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Rare variants in the *FBN1* gene are associated with sporadic dilated cardiomyopathy in a Chinese Han population

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Abstract

Introduction: Dilated cardiomyopathy (DCM) represents a diverse set of myocardial diseases characterized by notable genetic heterogeneity. Although over 50 genes have been associated with DCM, these collectively explain 35% of idiopathic DCM cases. Variants in the *FBN1* gene encoding fibrillin-1 are primarily linked to connective tissue disorders. Considering the potential of these disorders to impact myocardial tissue, this study probes into the possible association between *FBN1* variants and DCM.

Aim: The objective of this study was to investigate the association between *FBN1* variants and DCM in a Chinese Han population.

Methods and Results: We performed whole-exome sequencing (WES) to identify rare *FBN1* variants among 1,059 DCM cases and 514 controls. Utilizing a case-control strategy and the optimal sequence kernel association test (SKAT-O), we found a significant enrichment of rare deleterious *FBN1* variants in DCM patients (19 of 1,059 *vs.* 0 of

514, P_{SKAT-O} = 7.49E-04). Clinical characteristics analysis indicated a higher occurrence of atrial fibrillation and a higher rate of implantable cardioverter-defibrillator (ICD) implantation among DCM patients carrying *FBN1* variants (*FBN1*$^+$) compared to non-carriers (*FBN1*$^-$). However, these *FBN1* variants did not significantly affect primary endpoints, defined as cardiac mortality or heart transplantation, yet appeared to increase the risk of secondary endpoints, including all-cause mortality or heart failure recurrence.

Conclusion: The findings suggest an association between rare deleterious variants in the *FBN1* gene and DCM in a Chinese Han population. Our findings underline the importance of further research to validate these results and elucidate the role of *FBN1* in DCM.

Potential Impact of the findings: This research provides fresh insights into the potential role of *FBN1* rare variants in DCM, pointing to new directions for future genetic studies and potential therapeutic strategies in DCM management.

Keywords: Dilated cardiomyopathy, whole-exome sequencing, gene-based association test, case-control study

INTRODUCTION

Dilated cardiomyopathy (DCM) is defined as left ventricular (LV) or biventricular dilatation and systolic dysfunction in the absence of coronary artery disease or abnormal load proportional to the degree of LV injury[1]. DCM is the second-most common cause of heart failure (after coronary artery disease) and the most common indicator for heart transplantation, accounting for 36% of all heart failure cases; the prevalence of DCM in the general population is estimated to be > 0.4%[2,3]. Reports show that approximately 25%-35% of patients with idiopathic DCM have a positive family history, indicative of a major genetic cause[2,4]. Most familial DCMs are inherited in an autosomal dominant pattern. In addition, DCM can be inherited in autosomal recessive, X-linked recessive, and mitochondrial manners[5]. Over 50 genes associated with DCM have been identified[6]. However, the combined genetic contribution of these genes explains about 35% of idiopathic dilated cardiomyopathy cases[7]. This suggests the existence of as-yet-undiscovered genes or mechanisms associated with DCM.

FBN1, the gene that encodes fibrillin-1, a major component of extracellular microfibrils, has been identified as the causal gene for Marfan syndrome (MFS). A total of 1,847 different variants have been reported in the *FBN1* mutation database[8]. The "Revised Ghent nosology for the Marfan syndrome" posits that the primary basis for diagnosing MFS is the identification of specific clinical signs, predominantly involving the cardiovascular, musculoskeletal, and ocular systems. Confirmation of a pathogenic *FBN1* variant would further substantiate the diagnosis[9]. The primary cardiovascular symptom of MFS, caused by *FBN1* variants, is the progressive dilation of the ascending aorta, which can lead to aortic aneurysm or dissection, thus placing patients at a significant risk of cardiovascular mortality[10,11].

While the impact of MFS is most notable on the ascending aorta within the cardiovascular system, there is also evidence suggesting a potential association between *FBN1* variants and myocardial dysfunction. Campens *et al.* (2015) found that mice with *FBN1* variants exhibited left ventricular systolic dysfunction[12]. Further *ex-vivo* studies on the myocardium of these mutant mice suggest a role for microfibrils in influencing the mechanical properties of the myocardium[12]. A recent study by Connor *et al.*, which involved 241 MFS patients without severe valvular disease, revealed evidence of left ventricular (LV) dysfunction in 12% of the participants, suggesting the potential for primary cardiomyopathy in MFS[13].

