JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 8, NO. 7, 2023

ORIGINAL RESEARCH - CLINICAL

Multimodal Detection and Targeting of Biopsy-Proven Myocardial Inflammation in Genetic Cardiomyopathies

A Pilot Report

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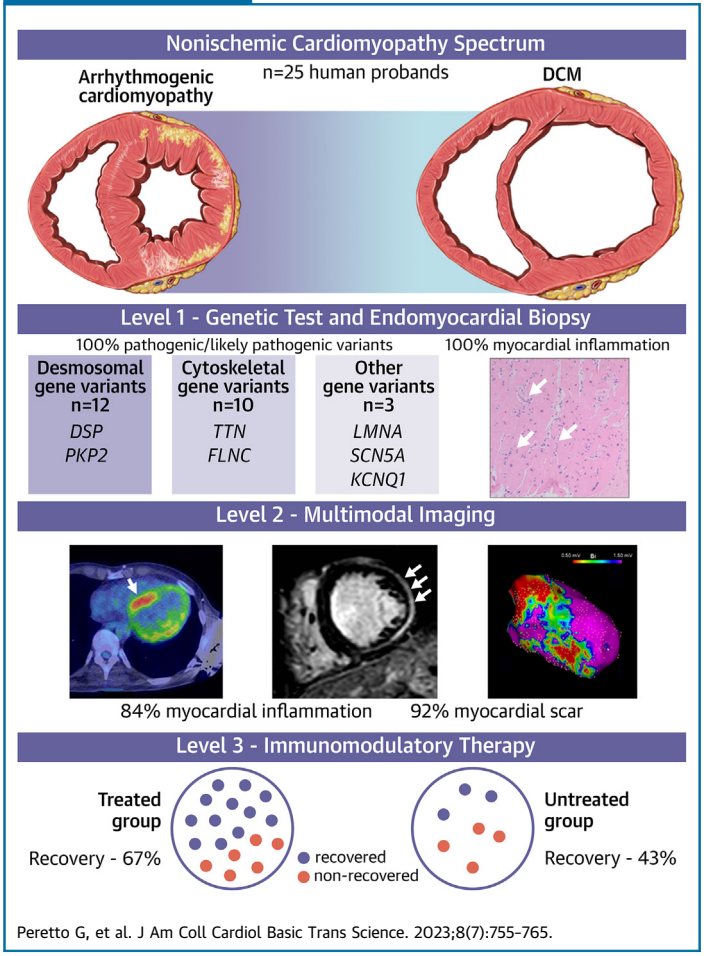
VISUAL ABSTRACT



Peretto G, et al. J Am Coll Cardiol Basic Trans Science. 2023;8(7):755-765.

HIGHLIGHTS

- **M-Infl proven using EMB may be found in patients with a spectrum of genetic cardiomyopathies.**
- **Multimodal imaging, including CMR and PET, may allow noninvasive assessment of cardiomyopathy-associated M-Infl.**
- **IMT is a feasible and promising strategy to target cardiomyopathy-associated M-Infl.**

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2023.02.018

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 8, NO. 7, 2023
JULY 2023:755–765

ABBREVIATIONS AND ACRONYMS

CGV = cytoskeletal gene variants

CMR = cardiac magnetic resonance

DGV = desmosomal gene variants

EMB = endomyocardial biopsy

FDG-PET = ^{18}F-fluorodeoxyglucose-positron emission tomography

GV = gene variant

HPB = hot-phase bursts

ICD = implantable cardioverter defibrillator

IMT = immunomodulatory therapy

LGE = late gadolinium enhancement

LLC = Lake Louise criteria

M-Infl = myocardial inflammation

VA = ventricular arrhythmia

VT = ventricular tachycardia

SUMMARY

The authors present a clinical report focused on the overlap between myocarditis and genetic cardiomyopathies of the dilated and arrhythmogenic spectrum. Our cohort was composed of 25 patients undergoing extensive baseline characterization and prospective reassessment by a dedicated multidisciplinary disease unit during a median follow-up of 69 months. We showed that the use of multimodal imaging allowed both discrimination of specific genotypes and identification of myocardial inflammation proven using endomyocardial biopsy. In addition, we showed that the use of immunomodulatory therapy was beneficial for most patients. (J Am Coll Cardiol Basic Trans Science 2023;8:755–765)

Preclinical data support the rationale for identifying and targeting myocardial inflammation (M-Infl) in genetic nonischemic cardiomyopathies.[1-3] However, no consistent clinical reports have been provided so far. The issue is demanding because primary cardiomyopathies account for a relevant proportion of sudden deaths and heart transplantation procedures in the young population.[4,5] Remarkably, lymphocytic inflammatory infiltrates have been described in histology specimens of autoptic or explanted cardiomyopathy hearts.[6,7] Furthermore, hot-phase bursts (HPB) of chest pain and troponin peaks mimicking myocarditis have been recently reported in patients carrying pathogenic mutations in cardiomyopathic genes.[8-10] Although myocarditis overlapping with cardiomyopathy has been already described,[11] no prior reports to our knowledge focused on a uniform cohort of patients with genetic cardiomyopathy and M-Infl proven using endomyocardial biopsy (EMB). In this setting, the diagnostic role of noninvasive multimodality imaging for detecting M-Infl and characterizing phenotypes is unknown. Most importantly, no clinical data currently support the use of immunomodulatory therapy (IMT) in this population. We hereby provide a pilot report on patients with genetic cardiomyopathy and EMB-proven M-Infl, aimed at: 1) detecting M-Infl and characterizing phenotypes by multimodal imaging; and 2) targeting M-Infl using IMT.

