (online supplementary *Appendix S1*), with the carrier status defined using the set of mutational models described above. Rare truncating pLoF carriers in tier-1 DCM-related genes were examined firstly alone, secondly incorporating truncating pLoF carriers in tiers-2 and -3 DCM-related genes and thirdly with the predicted missense carrier status as an additive term in the linear models (online supplementary *Appendix S1*).

Frequencies were compared using Fisher's exact test. As comparison of carriers versus non-carriers was exploratory, nominal p-values with threshold set at 0.05 were used as a criterion for statistical significance. The reported nominal p-values were further corrected for multiple testing using the Benjamini–Hochberg method. Multiple testing adjusted p-values (p_{adj}) < 0.05 was considered as an additional indicator for significance. All analyses were conducted using R 4.1.2.[28]

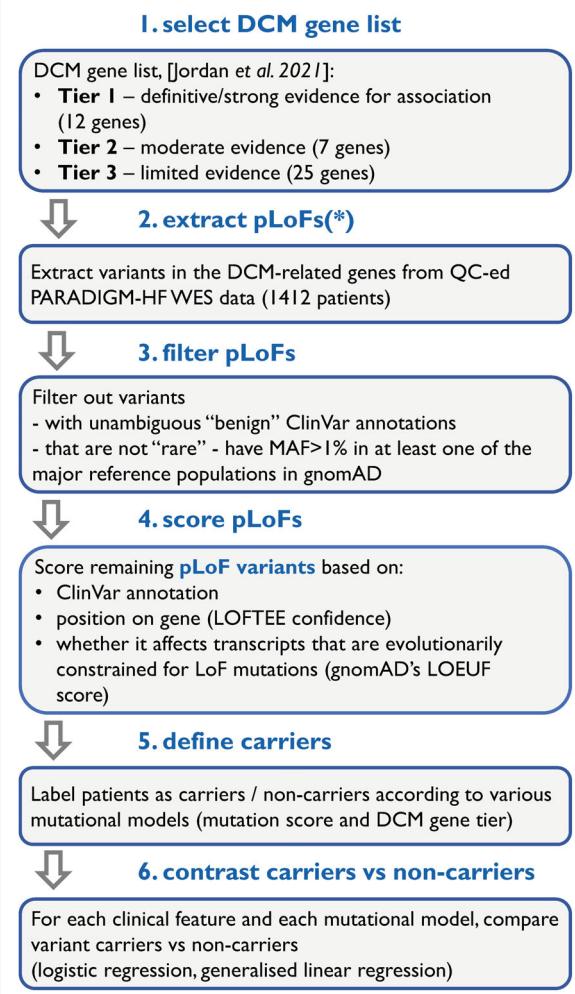


Figure 1 Summary of methodology used to extract, filter, and score rare variants. A similar approach was utilized to extract and filter rare missense mutations and various approaches were used to score them according to priority in this analysis, using different *in silico* methods that predict missense mutation pathogenicity. DCM, dilated cardiomyopathy; LOEUF, loss of function observed/expected upper-bound fraction; LOFTEE; Loss of Function Transcript Effect Estimator; MAF, minor allele frequency; pLoF, predicted loss-of-function; WES, whole exome sequencing.

Results

In PARADIGM-HF, WES data were available for 1412 out of 8399 participants (*Table 1*). The participants with WES data were more likely White, less likely female, had higher body mass index, and were more likely to have HFrEF of ischaemic aetiology, and comorbidities such as hypertension, diabetes, atrial fibrillation, history of MI than the rest of the PARADIGM-HF participants. Most participants (66%) had HFrEF of ischaemic aetiology, as classified by history of prior coronary heart disease (i.e. MI, stable and/or unstable angina, coronary artery bypass graft or percutaneous coronary intervention). Among participants with HFrEF of non-ischaemic primary aetiology, 47% had aetiology classified as idiopathic, 29% as hypertensive and 24% had other aetiologies (*Table 1*), similar to the non-ischaemic category in the overall PARADIGM-HF study.[29]

Prevalence of rare pathogenic variants

Among the 44 DCM-associated genes evaluated, heterozygous truncating pLoF rare variants were identified in 106 participants. Of these, 68 (4.8%) had a truncating pLoF variant in eight of the 12 genes classified as tier-1 (definitive/strong evidence for association with DCM), seven (0.5%) had variants in three of the seven tier-2 (moderate evidence) genes and 33 (2.7%) had variants in nine of the 25 tier-3 (limited evidence) genes (*Table 2*). All DCM genes truncating variants scoring 1–3 had sufficient evidence to be scored as at least 'likely pathogenic' according to the ACMG criteria (conservatively selected truncating variants that are not present or very rare in gnomAD, formerly ExAC). Indeed, the maximum MAF was 0.034% for tiers-1 and -2 truncating variants and 0.15% for tier-3. No homozygous truncating variants were found. Only two participants had pLoF variants in more than one DCM gene (*TTN* and *VCL*; *NEXN*, and *ANKRD1*). Overall, 18 (26.5%) of the truncating pLoF variants were annotated as unambiguously pathogenic in ClinVar (priority score 1). A majority of the pLoF variants were in the *TTN* gene, accounting for 79.6% of the unique variants observed in tier-1 genes.

