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ORIGINAL ARTICLE

Genetic Determinants and Genotype-Phenotype Correlations in Vietnamese Patients With Dilated Cardiomyopathy

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Background: Dilated cardiomyopathy (DCM) is an important cause of heart failure and cardiac transplantation. This study determined the prevalence of DCM-associated genes and evaluated the genotype-phenotype correlation in Vietnamese patients.

Methods and Results: This study analyzed 58 genes from 230 patients. The study cohort consisted of 64.3% men; age at diagnosis 47.9±13.7 years; familial (10.9%) and sporadic DCM (82.2%). The diagnostic yield was 23.5%, 44.0% in familial and 19.6% in sporadic DCM. *TTN* truncating variants (*TTN*tv) were predominant (46.4%), followed by *TPM1*, *DSP*, *LMNA*, *MYBPC3*, *MYH6*, *MYH7*, *DES*, *TNNT2*, *ACTC1*, *ACTN2*, *BAG3*, *DMD*, *FKTN*, *PLN*, *TBX5*, *RBM20*, *TCAP* (2–6%). Familial DCM, genotype-positive and *TTN*tv-positive patients were younger than those with genotype-negative and sporadic DCM. Genotype-positive patients displayed a decreased systolic blood pressure and left ventricular wall thickness compared to genotype-negative patients. Genotype-positive patients, particularly those with *TTN*tv, had a family history of DCM, higher left atrial volume index and body mass index, and lower right ventricle-fractional area change than genotype-negative patients. Genotype-positive patients reached the combined outcomes more frequently and at a younger age than genotype-negative patients. Major cardiac events occurred more frequently in patients positive with genes other than *TTN*tv.

Conclusions: The study findings provided an overview of Vietnamese DCM patients' genetic profile and suggested that management of environmental factors may be beneficial for DCM patients.

Key Words: Dilated cardiomyopathy; Genetic mutations; Next-generation sequencing; Phenotype; Vietnamese

Dilated cardiomyopathy (DCM) was characterized by left or biventricular dilatation and systolic dysfunction in the absence of secondary causes such as coronary artery disease.[1] With a prevalence of 1 in 250 in the population, DCM is a common cause of heart failure and the leading indication for cardiac transplantation.[2] DCM can be attributed to genetic and non-genetic causes, with approximately 40% of DCM cases having a genetic cause.[3] Rare variants in multiple genes encoding cardiac sarcomeric, cytoskeletal, desmosomal, nuclear lamina, mitochondrial and ion flux-handling proteins have been linked to disease manifestations.[4] The relationship between mutations in DCM-related genes and abnormalities of cardiac morphology and functions were investigated mostly in Western populations; data from Asian countries were scarce. With next-generation sequencing, multiple genes can be analyzed simultaneously, making genetic testing a powerful tool for disease management as for hypertrophic cardiomyopathy (HCM). However, the clinical utility of DCM genetic testing still needs to be established. Furthermore, a focused panel comprising the most prevalent DCM-associated genes in the Vietnamese population would enable a cost-effective DCM genetic testing program in Vietnam.

Our study aimed to determine the prevalence of rare variants from 58 DCM-related genes in 230 well-phenotyped DCM Vietnamese patients, and to analyze genotype-

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phenotype correlations in the study cohort.

Methods

Patient Population

A total of 230 unrelated patients admitted to the Heart Institute and Tam Duc Heart Hospital between September 2019 and August 2020 were enrolled in this study. All patients provided informed written consent and received genetic counseling prior to genetic testing. The study was approved by ethics committees of the 2 participating hospitals according to local regulations and followed the Declaration of Helsinki on human experimentation.

Diagnosis of DCM was issued based on left ventricular end-diastolic (LVED) volumes or diameters >2 SD from normal according to normograms (Z scores >2 SD) corrected by body surface area (BSA) and age, or BSA and gender and left ventricular ejection fraction (LVEF) ≤50%, not explained by abnormal loading conditions or coronary artery disease, valvular heart diseases, congenital heart lesions, and other systemic diseases.[1] Before enrollment in the study, all patients were subjected to a physical examination, chest radiography, electrocardiography (ECG), echocardiography, 24-h ambulatory ECG monitoring, coronary artery angiography or coronary multislice computed tomography angiography. Patient information included family and personal history of DCM, and family history of sudden cardiac death (SCD). Familial DCM was assigned when confirmed disease and confirmed or probable disease was observed in the proband and in at least one relative.[5]

Targeted Next-Generation Sequencing and Bioinformatics Data Processing

We used the Illumina TruSight Cardio panel and selected 58 genes (**Supplementary Table 1**) either with ≥1 variant reported as pathogenic (P) for DCM in the Human Gene Mutation Database or having a DCM phenotype number in online mendelian inheritance in man (OMIM) for inclusion in the analysis.

DNA was extracted from peripheral blood using a QIAamp DNA Blood Mini Kit (Qiagen). Target enrichment was performed with the TruSight Rapid Capture kit (Illumina). Captured libraries were sequenced with 2×150 bp reads on a MiSeq/Miniseq platform (Illumina). Sequence reads were mapped onto the human reference genome, hg38, using the burrows-wheeler alignment (BWA) tool.[6] The Genome Analysis ToolKit was used for variants (single nucleotide polymorphisms (SNPs) and indels) calling.[7] Analytic validation of the gene panel used has been submitted elsewhere with a sensitivity of 100.0% for SNPs and 75.0% for indels; a specificity of 100.0% and 83.3% for SNPs and indels, respectively. Identified variants were annotated using Annotate variation (ANNOVAR).[8]