METHODS

PATIENT SELECTION. Consecutive symptomatic probands (n = 25) bearing class 4 or 5 mutations[12] in cardiomyopathic genes and undergoing multidisciplinary assessment for M-Infl were retrospectively selected at a third level center from January 2007 to June 2020. Mutations in cardiomyopathic genes were uniformly identified using next-generation sequencing by the Illumina TruSight One-Sequence panel (Illumina). All patients gave written informed consent for enrolment in a research registry approved by the local Institutional Review Board. The study flowchart is shown in **Figure 1**.

MULTIMODAL DIAGNOSTIC WORK-UP. The standard diagnostic work-up included 12-lead electrocardiogram telemonitoring, transthoracic echocardiogram, and assessment of cardiac biomarkers (troponin, brain natriuretic peptide). Furthermore, all patients underwent both EMB and noninvasive imaging techniques to detect M-Infl. At EMB, M-Infl was defined as an inflammatory infiltrate containing ≥14 leukocytes/mm^2 and ≥7 CD3-positive T lymphocytes/mm^2 after histologic and immunohistochemical analyses.[13] The

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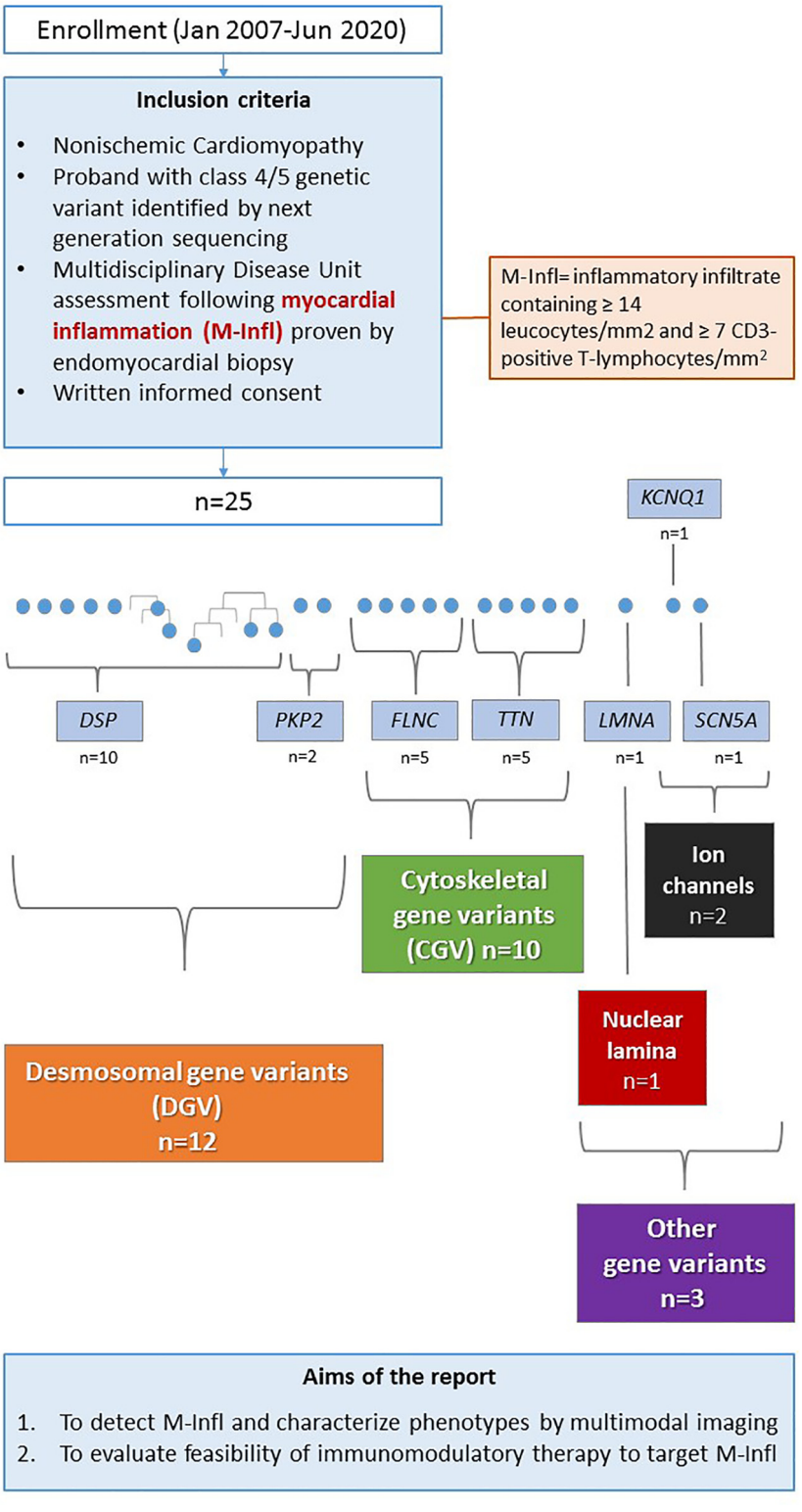
Manuscript received December 12, 2022; revised manuscript received February 15, 2023, accepted February 15, 2023.

FIGURE 1 Study Flowchart



The study flowchart, together with classification of genotypes, is shown for the case series of 25 patients. Five *DSP* probands were from 2 families. CGV = cytoskeletal gene variants; DGV = desmosomal gene variants; DSP = desmoplakin gene; FLNC = filamin C gene; KCNQ1 = potassium channel subunit Q1 gene; LMNA = lamin A/C gene; PKP2 = plakophilin-2 gene; M-Infl = myocardial inflammation; SCN5A = voltage-gated sodium channel subunit 5A gene; TTN = titin gene.

presence of myocyte necrosis and/or degeneration pointed to a diagnosis of myocarditis. Viral genomes were also analyzed using polymerase chain reaction. At cardiac magnetic resonance (CMR), the standard and updated Lake Louise criteria (LLC) were applied to identify M-Infl in patients enrolled before and after 2016, respectively.[14,15] At ^{18}F-fluorodeoxyglucose-positron emission tomography (FDG-PET), M-Infl was defined in the presence of focal or focal-on-diffuse pathologic FDG uptake within the myocardium.[16] Further detail about multimodal imaging is reported in the Supplemental Material.