Given these findings, we employed Whole-exome Sequencing (WES) to scrutinize the variation profile of the *FBN1* gene in a cohort of Chinese Han DCM patients, thereby further investigating the potential association between *FBN1* variants and DCM.

METHODS

Study population

This study was conducted with the approval of the institutional ethics committee and complied with the International Conference on Harmonization Guidelines for Good Clinical Practice and the Declaration of Helsinki (Ethics Approval Document ID: TJ-C20181101). All participating individuals provided their written informed consent. This investigation is part of an ongoing prospective registry study (NCT03754101) aimed at identifying genetic variants or genes potentially linked with the onset and prognosis of DCM.

A total of 1,059 sporadic DCM patients and 514 ethnically matched controls were recruited from Tongji Hospital, Wuhan, China, between July 2007 and December 2020. The criteria for DCM diagnosis was the presence of left ventricular or biventricular dilatation, characterized by a left ventricular end-diastolic diameter (LVEDD) exceeding 33 mm/m^2 in men or 32 mm/m^2 in women, coupled with systolic dysfunction, represented by a left ventricular ejection fraction (LVEF) less than 50%[14].

Patients with heart failure due to ischemic heart disease, uncontrolled hypertension, major valvular disease, severe systemic infections, excessive alcohol consumption, insulin-treated diabetes, endocrine disorders such as pheochromocytoma, acromegaly, and thyroid disease, systemic diseases, or previous cancer treatment including radiotherapy were excluded from the study[15].

The control group, despite some individuals with managed hypertension considered as a systemic disease, was free of any specific cardiovascular disease or dilated cardiomyopathy. Detailed characteristics of the participants are presented in [Table 1].

Whole-exome sequencing and variants quality control

Whole-exome sequencing (WES) was conducted on both DCM cases (n = 1,059) and controls (n = 514). Genomic DNA, extracted from peripheral blood leukocytes, was measured for concentration using a Nanodrop 2000 (Thermo Fisher Scientific). We employed the SureSelectXT exon V6 kit (Agilent Technologies) to fragment this DNA into pieces of approximately 300 bp. After adaptor ligation and end repair, these fragments underwent targeted region capture. This captured library was amplified to create clusters suitable for sequencing on a HiseqXten sequencer (Illumina), yielding paired-end reads of 150 bp. Following adapter sequence removal, we aligned the reads to the human reference genome (hg19) using the Burrows-Wheeler algorithm (BWA)[16]. PCR duplicates were marked with Picard, and SAMtools facilitated the classification and management of post-alignment SAM or BAM files[17].

The Genome Analysis Toolkit (GATK) was used for the final steps of base quality score recalibration, indel realignment, and variant calling, all following GATK best-practice guidelines[18]. After a collective call for the study cohort, variants were documented in a variant call format (VCF) employing VCFtools[19]. Any variant with a read depth of less than 20 or a missing rate above 20% across the entire cohort was discarded. The remaining variants were annotated using ANNOVAR[20].

Variants identification and pathogenicity assessment

Rare variants, defined as those with a minor allele frequency (MAF) < 0.001 in East Asian populations

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Table 1. Comparative analysis of baseline clinical characteristics between DCM and control groups, as well as *FBN1* variants carriers and non-carriers within DCM patients