We also identified a higher prevalence of rare truncating pLoF variants in tier-1 DCM-related genes in participants with suspected non-ischaemic investigator-reported aetiology compared to those with suspected ischaemic HFrEF (8.9% [43/483] vs. 2.7% [25/929]; $p = 6.3 \times 10^{-7}$). The prevalence rates of truncating pLoF variants in tier-2 and -3 DCM-related genes were similar between the primary aetiology subgroups (2.3% vs. 3%; $p = 0.5$) (*Table 3*). Among participants with non-ischaemic idiopathic aetiology, rare variants in tier-1 DCM genes were identified in 10% (23/229), while 2.6% (6/229) had at least one variant in tier-2 or -3 DCM genes.

Only one rare unambiguously pathogenic missense variant, affecting the *RBM20* gene from tier-1, was identified. All other missense variants had no or ambiguous ClinVar annotations, which were also substantiated by *in silico* algorithm predictions (online supplementary *Appendix S1*).

Baseline clinical characteristics for variant carriers versus non-carriers

Compared to non-carriers, participants with at least one truncating pLoF variant in a tier-1 DCM-related gene were 4.1 years younger (mean age of 60.9 vs. 65.0 years; $p_{adj} = 4 \times 10^{-3}$), less likely to present with HF of ischaemic origin (37.3% vs. 67.4%; $p_{adj} = 4 \times 10^{-4}$), had lower body mass index (27.8 vs. 29.4 kg/m^2; $p_{adj} = 3 \times 10^{-3}$), lower ejection fraction (27.3% vs. 29.8%; $p_{adj} = 6 \times 10^{-3}$), and were less likely to have a history of MI (19.4% vs. 48.1%; $p_{adj} = 4.5 \times 10^{-4}$) or hypertension (52.2% vs. 76.1%; $p_{adj} = 4.5 \times 10^{-4}$) in regression models adjusted for age, sex, smoking status and genetic principal components (*Table 4* and *Figure 2*). In addition, carriers of truncating variants in tier-1 DCM-related gene were more likely to be hospitalized for HF than

Table 1 Patient characteristics and demographics of the PARADIGM-HF cohort

Characteristics	WES cohort ($n = 1412$)	Non-WES cohort ($n = 6987$)	Overall PARADIGM-HF ($n = 8399$)	p-value WES vs. non-WES cohort[a]
Age (years)	64.8 ± 10.7	63.6 ± 11.5	63.8 ± 11.4	< 0.001
Female sex	18.7 (265)	22.4 (1567)	21.8 (1832)	0.002
Race				< 0.001
White	82.9 (1171)	62.6 (4373)	66.0 (5544)	
Black	1.8 (26)	5.8 (402)	5.1 (428)	
Asian	12.7 (179)	19.0 (1331)	18.0 (1510)	
Other	2.5 (36)	12.6 (881)	10.9 (917)	
SBP (mmHg)	121.4 ± 15.7	121.4 ± 15.3	121.4 ± 15.3	NS
DBP (mmHg)	73.3 ± 10	73.6 ± 10.0	73.6 ± 10.1	NS
Pulse (bpm)	72.5 ± 12.2	72.3 ± 12.0	72.4 ± 12.0	NS
BMI (kg/m^2)	29.3 ± 5.4	27.9 ± 5.5	28.1 ± 5.5	< 0.001
Serum creatinine, mg/dl	100.8 ± 25.4	98.7 ± 26.4	99.0 ± 26.2	0.01
Clinical features of HF				
EF (%)	29.7 ± 6.0	29.4 ± 6.3	29.5 ± 6.2	NS
NYHA class				
I	4.7 (66)	4.6 (323)	4.6 (389)	NS
II	71.9 (1015)	70.3 (4912)	70.6 (5927)	NS
III	22.8 (322)	24.3 (1701)	24.1 (2023)	NS
IV	0.6 (9)	0.7 (51)	0.7 (60)	NS
Aetiology				
Ischaemic	65.8 (929)	58.8 (4107)	60 (5036)	< 0.001
Non-ischaemic	34.2 (483)	41.2 (2880)	40 (3363)	< 0.001
Hypertensive	28.8 (139/483)	28.8 (829/2880)	28.9 (968/3363)	NS
Idiopathic	47.4 (229/483)	47.4 (1366/2880)	47.4 (1595/3363)	NS
Other	23.8 (115[b]/483)	23.8 (685/2880)	23.8 (800[c]/3363)	NS
Medical history				
Hypertension	75.0 (1059)	69.9 (4881)	70.7 (5940)	< 0.001
Diabetes	39.1 (552)	33.5 (2344)	34.5 (2896)	< 0.001
Atrial fibrillation	43.1 (608)	35.5 (2483)	36.8 (3091)	< 0.001
Prior HF hospitalization	60.7 (857)	63.3 (4417)	62.8 (5274)	NS
Myocardial infarction	46.7 (659)	42.6 (2975)	43.3 (3634)	0.005
Stroke	9.8 (139)	8.4 (586)	8.6 (725)	NS
COPD	14.7 (207)	12.5 (873)	12.9 (1080)	0.03
Cancer	6.4 (90)	4.6 (323)	4.9 (413)	0.007