TREATMENT STRATEGIES. Before treatment, all patients underwent multidisciplinary assessment at a dedicated "disease unit" for myocarditis.[17] Treatment strategies were patient-tailored, and included optimal cardiologic therapy, implantation of cardiac devices, and catheter ablation of arrhythmias. On top of the standard treatment, IMT was started to target M-Infl, provided lack of contraindications and absent intramyocardial pathogenic viral genomes. The criteria for both patient selection and IMT choice mirrored the current standards for immunosuppression in autoimmune myocarditis,[13,18] integrated with the experience of a referral center.[19] In detail, immunomodulatory agents included prednisone, azathioprine, mofetil mycophenolate, colchicine, and anakinra.

OUTCOMES. All patients were periodically evaluated at a dedicated multidisciplinary outpatient facility. In particular, follow-up monitoring occurred every 3 months during IMT, and every 6 months otherwise. Multimodal work-up included blood examinations, echocardiogram, 24-hour Holter electrocardiogram, cardiac device telemonitoring whenever applicable, and either invasive or noninvasive reassessment of M-Infl. Surveillance of IMT toxicity was regularly performed as in autoimmune myocarditis.[19] Beyond cardiac death and heart transplantation, cardiac adverse events during follow-up included HPB (defined as acute chest pain accompanied by troponin peak at least 12 months after the clinical presentation), severe left ventricular systolic dysfunction (defined as left ventricular ejection fraction <35% as currently recommended for the primary prevention of sudden cardiac death),[5] and major ventricular arrhythmias (namely, sustained ventricular tachycardia, ventricular fibrillation or appropriate antitachycardia pacing, or shock by implantable cardioverter defibrillator).

STATISTICAL ANALYSIS. SPSS version 20 (IBM Corp) was used for analysis. Continuous variables are expressed as mean ± SD or median (range), depending on the distribution of data, as assessed using the Shapiro-Wilk test. Accordingly, they were compared using parametric (Student *t*) or nonparametric (Mann-Whitney *U*) tests, respectively. Categorical variables are reported as counts and percentages, and were compared using the Fisher exact test, or using the McNemar test for paired dichotomous data, namely, before and after IMT conditions, or first and last follow-up in untreated patients. Survival curves for familial and nonfamilial cardiomyopathies were generated using the Kaplan-Meier method and compared using the log-rank test. Chi-squared automatic interaction detection algorithms were used to generate classification trees. Two-sided *P* values <0.05 were set as statistically significant.

RESULTS

BASELINE CLINICAL FEATURES. All patients in our series had either pathogenic or likely pathogenic gene variants (GV) associated with cardiomyopathy. In detail, 12 patients (48%) had desmosomal GV (DGV; including n = 10 desmoplakin [*DSP*]; and n = 2 plakophilin-2 [*PKP2*]), and 10 (40%) cytoskeletal GV (CGV) (including n = 5 filamin C [*FLNC*]; and n = 5 titin [*TTN*]). The remaining 3 patients (12%) had other GVs, involving nuclear lamina (lamin A/C [*LMNA*]; n = 1) and ion channels (voltage-gated sodium channel [*SCN5A*]; n = 1; and potassium channel [*KCNQ1*]; n = 1). As shown in **Figure 1**, we included 5 patients from 2 strains, who independently presented with cardiac symptoms before the time of familial screening. The clinical presentation included myocarditis-like chest pain (n = 9), acute heart failure (n = 4), and ventricular arrhythmias (n = 12). The full list of genotypes, clinical presentations, and subsequent work-up are summarized in **Table 1**. Family trees are shown in Supplemental Figure 1.

DETECTION OF M-INFL. As a uniform finding in the series, M-Infl was detected using EMB. In detail, all patients had lymphocytic inflammatory infiltrates, with interstitial edema and low prevalence of necrosis (ie, definite criteria for myocarditis were met only in n = 4 of 25 cases; 16%). Replacement fibrosis was extensively documented (n = 21 of 25; 84%). No intramyocardial viral genomes were found, except for low-load (<500 copies) parvovirus B19 in 3 patients (12%).