Variant Interpretation

Variants with at least 20× coverage were analyzed using Alamut Visual (Interactive Biosoftware) and interpreted according to recent the American college of medical genetics and genomics (ACMG) guidelines.[9] Synonymous variants, intronic variants outside of the flanking regions, and variants with a minor allelic frequency (MAF) ≥0.1% in the Genome Aggregation (gnomAD) databases were excluded.[10] The 1,000 Genomes database, including data from 99 Vietnamese subjects, was used to check for the presence of all P and likely pathogenic (LP) variants identified.[11] For disease-specific refinement of the ACMG guidelines, we adopted CardioClassifier, a disease- and gene-specific computational decision support tool, which defines more specific thresholds for inherited cardiac disorders. According to CardioClassifier, the maximum credible population allele frequency for any DCM causative variant was set at 0.0056%; therefore, in this study, variants with a frequency less and greater than 0.0056% were categorized as PM2 and BS1, respectively.[12] Various in silico prediction programs, including SIFT, PolyPhen-2, AlignGVGD, MutationTaster, Mutation Assessor, CADD, and REVEL were used to analyze missense variants.[13,14] The analysis of intronic changes was performed with MaxEntScan, and Splice Site Finder-like;[15] and GERP++ was used to explore nucleotide-specific estimates of evolutionary constraint.[16] TTN missense variants were classified as benign variants.[17] All detected P and LP variants were confirmed by Sanger sequencing. Patients harboring P/LP variants were classified as genotype positive. Non-carriers of P/LP variants were considered genotype negative.

Statistical Analysis

Normally distributed, continuous variables were expressed as mean±SD and non-parametric as median (interquartile range). Categorical variables were depicted using numbers (proportions). Independent sample's t-test combined with Levene's tests was used for comparison between the groups for all continuous variables, and Mann-Whitney U-tests were used for non-parametric variables. Categorical variables were compared using the chi-squared test or Fisher's exact test. The Cox proportional hazard model was used for event-free survival analyses by comparing patients with and without mutation, *TTN*tv mutation and no mutation, and *TTN*tv mutation and mutation in other genes. Event-free survival was adjusted for gender, hypertension, and arrhythmia. The events used included death from any cause, heart transplantation, non-fatal stroke, life-threatening arrhythmia requiring implantable cardioverter-defibrillator (ICD) implant. Two-sided probability values were considered significant at $P<0.05$. All statistical analyses were performed with SPSS version 25.0 (SPSS Inc., Chicago, IL, USA).

Results

Clinical Profile of Patients

Our study cohort consisted of 230 unrelated DCM patients of Vietnamese origin; 148 males (64.3%), mean age 51.3±14.0 years, mean age at diagnosis 47.9±13.7 years; 25 with familial DCM (10.9%) and 189 with sporadic DCM (82.2%). The mean LVEF and LV ED diameter index (LVEDDi) were 28.3±7.5 and 40.6±5.2, respectively. Patients characteristics stratified by genetic status are summarized in **Table 1**.

Prevalence of P Variants in DCM-Associated Genes

The analysis of 58 DCM-related genes in 230 patients revealed a total of 56 variants classified as P/LP in 54 patients. The overall diagnostic yield was 23.5%; higher in familial than in sporadic DCM (44.0% vs. 19.6%). Among P variants identified, 17 were missense, 13 were nonsense, 7 were splice-site, 16 were frameshift indels and 3 were in-frame indels. Thirty (53.6%) disease-causing variants were known variants and 26 (46.4%) were novel. Among 56 P