Values are given as % (n), or mean ± standard deviation.
BMI, body mass index; COPD, chronic obstructive pulmonary disease; DBP, diastolic blood pressure; DCM, dilated cardiomyopathy; EF, ejection fraction; HF, heart failure; MI, myocardial infarction; NYHA, New York Heart Association; SBP, systolic blood pressure; WES, whole exome sequencing.
[a]Fisher exact test for contingency tables and t-test for comparing age between the two groups.
[b]The 115 WES PARADIGM-HF with other aetiologies included: 38 infective/viral, 26 alcoholic, 18 primary valvular, 7 diabetic, 5 drug-related, 1 peripartum-related, and 20 other.
[c]The 800 PARADIGM-HF with other aetiologies included: 185 infective/viral, 158 alcoholic, 110 valvular, 66 diabetic, 30 drug-related, 14 peripartum-related, and 237 other[29].

non-carriers (74.6% vs. 60%, $p = 0.04$); however, this association was not significant after correction for multiple testing ($p_{adj} = 0.14$) (*Table 4*). When carriers of tiers-2 and -3 DCM-related genes were considered in addition to carriers of tier-1, the baseline clinical characteristics of carriers of rare truncating pLoFs of tier-2 and -3 genes were similar to those with no variants in DCM-related genes (online supplementary *Appendix S1*).

Finally, in analyses including carrier status for rare missense variants as a separate variable to the truncating pLoF carrier status, carriers of predicted pathogenic missense variants were less likely to have a history of MI ($p_{adj} < 0.05$) (online supplementary *Appendix S1*).

Clinical outcomes for variant carriers versus non-carriers

Among the 1412 participants with WES data, over a median follow-up period of 27 months, there were 150 cardiovascular deaths, and 196 participants were hospitalized for HF. The incidence of cardiovascular death or HF rehospitalization (primary

Table 2 Distribution of truncating predicted loss-of-function (pLoF) variant carriers stratified by genes, dilated cardiomyopathy (DCM)-gene tiers and pLoF prioritization scores, with estimated prevalence of genetic heart failure with reduced ejection fraction across the three tiers of DCM genes

Tier	Genes	Proteins	Scores			% carriers ($n = 1412$)
			1 (LoF)	2 (pLoF)	3 (pLoF)	
1	*BAG3*	BCL2-associated athanogene 3	1	0	0	0.07
1	*TNNT2*	Cardiac troponin T	0	0	3	0.21
1	*SCN5A*	Voltage gated sodium channel, Type V, Alpha	0	1	0	0.07
1	*RBM20*	RNA-binding protein 20	0	1	0	0.07
1	*LMNA*	Lamin A/C	1	0	0	0.07
1	*FLNC*	Filamin C	0	3	0	0.21
1	*DSP*	Desmoplakin	1	1	1	0.21
1	*TTN*	Titin	14	41	0	3.90
1	*DES*, *MYH7*, *PLN*, *TNNC1* – no pLoFs					
1	Tier-1		17	47	4	**4.82**
2	*NEXN*	Nexilin F-actin-binding protein	1	0	4	0.35
2	*TNNI3*	Cardiac troponin I	0	0	1	0.07
2	*VCL*	Vinculin	0	1[a]	0	0.07
2	*ACTC1*, *ACTN2*, *JPH2*, *TPM1* – no pLoFs					
2	Tier-2		1	1[a]	5	**0.42**
	Tier-1, -2		18	47 + 1[a]	9	**5.24**
3	*ABCC9*	ATP-binding cassette, subfamily C, member 9	0	0	5	0.35
3	*ANKRD1*	Cardiac ankyrin repeat protein	0	0	2 + 1[b]	0.14
3	*DSG2*	Desmoglein 2	0	0	1	0.07
3	*LAMA4*	Laminin, Alpha 4	0	0	1	0.07
3	*MYH6*	α-Myosin heavy chain	0	0	3	0.21
3	*MYPN*	Myopalladin	0	0	1	0.07
3	*NEBL*	Nebulette	0	0	13	0.92
3	*OBSCN*	Obscurin	0	0	4	0.28
3	*TNNI3K*	TNNI3-interacting kinase	0	0	2	0.14
3	*CSRP3*, *CTF1*, *DTNA*, *EYA4*, *GATAD1*, *ILK*, *LDB3*, *MYBPC3*, *MYL2*, *NKX2-5*, *PLEKHM2*, *PRDM16*, *PSEN2*, *SGCD*, *TBX20*, *TCAP* – no pLoFs					
3	Tier-3		0	0	32 + 1[b]	**2.27**
	Tier-1, -2, -3		18	47 + 1[a]	41 + 1[b]	**7.51**

DCM, dilated cardiomyopathy; LOEUF, loss of function observed/expected upper-bound fraction; LoF, loss-of-function; LOFTEE, Loss of Function Transcript Effect Estimator; pLOF, predicted loss-of-function.