TABLE 1 Genotypes, Baseline Features, Diagnostic Work-Up, and Management

PID	Age (y)	Gender	Genotype	Phenotype	LVEF (%)	Diagnostic Work-Up	Cardiac Device	Treatment	IMT
P01	20	Male	*DSP*, c.5428C>T, class 5	AM-like	58	EMB(+), CMR(+), PET(-)	ILR-ICD	Ramipril, sotalol	Prednisone, azathioprine
P02	54	Female	*DSP*, c.7903 G>T, class 5	AM-like	50	EMB(+), CMR(+)	ILR	Ramipril, metoprolol	No
P03	36	Male	*FLNC*, c.2971C>T, class 5	AM-like	55	EMB(+), CMR(+), PET(+)	ILR	Ramipril, metoprolol	Prednisone, azathioprine
P04	30	Female	*FLNC*, c.7002_7003insGG, class 4	Minor VA	50	EMB(+), CMR(-)	ILR-ICD	Ramipril, sotalol	No
P05	29	Female	*DSP*, c.4198 C>T, class 4	AM-like	60	EMB(+), CMR(+)	ILR	Ramipril, metoprolol	Prednisone, azathioprine, mofetil mycophenolate, anakinra, colchicine
P06	23	Female	*DSP*, c.3155_3156del, class 5	Minor VA	44	EMB(+), CMR(-), PET(+)	ILR-ICD	Enalapril, metoprolol, sotalol	Prednisone, azathioprine
P07	40	Male	*TTN*, c.3729+1G>A, class 4	Acute HF	38	EMB(+), CMR(-), PET(+)	No	Ramipril, bisoprolol	Anakinra
P08	52	Male	*FLNC*, c.6561_6564del, class 4	Minor VA	55	EMB(+), CMR(+), CT, PET(-), EAM	ILR	Enalapril, bisoprolol, flecainide, amiodarone	Prednisone, azathioprine
P09	34	Female	*DSP*, c.313C>T, class 5	Major VA	71	EMB(+), CMR(-), PET(-), EAM	ILR	Ramipril, sotalol	Prednisone, azathioprine
P10	42	Female	*FLNC*, c.7233_7236del, class 4	Minor VA	47	EMB(+), CMR(-), PET(-)	ICD	Zofenopril, metoprolol	Prednisone, mofetil mycophenolate
P11	23	Female	*PKP2*, c.1440_1444del, class 4	Major VA	43	EMB(+), CMR(+), CT, PET(+), EAM	ICD	Ramipril, metoprolol, flecainide	Prednisone, azathioprine
P12	45	Female	*DSP*, c.7899dup, class 4	Major VA	48	EMB(+), CMR(+), PET(-)	ICD	Ramipril, bisoprolol	Prednisone, azathioprine
P13	20	Male	*DSP*, c.7903 G>T, class 5	AM-like	62	EMB(+), CMR(+)	No	Zofenopril, bisoprolol	Prednisone, azathioprine
P14	62	Male	*KCNQ1*, c.1031C>T, class 5	AM-like	60	EMB(+), CMR(+)	ILR	Ramipril, metoprolol	No
P15	38	Female	*FLNC*, c.5142C>G, class 4	Acute HF	20	EMB(+), CMR(+)	ILR	Ramipril, bisoprolol	Prednisone, azathioprine, anakinra
P16	52	Male	*TTN*, c.52021C>T, class 5	Minor VA	45	EMB(+), CMR(-), PET(-)	ILR-ICD	Enalapril, bisoprolol, amiodarone	Prednisone, azathioprine, anakinra
P17	22	Male	*DSP*, c.3155_3156del, class 5	AM-like	63	EMB(+), CMR(+)	ILR	Ramipril	No
P18	35	Male	*PKP2*, c.368G>A, class 5	Major VA	60	EMB(+), CMR(-), PET(-)	ICD	Ramipril, sotalol	Prednisone, azathioprine
P19	57	Male	*TTN*, c.72690_72691dup, class 4	Acute HF	23	EMB(+), CMR(+), PET(+)	ICD	Ramipril, sotalol	Prednisone, azathioprine, anakinra
P20	52	Female	*DSP*, c.6496C>T, class 5	Major VA	36	EMB(+), CMR(+)	ILR	Ramipril, bisoprolol, amiodarone	Prednisone, mofetil mycophenolate
P21	36	Male	*SCN5A*, c.659C>A, class 4	AM-like	64	EMB(+), CMR(+)	No	Ramipril, bisoprolol	No
P22	50	Male	*TTN*, c.41641C>T, class 4	Acute HF	25	EMB(+), PET(+)	ICD	Ramipril, bisoprolol, amiodarone	Prednisone, azathioprine, anakinra
P23	64	Male	*TTN*, c.54181G>T, class 4	Major VA	15	EMB(+), CMR(+), PET(+)	ICD	Enalapril, metoprolol	No
P24	15	Female	*DSP*, c.3155_3156del, class 5	AM-like	59	EMB(+), CMR(+)	ILR	Ramipril, metoprolol	No
P25	38	Female	*LMNA*, c.1262_1263del, class 4	Major VA	48	EMB(+), CMR(+), PET(+), EAM	S-ICD-ICD	Metoprolol, amiodarone	Anakinra

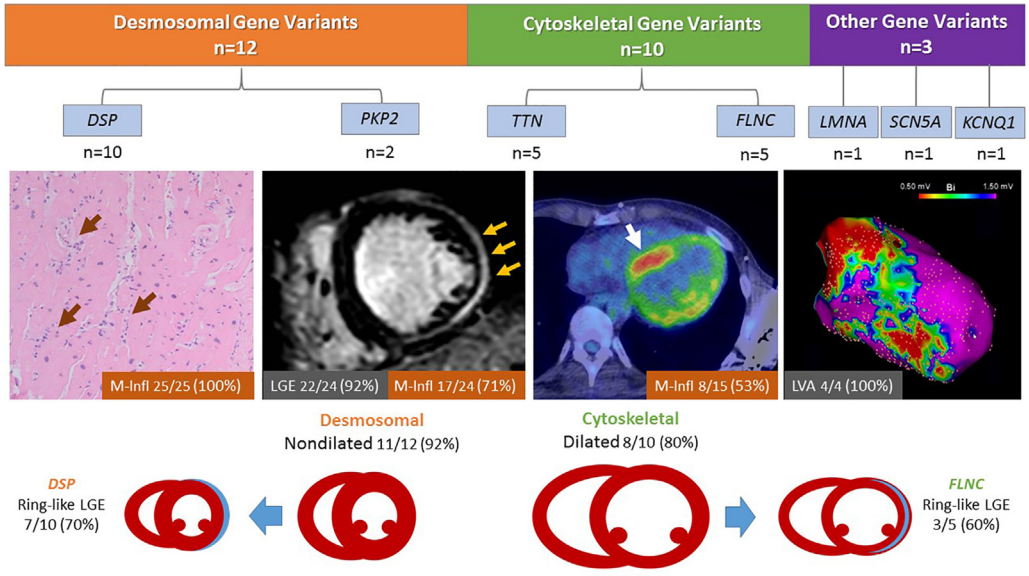
Clinical details about the case series are shown, with patients (P01-P25) listed in chronological order. For each diagnostic examination capable of detecting M-Infl, the symbols (+) and (-) indicate positive and negative results, respectively.