Table 1. Clinical Characteristics of the Study Cohort

Characteristics	All (n=230)	Mutation-positive (n=54)	No mutation (n=176)	P value[a]	*TTN*tv-positive (n=26)	P value[b]	Other gene-positive (n=28)	P value[c]	Familial DCM (n=25)	Sporadic DCM (n=189)	P value[d]
Patient age, years	51.3±14.0	47.0±12.7	52.6±14.1	0.010*	48.2±14.0	0.135	46.0±11.5	0.539	45.6±13.3	52.2±14.1	0.028*
Age of diagnosis, years	47.8±13.7	43.0±11.4	49.3±14.0	0.003*	43.1±12.8	0.033*	42.9±10.3	0.963	41.7±12.5	48.7±13.8	0.017*
BMI, kg/m^2	23.9±4.3	24.6±4.1	23.6±4.4	0.042*	25.8±4.1	0.007*	23.5±3.8	0.051	23.8±2.9	23.8±4.4	0.591
	23.0 (5.4)	24.4 (4.8)	22.8 (5.1)		25.7 (5.3)		23.2 (4.5)		24.0 (5.3)	22.9 (5.3)	
SBP, mmHg	116.8±17.1	111.5±15.5	118.4±17.2	0.009*	113.7±16.3	0.194	109.4±14.7	0.313	115.6±16.9	116.7±17.4	0.771
NYHA class at onset	2.1±0.4	2.1±0.5	2.1±0.4	0.315	2.1±0.6	0.645	2.2±0.4	0.642	2.1±0.4	2.1±0.4	0.817
LVEF, %	28.2±7.3	27.2±7.1	28.5±7.4	0.234	27.1±7.3	0.368	27.2±7.0	0.969	27.1±6.1	28.2±7.5	0.501
LV maximal wall thickness, mm	10.5±2.1	10.0±1.9	10.6±2.2	0.046*	10.0±1.8	0.138	10.0±2.0	0.972	9.7±1.4	10.5±2.1	0.024*
LVEDD index, mm/m^2	40.6±5.2	39.6±4.6	40.9±5.4	0.122	38.8±3.3	0.008*	40.4±5.6	0.207	39.9±4.4	40.9±5.0	0.365
LAV index, mL/m^2	49.0±28.2	55.7±21.1	47.0±29.8	0.001*	58.4±19.4	0.001*	53.4±22.7	0.322	42.2±21.2	49.5±29.5	0.181
	39.0 (29.0)	58.0 (38.0)	38.0 (21.0)		59.0 (33.3)		45.0 (42.0)		36.5 (22.7)	39.0 (29.5)	
RV-TAPSE, mm	18.5±4.1	17.7±5.0	18.7±3.8	0.134	18.6±6.1	0.894	16.8±3.3	0.218	17.4±3.7	18.6±4.1	0.189
RV-FAC, %	35.3±11.9	30.8±13.1	36.7±11.2	0.005*	31.9±12.8	0.052	29.7±13.6	0.560	32.2±10.7	35.7±12.1	0.218
Patient gender, male	148 (64.3)	40 (74.1)	108 (61.4)	0.105	23 (88.5)	0.007*	17 (60.7)	0.030*	18 (72.0)	118 (62.4)	0.387
Family history of SD	27 (11.7)	11 (20.4)	16 (9.1)	0.134	6 (23.1)	0.188	5 (17.9)	0.741	11 (44.0)	12 (6.3)	<0.001*
Family history of DCM	25 (10.9)	11 (20.4)	14 (8.0)	0.024*	6 (23.1)	0.003*	5 (17.9)	0.246	–	–	–
Hypertension	55 (23.9)	8 (14.8)	47 (26.7)	0.136	4 (15.4)	0.421	4 (14.3)	1.000	6 (24.0)	46 (24.3)	0.934
Palpitation	67 (29.1)	19 (35.2)	48 (27.3)	0.095	10 (38.5)	0.014*	9 (32.1)	0.484	8 (32.0)	53 (28.0)	0.865
Dyspnea	222 (96.5)	52 (96.3)	170 (96.6)	0.166	25 (96.2)	0.022*	27 (96.4)	0.367	25 (100)	182 (96.3)	0.620
Syncope	9 (3.9)	3 (5.6)	6 (3.4)	0.148	1 (3.8)	0.033*	2 (7.1)	0.511	1 (4.0)	7 (3.7)	0.934
Left bundle branch block	134 (58.3)	31 (57.4)	103 (58.5)	0.714	18 (69.2)	0.533	13 (46.4)	0.107	9 (36.0)	115 (60.8)	0.045*
Atrial fibrillation	19 (8.3)	8 (14.8)	11 (6.3)	0.104	5 (19.2)	0.065	3 (10.7)	0.460	1 (4.0)	16 (8.5)	0.640
Arrhythmia	58 (25.2)	19 (35.2)	39 (22.2)	0.123	8 (30.8)	0.553	11 (39.3)	0.577	3 (12.0)	52 (27.5)	0.206
Mutation	–	–	–	–	–	–	–	–	11 (44.0)	37 (19.6)	0.010*

Data are presented as mean±SD (normal distribution) or median (interquartile range) (non-parametric distribution) or n (%). *Statistically significant difference between compared groups. [a]Mutation-positive and no-mutation. [b]*TTN*tv-positive and no-mutation. [c]*TTN*tv-positive and other gene-positive. [d]Familial DCM and sporadic DCM. BMI, body mass index; DCM, dilated cardiomyopathy; FAC, fractional area change; LAV, Left atrial volume; LV, left ventricular; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association functional class; RV, right ventricle; RV-FAC, right ventricle-fractional area change; SBP, systolic blood pressure; SD, sudden death; TAPSE, tricuspid annular plane systolic excursion; –, no information.

variants identified in this study, 4 were previously described in HCM Vietnamese patients. All DCM-associated variants are listed in **Table 2**.

The distribution of disease-causing mutations are presented in **Figure 1**. *TTN* gene mutations were predominant, found in 26/230 patients (11.3%). *TPM1*, *DSP*, *LMNA*, *MYBPC3*, *MYH6*, *MYH7*, *DES*, *TNNT2*, *ACTC1*, *ACTN2*, *BAG3*, *DMD*, *FKTN*, *PLN*, *TBX5*, *RBM20*, *TCAP*, contributed 2–6% each to the overall genotype-positive status. *TTN* truncating variants (*TTN*tv) accounted for 24% (6/25) and 7.9% (15/189) of familial and sporadic DCM, respectively. *TTN*tv were mostly found in the A band of the protein (17/23) (**Supplementary Figure**). The frequency of P variants in genes other than *TTN* was not sufficient for statistical analysis. Among 43 relatives of 21/56 genotype-positive index patients who accepted to participate in this study, 27 (62.8%) harbored the same mutation found in the proband.

The *RBM20* P variant, c.1907G>A, identified in one proband was found in 5 of 8 of his relatives. SCD was recorded for this proband; his mother and uncle who were diagnosed with DCM, all at a young age (**Figure 2**).

Genotype-Phenotype Correlation

To establish genotype-phenotype correlations, we compared clinical characteristics of genotype-positive and genotype-negative patients. Similar comparisons were made between patients harboring a *TTN*tv and genotype-negative patients. Familial and sporadic DCM patients were also analyzed for possible distinctive clinical manifestations. Correlation analyses are presented in **Table 1**.