The totals are computed only considering unique individuals; the two patients bearing truncating variants in distinct DCM genes are counted only once, for the highest evidence tier respectively, and not counted in the lines noted with (a) and (b).

[a]Denotes a patient with concomitant truncating pLoF variants in *VCL* (tier-2) and *TTN* (tier-1) – this patient is counted only once for tier-1.

[b]Denotes a patient with concomitant truncating pLoF variants in and *ANKRD1* (tier-3) and *NEXN* (tier-2) – this patient is counted once for tier-2.

Prioritization scores assigned to variants:

Score 1 – unambiguous ClinVar 'pathogenic' or 'likely pathogenic' annotation.

Score 2 – all the following apply: ambiguous or no ClinVar annotations; variant affects most LoF depleted and evolutionarily constrained genes/transcripts (within the first and second LOEUF decile); high confidence annotation by LOFTEE.

Score 3 – ambiguous or no ClinVar annotations; variant affects either (i) most evolutionarily constrained genes/transcripts (within the first and second LOEUF decile) with a low confidence annotation by LOFTEE, or (ii) least evolutionarily constrained genes (LOEUF decile is higher than second) with a high confidence annotation by LOFTEE.

composite outcome of PARADIGM-HF) as well as the rates of cardiovascular death, HF rehospitalization, all-cause death or sudden death were comparable among participants with and without rare truncating pLoF variants in tier-1 genes (*Table 4*).

Discussion

In this *post hoc* analysis of PARADIGM-HF, we report the prevalence of rare variants in 44 genes assigned to three tiers according to definitive (or strong), moderate, or limited evidence for a causal role in DCM,[14] in order to increase our understanding of the genetic basis of HF in a classic HFrEF population. Overall, 4.8% of participants with HFrEF had rare truncating pLoF variants in genes with definitive or strong evidence (tier-1) for association with DCM, with the rare pLoF variant prevalence increasing to ~7.5% when all 44 DCM genes were considered.

In addition to *TTN* (accounting for ~80% of the tier-1 variants), pathogenic/deleterious variants were also detected, albeit infrequently, in other tier-1 DCM genes[6,7,15,17] (*FLNC*, *DSP*, *TNNT2*, *BAG3*, *SCN5A*, *RBM20*, and *LMNA*), although we did not identify

Table 3 Prevalence of predicted loss-of-function rare mutations according to aetiology of heart failure, in PARADIGM-HF and in other cohorts

Cohort	Gene list	Prevalence in entire cohort, % (*n*)	Prevalence in ischaemic primary HF aetiology, % (*n*)	Prevalence in non-ischaemic primary HF aetiology, % (*n*)	Prevalence in non-ischaemic primary HF aetiology groups, % (*n*)		
					Idiopathic	Hypertensive	Other
PARADIGM-HF, HFrEF	*n*	1412	929	483	229	139	115
	tier-1 (truncating pLoFs)	**4.82 (68)**	**2.7 (25)**	**8.9 (43)**	**10.0 (23)**	**7.9 (11)**	**7.8 (9)**
	tier-1 (pathogenic missense)	**0.071 (1)**	**0.1 (1)**	**0**	**0**	**0**	**0**
	tier-1 (all variants)	**4.9 (69)**	**2.8 (26)**	**8.9 (43)**	**10.0 (23)**	**7.9 (11)**	**7.8 (9)**
	tier-2, -3 (all variants)[c]	2.7 (38)	3.0 (27)	2.3 (11)	2.6 (6)	2.2 (3)	1.7 (2)
	tier-1, -2, -3 (all variants)	7.6 (107)	5.7 (52)	11.2 (54)	12.6 (29)	10 (14)	9.6 (11)
CHARM and CORONA, HFrEF[a]	*n*	4776	4117	640	435	110	95
	tier-1 (all variants)	**3.0**	**NA**	**NA**	**NA**	**NA**	**NA**
	tier-2, -3 (8 genes from tier-3 and 14 other genes, all variants)	0.5	NA	NA	NA	NA	NA
	tier-1, -2, -3 (8 genes from tier-3 and 14 other genes, all variants)	3.5	2.8 (114)	8.1 (52)	9.9 (43)	3.6 (4)	5.3 (5)
Genomics England Limited (GEL), DCM[b]	*n*	324					
	tier-1 (truncating pLoFs)	**18.5 (60)**					
	tier-1 (pathogenic missense)	**2.5 (8)**					
	tier-1 (all variants)	**21 (68)**					
	tier-2, -3 (all variants)[c]	**3.4 (11)**					
	tier-1, -2, -3 (all variants)	24.4 (79)					