AM = acute myocarditis; CMR = cardiac magnetic resonance; DSP = desmoplakin gene; EAM = electroanatomical mapping; EMB = endomyocardial biopsy; FLNC = filamin C gene; HF = heart failure; ILR = implantable loop recorder; ICD = implantable cardioverter defibrillator; IMT = immunomodulatory therapy; KCNQ1 = potassium channel subunit Q1 gene; LMNA = lamin A/C gene; LVEF = left ventricular ejection fraction; PET = positron emission tomography; PID = patient ID; PKP2 = plakophilin-2 gene; S = subcutaneous; SCN5A = voltage-gated sodium channel subunit 5A gene; TTN = titin gene; VA = ventricular arrhythmias.

Using noninvasive imaging techniques, M-Infl was confirmed in 21 patients (84%). Of the 24 patients undergoing CMR, 22 (92%) had nonischemic late gadolinium enhancement (LGE), and 17 (71%) had abnormal T2-weighted sequences fulfilling the updated LLC. Furthermore, an abnormal FDG uptake was detected in 8 of the 15 patients undergoing FDG-PET scan (53%). Representative examples of the diagnostic work-up are shown in Figure 2.

CLUSTERING BY GENOTYPES. Table 2 summarizes patient features clustered by genotype classes. Compared with the other groups, patients with DGV were younger (mean age: 31 ± 13 years vs 46 ± 11 years), had greater prevalence of females (67% vs 31%), and uniformly presented with myocarditis-like chest pain and ventricular arrhythmias (100%). Conversely, heart failure presentation was exclusive of CGV carriers, who consistently showed higher

FIGURE 2 Genotype-Phenotype Relationships in Inflammatory Cardiomyopathies



The main study findings are summarized. **(Upper)** Study population clustered by genotype classes. **(Middle)** Results of the multimodal diagnostic work-up, including M-Infl detection rate. **(Left to Right)** EMB in a *FLNC* mutation carrier (P03), showing M-Infl with lymphocytic infiltrates (**arrows**, hematoxylin eosin assay), as a uniform finding in the whole cohort; CMR in a *DSP* mutation carrier (P13), showing non-ischemic ring-like pattern of LGE involving the inferolateral basal segments of the left ventricle **(arrows)**; FDG-PET in a *LMNA* mutation carrier (P25), showing abnormal metabolic activity at the interventricular septum **(arrow)**; endocardial bipolar electroanatomical map of the left ventricle in the same *LMNA* mutation carrier (P25), showing extensive LVA at the septum (spectrum of colors other than purple, right anterior oblique view). (Lower) Genotype-phenotype relationships, with prevalence of dilated cardiomyopathy among cytoskeletal gene variants carriers, and ring-like pattern of LGE found in most *DSP* and *FLNC* mutation carriers. CMR = cardiac magnetic resonance; EMB = endomyocardial biopsy; FDG-PET = 18F-Fluorodeoxyglucose positron emission tomography; LGE = late gadolinium enhancement; LVA = low-voltage areas; other abbreviations as in **Figure 1**.

prevalence of dilated cardiomyopathy phenotype (90% vs 7%), frequent N-terminal pro-B-type natriuretic peptide abnormalities (70% vs 40%), and higher median NYHA functional class (II vs I). No remarkable differences among groups were found at histology and cardiac imaging. However, CMR revealed a ring-like pattern of LGE in patients with either *DSP* or *FLNC* mutations (10/15; 67%), and in no alternative genotype carriers (0/7; 0%). Overall, classification trees identified dilated cardiomyopathy and ring-like pattern of LGE as the best discriminators of genotypes (Supplemental Figure 2).

THERAPEUTIC STRATEGIES. After presentation, 8 patients (32%) underwent implantable cardioverter-defibrillator implantation either for secondary (n = 5) or primary prevention (n = 3). In addition, 14 patients (56%) underwent continuous telemonitoring using implantable loop recorders. Standard medical treatment included renin-angiotensin-aldosterone system inhibitors, beta-blockers, and antiarrhythmic drugs, with no remarkable differences among groups (**Tables 1 and 2**). After dedicated multidisciplinary assessment, 18 patients (72%) underwent IMT in addition to optimal medical treatment to target EMB-proven M-Infl. As summarized in Supplemental Figure 3, IMT included 1-5 drugs per patient (**Table 1**), and had an average duration of 17 ± 6 months. No serious adverse events were reported after IMT (Supplemental Table 1).