In this study, familial DCM, genotype-positive and *TTN*tv-positive patients were younger than those with genotype-negative and sporadic DCM. Male gender was markedly associated with *TTN*tv-positive status (23/88.5%). Genotype-positive patients, particularly those with *TTN*tv had a higher BMI compared to genotype-negative patients. Genotype-positive, especially those with a *TTN*tv-positive status, was associated with a family history of DCM, whereas family history of SCD was significantly enriched in familial DCM. Genotype-positive patients displayed a significant slight decrease in systolic blood pressure (P=0.009). These patients also exhibited a decreased LV wall thickness compared to genotype-negative patients (P=0.013). A similar result, though not significant, was observed in *TTN*tv-positive patients (P=0.077), and familial DCM cases (P=0.057). Compared to genotype-negative patients, the right ventricle-fractional area change (RV-FAC) value was lower in genotype-positive and *TTN*tv-positive patients, although the difference was not significant for the latter

Table 2. List of DCM-Associated Variants in the Study Cohort

Gene	Transcript	Nucleotide change	Coding effect	Zygosity	MAF (%) gnomAD, ALL	MAF (%) gnomAD, EAS	MAF (%) 1,000G, KHV	ACMG class	Patient no.	ACMG criteria	References
ACTC1	NM_005159.4	c.941G>A	p.Arg314His	Het	0.0014	0	NA	LP	169	PS3 PS4 PM2 PP3 PP5	Olson et al (1998)[51]
BAG3	NM_004281.3	c.974delC	p.Pro325Glnfs*17	Het	0.0000	0	NA	P	004	PVS1 PM2 PM5 PP3 PP5	–
DES	NM_001927.3	c.83C>T	p.(Ser28Phe)	Het	0.0000	0	NA	LP	129	PM1 PM2 PP2 PP3	–
DES	NM_001927.3	c.193G>A	p.(Gly65Ser)	Het	0.0025	0	0	LP	197	PS4-M PM1 PM2 PP2 BP4	Walsh et al (2017)[52]
DMD	NM_004006.2	c.31+1G>T	–	Het	0.0000	0	NA	P	161	PVS1 PS4-M PM2 PP3 PP5	Milasin et al (1996)[53]; Feng et al (2002)[54]; Cho et al (2017)[55]
DSP	NM_004415.2	c.748C>T	p.(Gln250*)	Het	0.0000	0	NA	P	085	PVS1 PM2 PP3	–
DSP	NM_004415.2	c.2848dup	p.(Ile950Asnfs*3)	Het	0.0000	0	NA	P	160	PVS1 PS4-M PM2 PP3 PP5	Pugh et al (2014)[56]; Walsh et al (2017)[52]
DSP	NM_004415.2	c.5428del	p.Gln1810ArgfsTer8	Het	0.0000	0	NA	LP	263	PVS1 PM2	–
FKTN	NM_001351497.1	c.538C>T	p.(Arg180*)	Het	0.0008	0	NA	P	147	PVS1 PS4 PM2 PP3 PP5	Lévesque et al (2016)[57]
FKTN	NM_001351497.1	c.-2-2A>G	–	Het	0.0086	0.0099	0	P	147	PVS1 PM2 PM3 PP3	–
LAMA2	NM_000426.3	c.7525_7528dup	p.(Ser2510Thrfs*3)	Het	0.0000	0	NA	LP	182	PM1 PM2 PM4	–
LAMP2	NM_002294.2	c.139C>T	(p.Gln47Ter)	Het	0.0000	0	NA	P	016	PVS1 PM1 PM2 PP3	–
LAMP2	NM_002294.2	c.35_52del	p.(Ser12_Val17del)	Het	0.0000	0	NA	LP	082	PVS1 PM2	–
LMNA	NM_170707.3	c.566G>A	p.(Arg189Gln)	Het	0.0032	0	NA	LP	087	PM1 PM2 PM5 PP3 PP3	–
LMNA	NM_170707.3	c.3G>A	–	Het	0.0000	0	NA	P	143	PVS1 PS4-M PM1 PM2 PP3 PP5	–
MYBPC3	NM_000256.3	c.2308G>A	p.(Asp770Asn)	Het	0.0016	0	NA	LP	035	PVS1 PS4-M PM1 PM2 PP3 PP5	Van Driest et al (2004)[58]
MYBPC3	NM_000256.3	c.1504C>T	p.(Arg502Trp)	Het	0.0046	0	NA	LP	149	PM1 PM2 PM5 PP3 BP5	Richard et al (2003)[49]
MYH6	NM_002471.3	c.2040del	p.(Lys681Argfs*5)	Het	0.0000	0	NA	LP	037, 065	PVS1 PM2	–
MYH7	NM_000257.3	c.4823G>A	p.(Arg1608His)	Het	0.0008	0	NA	LP	036	PM1 PM2 PM5 PP2 PP3	–
MYH7	NM_000257.3	c.2155C>T	p.(Arg719Trp)	Het	0.0032	0	NA	P	116	PS3 PM1 PM2 PM5 PP2 PP5	Tesson et al (1998)[59]
MYH7	NM_000257.3	c.2207T>C	p.(Ile736Thr)	Het	0.0000	0	NA	P	149	PS3 PM1 PM2 PP2 PP3 PP5	Minoche et al (2019)[60]
PLN	NM_002667.4	c.40_42del	p.(Arg14del)	Het	0.0007	0	NA	P	006	PS3 PS4-M PM2 PM4 PP1 PP5	van der Zwaag et al (2012)[61]
RBM20	NM_001134363.2	c.1907G>A	p.(Arg636His)	Het	0.0000	0	NA	P	200	PS4 PM1 PM2 PM5 PP3 PP5	Brauch et al (2009)[44]
SDHA	NM_004168.3	c.1352G>A	p.(Arg451His)	Het	0.0004	0	NA	LP	215	PS3 PM2 PM5 PP3 BP1	Toledo et al (2017)[62]
SGCB	NM_000232.4	c.1A>G	–	Het	0.0000	0	0	P	131	PVS1 PS4-M PM2 PP3 PP5	Semplicini et al (2015)[63]
TBX5	NM_000192.3	c.652C>G	p.Gln218Glu	Het	0.0000	0	NA	LP	222	PM1 PM2 PP2 PP3	–
TCAP	NM_003673.3	c.472C>T	p.(Arg158Cys)	Het	0.0000	0	NA	LP	081	PM2 PM5 PP2 PP3	Hirtle-Lewis et al (2013)[64]
TNNT2	NM_001001430.2	c.620_622del	p.(Lys210del)	Het	0.0000	0	NA	P	043	PS3 PS4 PM2 PP5	Kamisago et al (2000)[65]

(**Table 2** continued the next page.)