DCM, dilated cardiomyopathy; HF, heart failure; HFrEF, heart failure with reduced ejection fraction; pLoF, predicted loss-of-function.
[a]List of 41 genes including tier-1 and -2 genes and 8 genes from tier-3. In the publication, truncating variants and unambiguous pathogenic missense mutations were included.[12] Only 6 variants of all 169 rare variants identified are on genes not on the tiered list.
[b]All patients in Genomics England were diagnosed with DCM, with aetiology unknown.
[c]Both in PARADIGM-HF and Genomics England only truncating pLoFs were found in genes from tier-2 and -3. No unambiguous ClinVar 'pathogenic' missense mutations were found.

pLoF variants (neither truncating nor unambiguously pathogenic missense) in *DES*, *MYH7*, *PLN*, and *TNNC1* in this PARADIGM-HF subgroup.

Rare, deleterious DCM-causing variants were identified in ~3.5% of patients in a large cohort of chronic/symptomatic HFrEF patients ($n = 4776$) mostly of ischaemic origin from the CHARM and CORONA trials.[9] The list of genes examined by Povysil *et al.*[9] had a high overlap to the list curated by Jordan *et al.*[14] and included genes in tier-1 and -2 and 8/25 genes from tier-3. Like in our observations, most of the rare variants were detected in tier-1 genes (affecting 3% of the patients), with variants in *TTN* being the most prevalent. The same study also found 2.6% rare variant carriers in 767 HFpEF patients and 0.7% in 13 156 controls (not screened for heart disease).[9] Similarly, 3% of participants with HF (irrespective of ejection fraction) from the UK Biobank ($n = 5344$) carried rare variants in tier-1 genes.[30] Finally, the prevalence of DCM-causing rare variants is low (0.3–1.2%) in broader population samples.[9,31,32]

As expected, the prevalence of pLoF variants in tier-1 DCM-related genes was significantly higher in patients with non-ischaemic as compared to those classified as having ischaemic primary HF aetiology ($p = 6.3 \times 10^{-7}$), but surprisingly not for pLoF variants in tier-2 and -3 genes ($p = 0.5$). Similar enrichments for rare variants in the non-ischaemic compared to the ischaemic group were also observed in the CHARM and CORONA studies[9] (8.1% vs. 2.8%, $p = 1.7 \times 10^{-9}$).

Within the non-ischaemic group, ~10% of idiopathic HFrEF patients carried variants in tier-1 genes in the subgroup of PARADIGM-HF, which was not significantly different from carrier prevalence in other non-ischaemic categories (7.9%, $p = 0.42$). A similar prevalence of carriers in tier-1, -2, and some genes from tier-3 was observed in the idiopathic category of HFrEF in the CHARM study.[9] The higher prevalence in the non-ischaemic idiopathic (10%) group in PARADIGM-HF is also largely comparable to observations made in DCM cohorts (e.g. 12% carriers in tier-1 genes in non-familial DCM,[18] 11% carriers in tier-1 genes in DCM from UK Biobank,[30] 17% carriers among 56 genes encompassing tiers-1, -2, -3 in a broad range outpatient DCM cohort[33]). Higher prevalence rates (~25–35%) were observed in DCM cohorts enriched for familial cases.[18,33] A DCM cohort ($n = 324$) from Genomics England (GEL), where recruitment was based on strong medical suspicion of a genetic component to the disease, was analysed using the same pipeline as for PARADIGM-HF. Indeed, for tier-1 DCM genes, a significantly higher prevalence of pathogenic variant carriers was observed in GEL compared to the non-ischaemic idiopathic components of the PARADIGM-HF cohort (18.5% truncating pLoF; 21.0% truncating pLoF and confirmed pathogenic missense variants). No significant enrichment was observed for variants tier-2 and -3 DCM genes (online supplementary *Appendix S1*).

The differences in prevalence and distribution patterns observed among the aetiologic categories of HFrEF of tier-1 DCM-related genes (enrichment in the non-ischaemic group of HFrEF cohorts and DCM cohorts) and tier-2 and -3 DCM-related genes (more equal distribution across aetiologic categories of HFrEF) may suggest that despite heterogeneity in genetic loci and penetrance, variants on tier-1 DCM-related genes can possibly act more often as independent contributors to HFrEF pathogenesis[9,30] than variants in tiers -2 and -3. This can be further supported by observations

Table 4 Carrier versus non-carrier comparison for various clinical features for the tier-1 of dilated cardiomyopathy genes