OUTCOMES. The median follow-up for the cohort was 71 months (range: 21-182 months). No patients died and no one underwent heart transplantation or had de novo left ventricular ejection fraction <35%. Patients with family history of sudden death or cardiomyopathy showed a trend toward significantly lower survival free from cardiac adverse events (Supplemental Figure 4). The full list of events is shown in **Table 3**. Symptomatic HPBs (1-10 episodes

TABLE 2 Comparison Between DGV and CGV Classes

	Total Gene Variants (n = 25)	DGV (*DSP*, *PKP2*) (n = 12)	CGV (*TTN*, *FLNC*) (n = 10)	*P* Value
Baseline features				
Age (y)	39 ± 14	31 ± 13	46 ± 11	**0.009**
Male	13 (52)	4 (33)	7 (70)	0.20
Caucasian	24 (96)	12 (100)	9 (90)	0.45
FH SCD	5 (20)	2 (17)	3 (30)	0.62
FH CM	8 (32)	5 (42)	3 (30)	0.68
Presentation				
AM-like	9 (36)	6 (50)	1 (10)	0.074
HF	4 (16)	0 (0)	4 (40)	**0.029**
VA	12 (48)	6 (50)	5 (50)	1.00
Viral infection <30 d	4 (16)	0 (0)	3 (30)	0.078
CCA	4 (116)	2 (17)	1 (10)	1.000
Syncope	2 (8)	2 (17)	0 (0)	0.48
Palpitation	11 (44)	6 (50)	4 (40)	0.69
NYHA functional class	1 (1-4)	1 (1-1)	2 (1-4)	0.088
Diagnostics				
Echocardiogram	25 (100)	12 (100)	10 (100)	1.00
LVEF (%)	48 ± 15	55 ± 10	37 ± 15	**0.003**
LVEF <50%	12 (48)	4 (33)	7 (70)	0.20
DCM	10 (40)	1 (8)	9 (90)	**<0.001**
RV dilation	1 (4)	0 (0)	1 (10)	0.45
RV dysfunction	2 (8)	0 (0)	2 (20)	0.20
CMR	24 (96)	12 (100)	9 (90)	0.46
LVEDVi (mL/m^2)	88 ± 26	75 ± 10	106 ± 38	**0.014**
LVEF (%)	50 ± 16	57 ± 7	39 ± 18	**0.005**
RVEDVi (mL/m^2)	71 ± 22	66 ± 12	75 ± 31	0.36
RVEF (%)	57 ± 16	60 ± 5	55 ± 23	0.47
Fatty replacement	2/24 (8)	1/12 (8)	1/9 (11)	1.00
T2W-STIR	17/24 (71)	10/12 (83)	4/9 (44)	0.16
Long T2	11/15 (73)	6/8 (75)	3/5 (60)	1.00
Long T1	11/15 (73)	5/8 (63)	4/5 (80)	1.00
High ECV	11/15 (71)	6/8 (75)	4/5 (80)	1.00
LGE, f	22/24 (92)	11/12 (92)	8/9 (89)	1.00
FDG-PET	15 (60)	5 (42)	9 (90)	**0.031**
Abnormal FDG uptake	8/15 (53)	2/5 (40)	5/9 (56)	1.00
EAM	4 (16)	2 (17)	1 (10)	1.00
Low-voltage areas	4/4 (100)	2/2 (100)	1/1 (100)	1.00
EMB	25 (100)	12 (100)	10 (100)	1.00
CD3+ TCL >7/mm^2	25 (100)	12 (100)	10 (100)	1.00
Necrosis	4 (16)	2 (17)	2 (20)	1.00
Replacement fibrosis	21 (84)	11 (92)	8 (80)	1.00
Fatty infiltration	7 (28)	4 (33)	2 (20)	0.65
Viral genome	3 (12)	2 (17)	1 (10)	1.00
Laboratory	25 (100)	12 (100)	10 (100)	1.00
High T-troponin	19 (76)	9 (75)	7 (70)	1.00
High NTproBNP	13 (52)	5 (42)	7 (70)	0.23
High C-reactive protein	7 (28)	2 (17)	3 (30)	0.62
Treatment				
RAAS-inhibitors	24 (96)	12 (100)	10 (100)	1.00
Beta-blockers	24 (96)	11 (92)	10 (100)	1.00
Antiarrhythmics	13 (52)	7 (58)	5 (50)	1.00
Immunosuppressants	18 (72)	9 (75)	8 (80)	1.00

Values are mean ± SD, n (%), median (range), or n/N (%). Baseline clinical features are compared between the main patient classes, namely, DGV and CGV. Significant differences are enhanced in **bold**.

CCA = cardiocirculatory arrest; CD = cluster of differentiation; CGV = cytoskeletal gene variants; CM = cardiomyopathy; CMR = cardiac magnetic resonance; DCM = dilated cardiomyopathy; DGV = desmosomal gene variants; ECV = extracellular volume; FDG-PET = ^{18}F-Fluorodeoxyglucose positron emission tomography; f = fraction; FH = family history; LGE = late gadolinium enhancement; LVEDVi = left ventricular end-diastolic volume; NTproBNP = N-terminal pro-B-type natriuretic peptide; RAAS = renin-angiotensin-aldosterone system; RVEDVi = right ventricular end-diastolic volume; RVEF = right ventricular ejection fraction; SCD = sudden cardiac death; T2W-STIR = T2-weighted short tau inversion recovery; other abbreviations as in Table 1.