Gene	Transcript	Nucleotide change	Coding effect	Zygosity	MAF (%) gnomAD, ALL	MAF (%) gnomAD, EAS	MAF (%) 1,000G, KHV	ACMG class	Patient (s)	ACMG criteria	References
TNNT2	NM_001001430.2	c.518G>A	p.(Arg173Gln)	Het	0.0000	0	NA	P	259	PS4 PM2 PM5 PP1 PP2 PP3	Van Acker et al (2010)[66]; Lakdawala et al (2012)[67]; Fernlund et al (2017)[68]
TPM1	NM_001018005.1	c.644C>T	p.(Ser215Leu)	Het	0.0004	0	NA	P	194	PS3 PM1 PM2 PP2 PP3 PP5	Cecconi et al (2016)[69]
TPM1	NM_001018005.1	c.598G>C	p.(Val200Leu)	Het	0.0000	0	NA	LP	208	PM1 PM2 PP2 PP3	–
TPM1	NM_001018005.1	c.842T>C	p.(Met281Thr)	Het	0.0004	0	NA	LP	271	PS4-M PM2 PP2 PP3	Van Driest et al (2003)[48]; Dorsch et al (2021)[70]
TTN	NM_001267550.2	c.40688_40689insT	p.(Arg13565Lysfs*7)	Het	0.0000	0	NA	LP	001	PVS1 PM2	–
TTN	NM_001267550.2	c.104974_104995dup	p.(Leu34999Glnfs*16)	Het	0.0000	0	NA	P	009	PVS1 PM2 PP3	–
TTN	NM_001267550.2	c.13898_13899del	p.(Lys4633Argfs*7)	Het	0.0000	0	NA	LP	013	PVS1 PM2	–
TTN	NM_001267550.2	c.49669A>T	p.(Lys16557*)	Het	0.0000	0	NA	P	025	PVS1 PM2 PP3	–
TTN	NM_001267550.2	c.68302A>T	p.(Lys22768*)	Het	0.0000	0	NA	P	026	PVS1 PM2 PP3	–
TTN	NM_001267550.2	c.86116C>T	p.(Arg28706*)	Het	0.0004	0	NA	P	040	PVS1 PS3 PM2 PP3 PP5	–
TTN	NM_001267550.2	c.71706del	p.(Ile23902Metfs*33)	Het	0.0000	0	NA	LP	055	PVS1 PM2	–
TTN	NM_001267550.2	c.42205C>T	p.(Arg14069*)	Het	0.0000	0	NA	P	057	PVS1 PM2 PP3	–
TTN	NM_001267550.2	c.92683C>T	p.Arg30895Ter	Het	0.0004	0	NA	P	066	PVS1 PM2 PP3	Roberts et al (2015)[37]
TTN	NM_001267550.2	c.59926+1G>A	–	Het	0.0004	0	NA	P	088	PVS1 PS4 PM2 PP3 PP5	Herman et al (2012)[71]
TTN	NM_001267550.2	c.52307_52310dup	p.(Glu17437Aspfs*2)	Het	0.0000	0	NA	P	107	PVS1 PM2 PP5	–
TTN	NM_001267550.2	c.94754T>G	p.(Leu31585*)	Het	0.0000	0	NA	P	109	PVS1 PM2 PP3	–
TTN	NM_001267550.2	c.54809del	p.(Ile18270Asnfs*22)	Het	0.0000	0	NA	P	141	PVS1 PM2 PP3	–
TTN	NM_001267550.2	c.41608+1G>T	–	Het	0.0000	0	NA	P	144	PVS1 PM2 PP3	–
TTN	NM_001267550.2	c.59460G>A	p.(Trp19820*)	Het	0.0032	0	NA	P	145	PVS1 PM2 PP3 PP5	–
TTN	NM_001267550.2	c.3073dup	p.(Ser1025Lysfs*15)	Het	0.0000	0	NA	P	166	PVS1 PM2 PP3	–
TTN	NM_001267550.2	c.58240_58244del	p.(Pro19414Alafs*2)	Het	0.0000	0	NA	P	177	PVS1 PM2 PP3	–
TTN	NM_001267550.2	c.47692C>T	p.(Arg15898*)	Het	0.0005	0	NA	P	190	PVS1 PS4-M PM2 PP3 PP5	Roberts et al (2015)[37]
TTN	NM_001267550.2	c.87459_87460dup	p.(Ser29154Lysfs*3)	Het	0.0000	0	NA	LP	207	PVS1 PM2	–
TTN	NM_001267550.2	c.67637-1G>C	–	Het	0.0000	0	NA	P	002, 140	PVS1 PM2 PP3 PP5	–
TTN	NM_001267550.2	c.59535del	p.(Asn19846Metfs*12)	Het	0.0000	0	NA	LP	087, 246	PVS1 PM2	–
TTN	NM_001267550.2	c.58870C>T	p.(Arg19624*)	Het	0.0000	0	NA	P	120, 213	PVS1 PM2 PP3 PP4	–
TTN	NM_001267550.2	c.81274C>T	p.(Gln27092*)	Het	0.0000	0	NA	P	251	PVS1 PM2 PP3	
TTN	NM_001267550.2	c.60359_60371del	p.(Lys20120Thrfs*19)	Het	0.0000	0	NA	LP	270	PVS1 PM2	–

ACMG, the American college of medical genetics and genomics; EAS, East Asia; Het, heterozygous; KHV, Kinh in Ho Chi Minh City, Vietnam; MAF, minor allele frequency; NA, not applicable. ACMG class: LP, likely pathogenic; P, pathogenic. ACMG criteria: _M, moderate; _P, supporting; _S, strong; PM, moderate pathogenicity evidence; PP, supporting pathogenicity evidence; PS, strong pathogenicity evidence; PVS, very strong pathogenicity evidence; –, no information.