Continuous clinical characteristic at baseline	Mean (min, max) among non-carriers	Mean (min, max) among carriers	Regression coefficient (β-value)	*p*-value	Adj. *p*-value
Age, years	65.0 (64.5, 65.4)	60.9 (58.7, 63.2)	−4.17 (−6.61, −1.72)	8.6×10^{-4}***	4.3×10^{-3}**
EF, %	29.8 (29.5, 30.1)	27.3 (26.0, 28.7)	−2.37 (−3.81, −0.92)	1.4×10^{-3}**	5.8×10^{-3}**
BMI, kg/m^2	29.4 (29.2, 29.7)	27.8 (27.1, 28.5)	−2.22 (−3.46, −0.97)	5.0×10^{-4}***	3.2×10^{-3}**
Systolic BP, mmHg	121.6 (120.9, 122.3)	118.9 (116.1, 121.7)	−2.44 (−6.19, 1.32)	NS	NS
Diastolic BP, mmHg	73.2 (72.8, 73.7)	74.9 (72.7, 77.0)	0.95 (−1.49, 3.39)	NS	NS
Pulse, bpm	72.5 (71.9, 73.1)	73.2 (70.8, 75.7)	0.10 (−2.89, 3.09)	NS	NS
eGFR, mL/min/1.73 m^2	65.8 (64.8, 66.8)	68.7 (64.2, 73.3)	0.63 (−4.36, 5.62)	NS	NS

Categorical clinical characteristic at baseline	Non-carriers ($n=1338$), % (n)	Carriers ($n=67$[a]), % (n)	OR (95% CI)	*p*-value	Adj. *p*-value
Ischaemic	67.4 (902)	37.3 (25)	0.32 (0.18–0.53)	2.4×10^{-5}***	4.5×10^{-4}***
Hypertension	76.1 (1018)	52.2 (35)	0.34 (0.2–0.58)	5.4×10^{-5}***	4.5×10^{-4}***
Diabetes	39.3 (526)	35.8 (24)	0.89 (0.52–1.48)	NS	NS
MI	48.1 (644)	19.4 (13)	0.27 (0.14–0.49)	3.8×10^{-5}***	4.5×10^{-4}***
Prior HF hospitalization	60 (803)	74.6 (50)	1.82 (1.05–3.31)	4.0×10^{-2}*	NS
Stroke	10 (134)	7.5 (5)	0.81 (0.28–1.88)	NS	NS
COPD	14.9 (199)	10.4 (7)	0.69 (0.28–1.46)	NS	NS
Cancer	6.4 (86)	6.0 (4)	1.39 (0.4–3.69)	NS	NS
AF	43 (575)	47.8 (32)	1.47 (0.87–2.51)	NS	NS
NYHA class			0.75 (0.41–1.33)	NS	NS
I	4.6 (61)	7.5 (5)			
II	71.8 (961)	71.6 (48)			
III	23.1 (309)	19.4 (13)			
IV	0.5 (7)	1.5 (1)			
Clinical outcomes					
CV death	10.9 (146)	6 (4)	0.57 (0.17–1.42)	NS	NS
Rehospitalization	13.7 (183)	19.4 (13)	1.67 (0.85–3.08)	NS	NS
CV death or rehospitalization	20.6 (276)	20.9 (14)	1.09 (0.57–1.96)	NS	NS
All-cause death	13.5 (181)	6.0 (4)	0.46 (0.14–1.14)	NS	NS
Resuscitated sudden death	0.7 (9)	0	NA	NA	NA
Sudden death	3.9 (52)	1.5 (1)	0.35 (0.02–1.66)	NS	NS
Non-fatal MI	2.8 (37)	0	NA	NA	NA
Non-fatal stroke	2.5 (34)	4.5 (3)	1.66 (0.38–4.98)	NS	NS

AF, atrial fibrillation; BMI, body mass index; BP, blood pressure; CI, confidence interval; COPD, chronic obstructive pulmonary disease; CV, cardiovascular; DCM, dilated cardiomyopathy; EF, ejection fraction; eGFR, estimated glomerular filtration rate; HF, heart failure; MI, myocardial infarction; NA, not available; OR, odds ratio.
*$p<0.05$.
**$p<0.01$.
***$p<0.001$.
[a]Data presented are from linear models adjusted for genetic principal components that were available for 1405 patients, age sex and smoking status.

with regard to the phenotypic characteristics of PARADIGM-HF HFrEF patients with variants in tier-1 genes. They were significantly younger than non-carriers, had lower body mass index and ejection fraction (even after adjusting for primary HF aetiology), had fewer comorbidities such as MI and hypertension, and trended towards increased prior HF hospitalizations. Interestingly, compared with non-carriers, carriers of rare mutations in DCM genes seem to present with lower ejection fraction across the population continuum including healthy volunteers to general population,[9,31,32] patients with HFrEF (present study) and in those with DCM.[32]

Carriers of tier-2 and -3 variants had phenotypic characteristics similar to non-carriers in our study. Of note, tier-2 genes *TPM1* and *VCL* have been associated with younger onset, but in our study no participants with variants in these genes and no concomitant variants in tier-1 were identified. Studies in larger cohorts may be needed to increase statistical power with regard to variants on tier-2 and -3 genes and related observations.