TABLE 3 Outcomes

	New Events During Follow-Up				Cumulative Events From Baseline to Follow-Up			
	All DGV (n = 12)	All CGV (n = 10)	All Others (n = 3)	Total (N = 25)	All DGV (n = 12)	All CGV (n = 10)	All Others (n = 3)	Total (N = 25)
HPB	3 (25)	2 (20)	0 (0)	5 (20)	6 (50)	2 (20)	2 (67)	10 (40)
Acute HF	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (40)	0 (0)	4 (16)
LVEF <35%	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (40)	0 (0)	4 (16)
Major[a] VA	2 (17)	1 (10)	1 (33)	4 (16)	6 (50)	2 (20)	1 (33)	9 (36)
ICD implant	2 (17)	2 (20)	1 (33)	5 (20)	5 (42)	6 (60)	1 (33)	12 (75)
VT ablation	1 (8)	0 (0)	1 (33)	2 (8)	2 (17)	0 (0)	1 (33)	3 (12)
NSVT	5 (42)	5 (50)	2 (67)	12 (48)	5 (42)	7 (70)	2 (67)	14 (56)
PVC $>10^3$	8 (67)	6 (60)	1 (33)	15 (60)	9 (75)	6 (60)	1 (33)	16 (64)
AVB	1 (8)	1 (10)	0 (0)	2 (8)	1 (8)	1 (10)	0 (0)	2 (8)
AF	0 (0)	1 (10)	0 (0)	1 (4)	1 (8)	1 (10)	0 (0)	2 (8)

Values are n (%). Outcomes of the case series are shown, together with relationships with genotype classes. [a]Major VA included sustained ventricular tachycardia, ventricular fibrillation of appropriate antitachycardia pacing, or shock by implantable cardioverter-defibrillator.

AF = atrial fibrillation; AVB = second- or third-degree atrioventricular block; DGV = desmosomal gene variants; HPB = hot-phase burst; NSVT = nonsustained ventricular tachycardia; PVC = premature ventricular complexes; other abbreviations as in Table 1.

per patient) were observed in 5 cases (20%), 4 of whom already presented with myocarditis-like chest pain; this group included only *DSP* (n = 3) and *FLNC* (n = 2) genotypes. New major ventricular tachycardia (VT) episodes were documented in 4 patients (16%) carrying mutations in *DSP* (n = 1), *PKP2* (n = 1), *LMNA* (n = 1), and *TTN* (n = 1) genes. Two of them subsequently underwent VT catheter ablation. Documentation of sustained VT was more common among DGV carriers (50% vs 23%). For most genotypes, paucity of adverse events was noted while on IMT as compared with the off-treatment period (Figure 3). In detail, a relative decrease was observed in HPBs, as well as nonsustained ventricular arrhythmia (VA). At last follow-up, signs of M-Infl were documented in 6/18 IMT receivers (33%) and 4/7 untreated cases (57%).

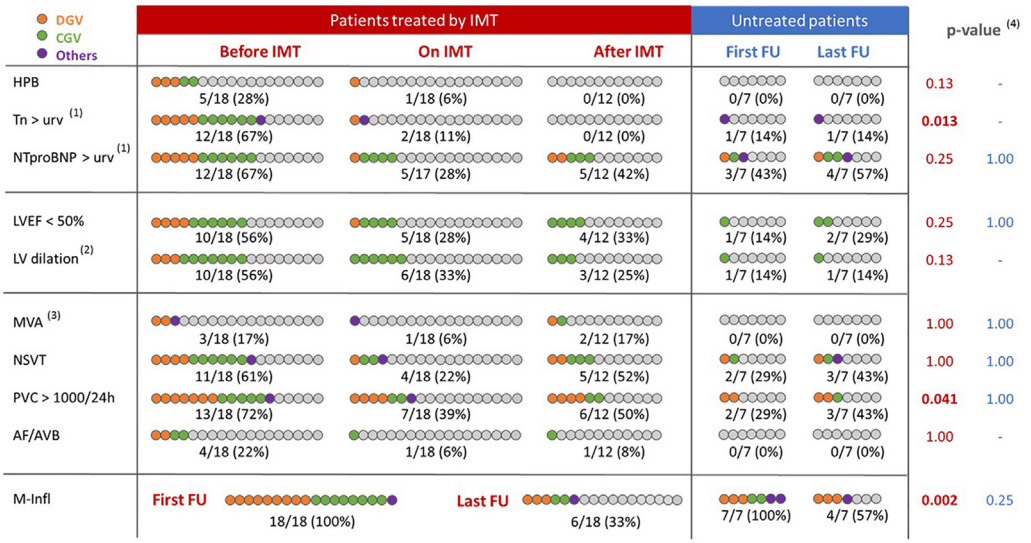
DISCUSSION

MAIN INSIGHTS FROM THE CASE SERIES. We described a cohort of patients with a spectrum of genetic cardiomyopathies[20,21] characterized by uniform documentation of EMB-proven M-Infl. In particular, we found that multimodal imaging is capable of detecting M-Infl in a sizable proportion of patients (84%), allowing further characterization of genotype-phenotype relationships. In addition, we showed targeting M-Infl using IMT is feasible in this population, and that it resulted in a relative reduction in the adverse events rate. Our experience provides preliminary evidence supporting new studies aimed at systematically applying IMT to patients with genetic cardiomyopathy-associated M-Infl.

GENOTYPES. DGV and CGV constituted the most represented genotypes in our cohort (88%). Based on our classification tree analysis (Supplemental Figure 2), dilated cardiomyopathy allowed identification of CGV, adding confirmatory evidence to prior reports about *TTN* and *FLNC* mutation carriers.[22,23] In keeping with recent findings,[24] a ring-like pattern of LGE was found only in *FLNC* and *DSP* mutation carriers, who also showed high prevalence of HPB. Results are consistent with the myocarditis-like presentation described in DGV,[25] and in particular at the very early onset of *DSP* cardiomyopathy.[9,26] On the other hand, all DGV carriers exhibited VA (Table 3), fulfilling the current diagnostic criteria for left ventricular arrhythmogenic cardiomyopathy[27] presenting as arrhythmic myocarditis.[28] Finally, we reported a minority of M-Infl cases associated with ion channels, as recently described in a *SCN5A* mutation carrier.[29] To the best of our knowledge, however, no prior reports described both FDG-PET- and EMB-proven M-Infl in *LMNA* cardiomyopathy (Figure 2), which is known for adverse arrhythmic outcomes.[30]