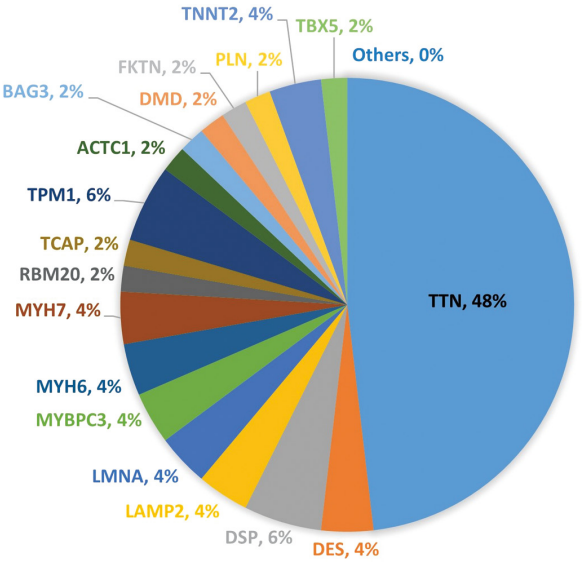

Figure 1. Distribution of pathogenic and likely pathogenic variants in Vietnamese dilated cardiomyopathy (DCM) patients.

group (P=0.052). Left atrial volume index (LAVi) was higher in genotype-positive and *TTN*tv-positive patients than in genotype-negative patients (P=0.001). Symptoms such as palpitation, dyspnea, and syncope were mostly observed in *TTN*tv-positive cases. Left bundle branch block was enriched in sporadic DCM compared to familial DCM. Interestingly, we noted no LV dilatation and no difference in LVEF values among all patient groups. Higher, though not significant, rates of atrial fibrillation and lower LVEDDi were observed in *TTN*tv-positive compared to genotype-negative patients (P=0.076) and in familial compared to sporadic DCM (P=0.078).

We defined major composite outcomes as death from any cause, heart transplantation, non-fatal stroke, life-threatening arrhythmia requiring ICD implant for the analysis of event-free survival curves. Event-free survival was measured from time of birth, and adjusted for gender, hypertension, and arrhythmia. Patients who did not have the outcome of interest were censored at the time of their last recorded follow up in this study. Genotype-positive patients reached the combined outcomes more frequently and at a younger age than genotype-negative patients (HR=3.4; 95% CI: 1.5–8.0; P=0.005) (**Figure 3A**). Major cardiac events occurred more frequently in patients with mutations in genes other than *TTN*tv (HR=9.2; 95% CI: 1.1–74.2; P=0.038) (**Figure 3B**). However, no difference in survival rate was observed between *TTN*tv-positive and genotype-negative patients (HR=0.7; 95% CI: 0.1–5.0; P=0.679) (**Figure 3C**).

Discussion

To the best of our knowledge, this is the first time disease-causing variants in DCM-associated genes were analyzed in Vietnamese patients diagnosed with DCM.

Mutation Prevalence

The overall diagnostic yield in our study was 23.5% (56/230); higher in familial (44%) than in sporadic DCM (19.6%), in line with previous observations.[18,19] Our diagnostic yield was lower than that of some previous studies, but comparable to others that showed a diagnostic yield of 17–26%.[18,19] A common point between these studies and ours was the use of a very stringent variant classification, whereas other reports were based on a less strict variant interpretation system. For example, a study in Han-Chinese DCM patients reported a diagnostic yield of 34.7%; however, the authors used less strict criteria for variant interpretation.[20] Furthermore, the ratio of familial DCM in our study (10.8%) was much lower than that found in other published studies. P variants in our study cohort were found in genes that have robust evidence for DCM association including *TTN*, *DSP*, *TPM1*, *LMNA*, *MYH7*, *TNNT2*, *BAG3*, *ACTC1*, and *RBM20*.[19] These P variants were not found in the 1,000 Genomes database and were determined using the strict ACMG guidelines combined with CardioClassifier, the computational tool specific for inherited cardiac conditions.

The number of P mutations found in a single patient

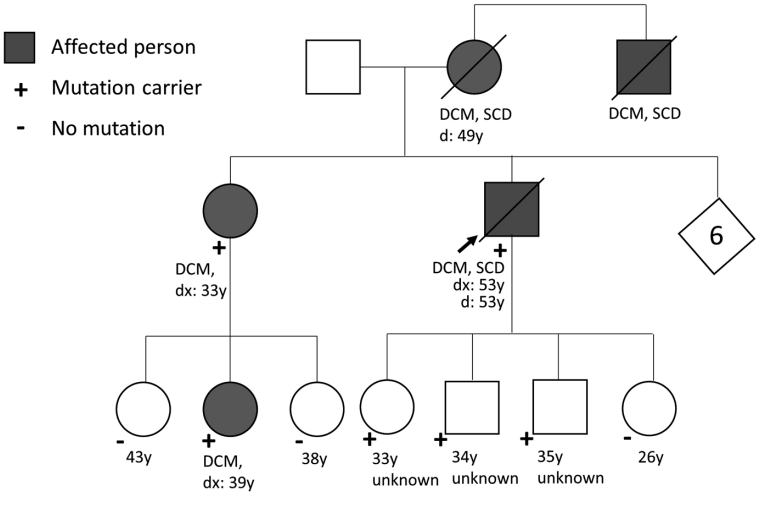

Figure 2. Pedigree of the family with dilated cardiomyopathy (DCM) due to *RBM20* c.1907G>A (p.Arg636His). Index patients are marked with an arrow. d, died; dx, diagnosis; SCD, sudden cardiac death; y, years old.