Higher percentages (~8%) of tier-1 truncating pLoF variants were identified in HFrEF patients with hypertensive and other non-ischaemic primary HF aetiology in PARADIGM-HF (e.g. viral, alcoholic, valvular, diabetic, drug, peripartum, etc.), in line with the key finding in a recent study[18] where 19% of patients with established, acquired or non-genetic causes of DCM still carried a (likely) pathogenic rare variant. Of interest, a small percentage of rare variants in tier-1 genes were identified in HFrEF patients of primary ischaemic aetiology in PARADIGM-HF (2.7%), similar to findings

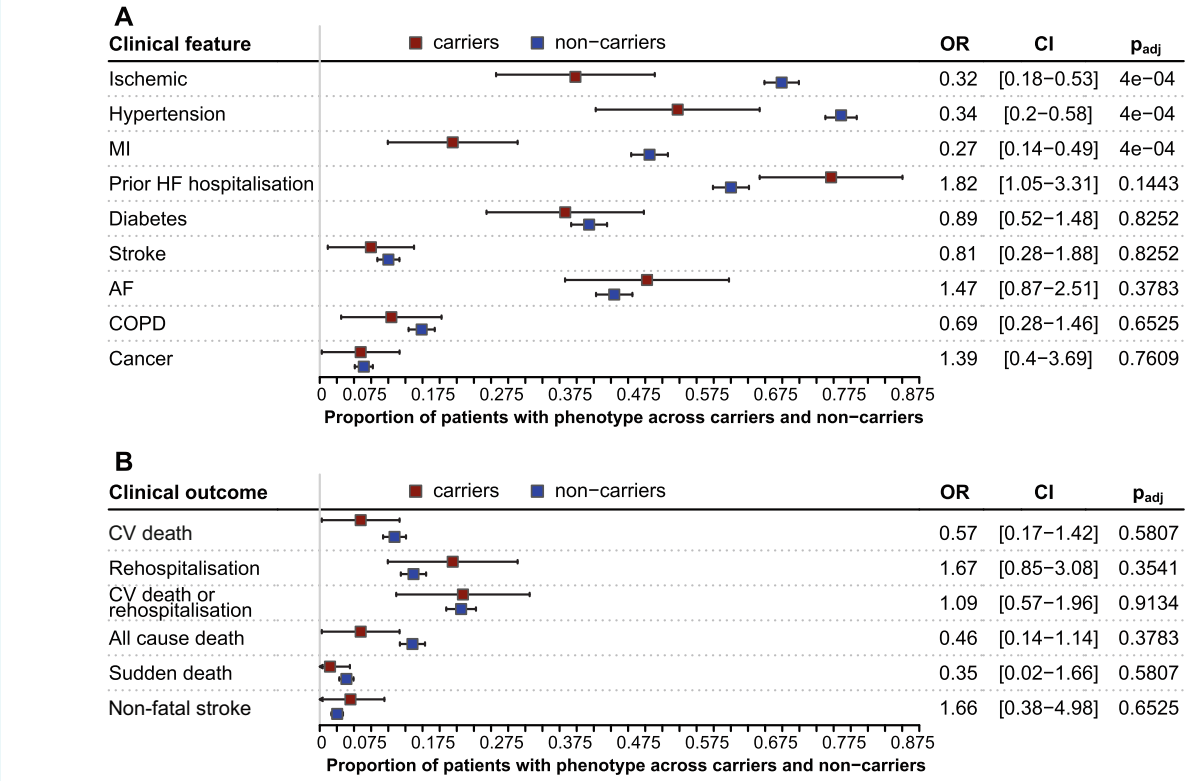


Figure 2 Forest plots for (*A*) baseline binary features and (*B*) clinical outcomes. AF, atrial fibrillation; CI, confidence interval; COPD, chronic obstructive pulmonary disease; CV, cardiovascular; HF, heart failure; MI, myocardial infarction; OR, odds ratio.

in CHARM and CORONA (2.8%).[9] These observations further support the hypothesis of the gene−environment-epigenetic interactions[14,17]: a genetic hit could increase susceptibility for adverse remodelling and HFrEF development in the presence of an environmental/physiological insult (such as an ischaemic event) by disrupting cardiomyocyte physiology[14,17] and consequently ventricular function and structural integrity (such as increased dilatation). Interactions with other contributors to risk may particularly be likely in the presence of pathogenic variants with a 'weak' phenotypic effect and low penetrance, such as those in tier-2 and -3 genes that are more equally distributed among the HF primary aetiology groups and have a less clear contribution specifically to DCM evolution.