MULTIMODAL WORK-UP. Multiple diagnostic techniques were adopted to detect M-Infl in our series. In keeping with its major diagnostic role in myocarditis,[13,31] EMB uniformly allowed detection of CD3-positive lymphocytic inflammatory infiltrates. Due to the lack of necrosis, however, the Dallas criteria for classic acute myocarditis were missed for most patients (84%). Borderline chronic myocarditis[13,31] was the dominant finding, and no viral genomes with a

FIGURE 3 **Outcomes in Patients Undergoing Immunomodulatory Therapy**



Multiparametric outcomes are shown for the case series, divided according to the use of IMT in treated (n = 18) and untreated patients (n = 7). Among treated patients, outcomes are shown before, during, and after IMT use. As for untreated patients, comparison is shown between first and last FU. The proportion of patients experiencing adverse outcomes is represented by **colored dots** clustered by genotypes. A relative decrease in the proportion of events was observed in IMT cases, in particular during the active treatment period. For both groups, the *P* values obtained using the McNemar test are shown on the **right**, with significant differences enhanced in **bold**. (1) urv were 14 ng/mL for T-troponin and 125 pg/mL for NTproBNP. (2) LV dilation was defined according to international reference standards using transthoracic echocardiogram.[37] (3) MVA included sustained ventricular tachycardia, ventricular fibrillation, or appropriate antitachycardia pacing, or shock by implantable cardioverter defibrillator. (4) *P* value compares before and after IMT in the treated group, or first and last FU in the untreated group. AF/AVB = atrial fibrillation and/or second- or third- degree atrioventricular block; FU = follow-up; HPB = hot-phase burst; IMT = immunomodulatory therapy; MVA = major ventricular arrhythmias; NSVT = nonsustained ventricular tachycardia; PVC = premature ventricular contractions; urv = upper reference values; other abbreviations as in **Figure 1**.

definite pathogenic role were identified. As a complementary technique, imaging was used to obtain panoramic and multiplanar evaluation of the myocardial inflammatory status, also serving as a baseline reference for noninvasive follow-up reassessment.[32] In this setting, CMR constituted the first-choice examination, as recommended for both myocarditis and cardiomyopathies.[4,5] In particular, M-Infl was assessed using the standard and updated LLC.[14,15] As an alternative technique particularly suitable for cardiac device carriers,[33] FDG-PET was also frequently applied.

IMMUNOMODULATORY THERAPY. Our report was original in showing widespread application of IMT to target cardiomyopathy-associated M-Infl. The issue is relevant because recent data on dilated cardiomyopathy suggest that guideline-directed medical therapy is less effective in patients carrying disease-causing genetic variants.[34] Consistent with prior reports on patients with myocarditis, after dedicated multidisciplinary assessment,[17,19] IMT was feasible and safe.[18] Although by no means could we directly assess IMT effectiveness, a number of clues support IMT's beneficial role: first, M-Infl clearance was more common among IMT receivers (67% vs 43%). Second, **Figure 3** shows that the occurrence of multiple adverse events was relatively lower during the treatment period. Notably, the proportion of HBPs associated with cardiac biomarker elevation, as well as left ventricular systolic dysfunction and VA, were all lower on IMT. These findings point to the possible role of immunomodulatory therapies in reverting the inflammatory pathways that lead to left ventricular dysfunction and arrhythmias.[1-3] Nonetheless, some patients showed a reversion of positive trend after IMT termination; likely, longer or even lifelong

treatment courses may be hypothesized to target a genetic disease, in contrast with the classic autoimmune myocarditis.[35]

STUDY LIMITATIONS. The series as collected retrospectively had a small sample size and lacked an appropriate control group. Diagnostic work-up and management reflect the experience of a single, third-level center, featuring dedicated multidisciplinary resources for myocarditis management.[36] Follow-up length was not homogeneous. Cohort entry at different disease stages as well as interpatient clustering due to unmeasured gene modifiers represent additional limiting factors. For CMR and FDG-PET even more, diagnostic accuracy (sensitivity, specificity) for the detection of cardiomyopathy-associated M-Infl may be lower as compared with classic myocarditis. Even modern parametric mapping on CMR may be subject to errors, especially in patients with thin walls, apical involvement, or arrhythmia-dependent motion abnormalities. Although IMT effectiveness could not be directly proven and several factors may have influenced our findings, we provided preliminary data supporting IMT feasibility for its subsequent investigation in the field of genetic cardiomyopathies. Larger studies are needed to provide guidance for patient selection and risk stratification.

CONCLUSIONS

We described a series of patients with a spectrum of genetic cardiomyopathies and evidence of EMB-proven M-Infl. In this setting, we showed that multimodal imaging allows further characterization of phenotypes and is capable of detecting M-Infl in most cases. In addition, our data suggest that IMT is a feasible and promising strategy to target cardiomyopathy-associated M-Infl. Studies are needed to verify our hypothesis and to confirm the preliminary findings of preclinical research in this area.[1,2]

FUNDING SUPPORT AND AUTHOR DISCLOSURES

The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: In patients with genetic cardiomyopathies of the dilated and arrhythmogenic spectrum, multimodal imaging allows identification of specific genotypes and detection of M-Infl subsequently enabling the use of IMT.

TRANSLATIONAL OUTLOOK: The systematic use of multimodal imaging, including CMR and PET, could be applied to a broad range of genetic nonischemic cardiomyopathies to detect and target M-Infl.

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KEY WORDS **cardiomyopathy, desmosomal gene variants, endomyocardial biopsy, genetics, immunosuppressive therapy, multimodal imaging, myocardial inflammation**

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