may affect the clinical severity.[21] In this study, 1 proband with compound mutations in *MYBPC3* and 1 with combined mutations in *MYBPC3* and *MYH7* were identified, a rate much lower than previously described.[22] Our finding was consistent with the predominant autosomal dominant inheritance of most DCM genes.[23]

Genotype-Phenotype Correlation

Genotype-phenotype correlations in DCM was a question for debate. Some studies showed no difference in term of clinical manifestations between patients with and without a mutation.[20,24] In contrast, genotype-positive status associated with more adverse outcomes were reported for other study populations.[19,25] Our genotype-positive and *TTN*tv-positive patients had a family history of DCM and an age at diagnosis significantly younger than genotype-negative patients, which is in agreement with previous research.[18] However, some abnormal cardiac features characterizing DCM such as LVEF, LVEDDi, arrhythmia, and especially atrial fibrillation displayed no difference between genotype-positive and genotype-negative probands, in contrast with previous studies.[19,25] The absence or very low prevalence of P variants in genes predominantly associated with arrhythmic DCM such as *SCN5A*, *LMNA* and *RBM20* in our study cohort may partly explain these findings. Systolic blood pressure together with ejection fraction were the 2 predictors for long-term survival in DCM patients.[26] Our genotype-positive patients displayed a slight though significant decrease in systolic blood pressure, even though it was still within the normal range. LV wall thinning and LV dilatation were factors that triggered LV remodelling.[27] In this study, genotype-positive patients displayed a marked decreased of LV wall thickness compared with genotype-negative patients, but no difference in LV dilatation was observed between the 2 groups. In DCM patients with reduced LVEF, LAV was a powerful predictive marker and increased LAV conferred an increased risk of cardiac death.[28,29] Findings that were replicated in this study with genotype-positive probands showed a marked increase in LAVi. RV systolic function was considered as a prognostic predictor of outcomes in DCM patients.[30,31] In this study, right ventricle-tricuspid annular plane systolic excursion (RV-TAPSE) and RV-FAC were lower in genotype-positive patients compared to genotype-negative patients, but only RV-FAC showed a significant difference. This observation, in line with our other results, showed a correlation between genotype-positive status and patients' adverse outcomes, as previously reported.[30,31]

Familial DCM was characterized by younger age and age at diagnosis, family history of SCD and was inversely correlated with the presence of left bundle branch block; findings that are in accordance with previous reports.[19,32] Diagnostic yield in familial DCM was higher than in sporadic DCM, as expected. A tendency for lower LVEDDi (P=0.078) and LV wall thickness (P=0.057) was observed in familial vs. sporadic DCM, although this was not significant and possibly due to insufficient data.

DCM is a complex disorder caused by genetic and environmental factors that combine to drive disease onset and outcomes. A significant though slight difference in BMI value was recorded between genotype-positive and genotype-negative patients. An association between high BMI and cardiomyopathies, in particular DCM, was observed in a large follow-up study in Sweden.[33] High BMI exacerbates genetic cardiac dysfunction, resulting in earlier onset of the disease. A prospective study showed that the risk of developing DCM in adulthood significantly increased with even mildly elevated body weight in late adolescence.[34]