Limitations

This *post hoc* exploratory analysis has several limitations. As in similar randomized clinical trials, primary aetiology of HFrEF in PARADIGM-HF was as reported by investigators, with no specific instructions provided as to how to identify aetiology. Patients may not have been exhaustively investigated for specific causes of HF, and hence some misclassification of primary aetiology is possible. The primary aetiology was attributed based on clinical judgment of an evident and traceable clinical event (e.g. MI or history of hypertension in absence of any other comorbidity) or a putative event emerging from clinical examination (e.g. myocarditis) that occurred before the manifestation of HFrEF; but no 'objective' data to confirm the clinical diagnosis was required (e.g. no coronary computed tomography angiography for confirming ischaemic aetiology). Additionally, the ischaemic event in a patient with reported ischaemic primary aetiology may be a secondary or 'bystander' event. We also carried out a sensitivity analysis for all patients classified with primary non-ischaemic idiopathic aetiology stratified by presence or absence of clinical evidence that *conservatively* suggest any potential atherosclerotic component at baseline. This analysis indicated similar prevalence of tier-1 truncating pLoF rare variants in both strata (one supplementary *Appendix S1*). Although a broad range of clinical characteristics and outcomes have been well documented in PARADIGM-HF,[19,29] WES data were available from a fraction (~17%) of the entire trial. The small sample size of this subgroup, the relatively short follow-up period and infrequent clinical events result in low power for the statistical tests contrasting carriers and non-carriers for clinical outcomes. Finally, the WES subgroup included largely White and male participants, limiting generalizability to broader populations.

Conclusions

In conclusion, our results shed further light on the genetic basis of HFrEF. This *post hoc* analysis of PARADIGM-HF shows the presence

of rare variants in DCM-associated genes in a proportion of chronic HFrEF patients, with ~5% harbouring a deleterious pLoF variant in 8 out of 12 genes definitively or strongly associated with DCM. Carrier patients are significantly enriched in the non-ischaemic group and present with different clinical baseline characteristics (HF at a younger age, lower ejection fraction and body mass index, less likely to have a history of MI and hypertension), but similar clinical outcomes to non-carriers, supporting an important role for these variants in HF pathogenesis (*Graphical Abstract*). Additional research is warranted to further our understanding of the roles of DCM-related genes in HF development, while signalling that tier-1 DCM-related genes could be prioritized in both early diagnostics and development of new personalized therapeutic interventions to prevent or delay HF onset.

Acknowledgements

Medical writing and editorial support was provided by Nagabhushana Ananthamurthy and Shivani Vadapalli of Novartis Healthcare Private Limited, India. Comparison to the DCM patients in Genomics England was made possible through access to the data and findings generated by the 100 000 Genomes Project. The 100 000 Genomes Project is managed by Genomics England Limited (a wholly owned company of the Department of Health and Social care). The 100 000 Genomes Project is funded by the National Institute for Health Research and NHS England. The Wellcome Trust, Cancer Research UK and the Medical Research Council have also funded research infrastructure. The 100 000 Genomes Project uses data provided by patients and collected by the National Health Service as part of their care and support.

Supplementary Information

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

Funding

The PARADIGM-HF study was funded by Novartis Pharma AG.

Conflict of interest: J.J.V.M. has received payments through Glasgow University from work on clinical trials, consulting and other activities from Alnylam, Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, BMS, Cardurion, Cytokinetics, DalCor, GSK, KBP Biosciences, Novartis, Pfizer, Theracos; and personal payments from Abbott, Hikma, Ionis, Sun Pharmaceuticals, Servier. M.P. repots consulting and other fee from Abbvie, Actavis, Amgen, Amarin, AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Casana, CSL Behring, Cytokinetics, Johnson & Johnson, Lilly, Moderna, Novartis, ParatusRx, Pfizer, Relypsa, Salamandra, Synthetic Biologics, Theravance. S.D.S. has received research grants from Actelion, Alnylam, Amgen, AstraZeneca, Bellerophon, Bayer, Bristol Myers Squibb, Celladon, Cytokinetics, Eidos, Gilead, GlaxoSmithKline, Ionis, Lilly, Lone Star Heart, Mesoblast, MyoKardia, the National Institutes of Health/National Heart, Lung, and Blood Institute, Neurotronik, Novartis, Novo Nordisk, Respicardia, Sanofi-Pasteur, and Theracos; and has consulted for Abbott, Action Akros, Alnylam, Amgen, Arena, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol Myers Squibb, Cardior, Cardurion, Corvia, Cytokinetics, Daiichi Sankyo, Gilead, GlaxoSmithKline, Ironwood, Lilly, Merck, Myokardia, Novartis, Roche, Takeda, Theracos, Quantum Genetics, Cardurion, AoBiome, Janssen, Cardiac Dimensions, Tenaya, Sanofi-Pasteur, Dinaqor, Tremeau, CellProThera, Moderna, and American Regent. A.S.D. has received research grant support from Abbott, Alnylam, AstraZeneca, Bayer, and Novartis; and has received consulting fees from Abbott, Alnylam, Amgen, AstraZeneca, Biofourmis, Boehringer Ingelheim, Boston Scientific, Cytokinetics, Lupin Pharma, Merck, Novartis, Relypsa, Regeneron, and Sun Pharma. J.L.R. has received grants and consulting fees from Novartis and consulting fee from AstraZeneca, BMS and Bayer. M.R.Z. has received consulting fee from Novartis for participation in the PARADIGM-HF executive committee and from Abbott, Boston Scientific, CVRx, EBR, Endotronics, Ironwood, Merck, Medtronic, and Myokardia V Wave. All other authors are employees of Novartis.

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