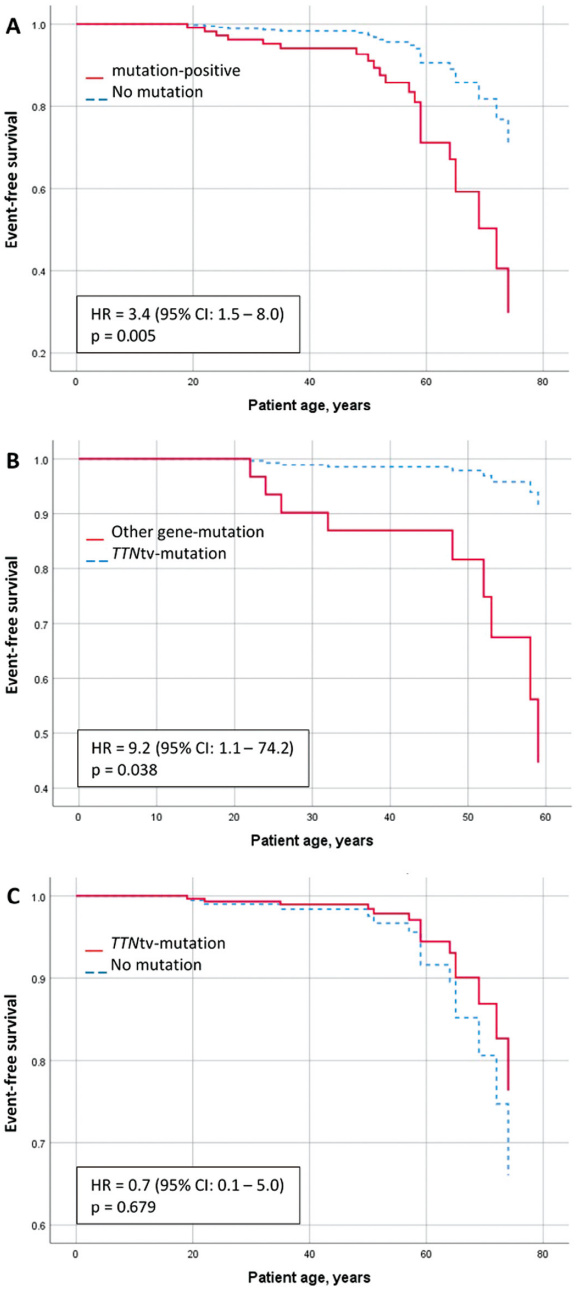

Figure 3. Survival curves show freedom from composite outcomes (death from any cause, heart transplantation, non-fatal stroke, life-threatening arrhythmia requiring implantable cardioverter-defibrillator implant). (**A**) Survival curve for patients with and without detected disease-causing mutation. (**B**,**C**) Comparison between patients with *TTN*tv vs. other gene mutation, and *TTN*tv vs. no mutation, respectively. Event-free survival is measured from time of birth, and adjusted for gender, hypertension, and arrhythmia. CI, confidence interval; HR, hazard ratio.

*TTN*tv-Positive Status

*TTN*tv accounted for 11.3% of the study cohort, and 48%

of all genotype-positive patients; 24% of familial cases and 7.9% of sporadic cases; these rates were similar to those determined in other published cohorts.[18,35] A significant male predominance was observed in our *TTN*tv patients, which is also in agreement with previous reports.[35,36] Clinical impacts of *TTN*tv depend on variant location in the entire gene. Variants situated in the A-band and the proximal or terminal part of the I-band were associated with higher cardiac expression and disease penetrance than those located in the Z-band and M-band regions.[37] The high percentage of *TTN*tv found in the A-band of our study cohort (73.9%) was in accordance with these findings.

*TTN*tv that are highly expressed in the heart (hiPSI *TTN*tv) were associated with severe cardiac phenotypes, largely driven by DCM in individuals with European ancestry.[36,38,39] In contrast, no difference in clinical manifestations or only a mild form of DCM were reported between *TTN*tv-positive and -negative patients.[20,40] A clear family history of DCM and significant increase of LAVi characterized our *TTN*tv-positive probands. They also manifested several heart conditions such as palpitations, dyspnea and syncope, indicators of morbidity and mortality risks when associated with cardiac structural diseases.[41] *TTN*tv-positive status created metabolic stress signaling, which can be accentuated by further increases in metabolic stressors, reflecting the age and BMI-induced onset of the disease.[42] Our *TTN*tv-positive probands exhibited a younger age and a significantly higher BMI than that of genotype-negative patients. However, despite all the unfavorable characteristics including male sex, family history of DCM, increased LAVi, and cardiac manifestations, probands positive with *TTN*tv in this study displayed a higher survival rate than those with mutations in other genes, finding in agreement with previous reports on the treatable nature and milder manifestations of *TTN*-positive status compared to *LMNA*, *SCN5A*, and *RBM20* genes.[24]

RBM20, a component of the RNA splicing machinery, regulates the splicing of at least 30 cardiac genes, including *TTN*.[43] The missense P *RBM20* variant c.1907G>A identified in this study was situated in the RS domain and considered as a mutational hotspot.[44] The *RBM20*-positive case exhibited a marked family history of DCM and SCD. These findings confirmed the early onset, rapidly progressive nature and high mortality associated with *RBM20* P variants.[44]

Overlapping DCM/HCM

Different mutations in the same gene cause either HCM or DCM, but no mutation can lead to both diseases.[45] Nevertheless, an overlapping spectrum of disease-causing mutations or DCM, HCM, arrhythmogenic right ventricular cardiomyopathy (ARVC) and channelopathies was also recognized.[22,46] In this study, 5 P variants identified in 4 DCM patients were previously reported as associated with other cardiomyopathies in previous studies, including a Vietnamese HCM study cohort.[47–49] Misdiagnosis of end-stage HCM was nearly excluded from our study because no long-standing history of HCM was recorded in any of these probands.[4] Furthermore, LV wall thickness of these patients (8–12.5 mm) was much lower than that reported for Vietnamese HCM patients (mean 22.5±4.8 mm)[47] (**Supplementary Table 2**).

Study Limitations

First, our study cohort was subjected to a selection bias because only patients with clear clinical symptoms were recruited from the 2 biggest heart hospitals in South Vietnam. Furthermore, due to limited awareness and capacity of access to health care of the general population, a certain number of DCM cases, especially those with milder manifestations, was probably missed. Second, the total number of analyzed cases was not sufficient to assess the penetrance of all identified variants. In cardiomyopathies including DCM, environmental epigenetic factors and common genetic variants also contributed to the manifestations of gene mutations. These factors were not considered in our study. Finally, the TruSight Cardio did not include *FLNC*, a gene with a strong association with DCM.

Conclusions

Determination of the most clinically relevant DCM genes and variants could provide evidence to increase the clinical utility through reducing the uncertainty associated with large number of variants of uncertain significance (VUS). Our findings, though, did not provide concluding results, but gave an overview of Vietnamese DCM patients' genetic profile. Further studies are required for more elaborated analyses. Previous reports showed that a clear improvement in all aspects of patients' quality of life can be obtained through early diagnosis leading to better risk stratification and follow up, and/or personalized therapy based on identification of etiological assessment.[32,50] Our findings suggested that management of environmental factors may be beneficial for DCM patients, especially *TTN*tv-positive ones. Data should be taken into consideration for genetic counseling of patients and families.

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Disclosures

The authors declare no conflicts of interest.

IRB Information

The study was approved by the ethics committees of the 2 participating hospitals according to local regulations (reference numbers: 1759/VT-HDDD, 19.18/QD-NC-TD).

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Supplementary Files

Please find supplementary file(s);
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