	Comparison between DCM and control			Comparison between *FBN1*$^+$ and *FBN1*- in DCM		
	DCM	Control		*FBN1*$^+$	*FBN1*$^-$	
	(*n* = 1,059)	(*n* = 514)	*P* value	(*n* = 19)	(*n* = 1,040)	*P* value
Male (%)	776 (73.3)	248 (48.2)	< 0.001	12 (63.2)	764 (73.5)	0.315
Age of onset (years)	52.04 ± 13.79	-	-	54.74 ± 19.57	51.99 ± 13.67	0.39
Age at enrollment (years)	54.97 ± 14.14	60.53 ± 14.18	< 0.001	57.42 ± 18.63	54.92 ± 14.05	0.445
NYHA III/IV (%)	727 (68.6)	0 (0.0)	< 0.001	14 (73.7)	713 (68.6)	0.633
Smoke (%)	449 (42.4)	107 (22.0)	< 0.001	5 (26.3)	444 (42.7)	0.152
Alcohol intake (%)	138 (15.9)	78 (16.0)	0.969	0 (0.0)	138 (16.2)	0.089
Non-fatal stroke (%)	47 (4.4)	7 (2.4)	0.125	1 (5.3)	46 (4.4)	0.861
Hypertension (%)	526 (49.7)	21 (4.1)	< 0.001	10 (52.6)	516 (49.6)	0.794
Hyperlipidemia (%)	106 (10.0)	12 (4.2)	0.002	1 (5.3)	105 (10.1)	0.487
Diabetes mellitus (%)	176 (16.6)	1 (0.2)	< 0.001	4 (21.1)	172 (16.5)	0.6
Renal insufficiency (%)	85 (9.0)	12 (4.2)	0.008	6 (33.3)	79 (8.5)	< 0.001
Body mass index (kg/m^2)	23.88 ± 4.46	22.83 ± 3.01	0.001	24.97 ± 3.36	23.84 ± 4.49	0.483
TC (mmol/L)	3.90 ± 0.99	4.43 ± 0.84	< 0.001	3.89 ± 1.25	3.91 ± 0.99	0.948
TG (mmol/L)	1.39 ± 0.94	1.29 ± 0.77	0.073	1.40 ± 0.75	1.39 ± 0.95	0.982
HDL-C (mmol/L)	0.96 ± 0.33	1.36 ± 0.34	< 0.001	0.98 ± 0.25	0.96 ± 0.33	0.855
LDL-C (mmol/L)	2.49 ± 0.82	2.46 ± 0.65	0.486	2.40 ± 1.15	2.50 ± 0.82	0.668
NT-proBNP (pg/mL)	3,813.00 [1,702.00, 8,915.50]	66.00 [32.00, 106.75]	< 0.001	3,368.00 [1,533.00, 1,0404.00]	3,817.00 [1,720.00, 8,899.75]	0.89
ALT (U/L)	26.00 [16.00, 47.00]	15.00 [11.00, 20.00]	< 0.001	30.00 [22.00, 45.50]	26.00 [16.00, 47.00]	0.34
AST (U/L)	27.00 [19.00, 41.00]	20.00 [17.00, 24.00]	< 0.001	27.00 [22.00, 34.50]	27.00 [19.00, 41.50]	0.895
Cr (μmol/L)	88.00 [73.00, 111.00]	66.00 [58.00, 77.00]	< 0.001	89.00 [73.00, 127.00]	88.00 [73.00, 111.00]	0.967
Atrial fibrillation (%)	237 (22.4)	1 (0.4)	< 0.001	8 (42.1)	229 (22.0)	0.037
Non-sustained ventricular tachycardia (%)	145 (13.7)	0 (0.0)	< 0.001	2 (10.5)	143 (13.8)	0.685
Left bundle branch block (%)	112 (10.6)	4 (1.4)	< 0.001	4 (21.1)	108 (10.4)	0.134
Any arrhythmia (%)	450 (42.5)	5 (1.8)	< 0.001	12 (63.2)	438 (42.1)	0.066
IVS (mm)	9.56 ± 1.42	9.13 ± 1.11	< 0.001	9.44 ± 1.34	9.56 ± 1.43	0.728
LVPW (mm)	9.55 ± 1.34	9.00 ± 1.02	< 0.001	9.44 ± 1.04	9.55 ± 1.35	0.738
LVEDD (mm)	66.64 ± 8.26	45.02 ± 3.76	< 0.001	65.42 ± 9.44	66.67 ± 8.24	0.515

LAD (mm)	45.66 ± 7.90	30.62 ± 3.90	< 0.001	46.16 ± 8.08	45.65 ± 7.90	0.784
LVEF (%)	31.41 ± 10.00	65.75 ± 4.96	< 0.001	33.32 ± 8.35	31.38 ± 10.03	0.403
E/A	1.74 ± 1.54	1.03 ± 0.35	< 0.001	1.45 ± 1.00	1.74 ± 1.54	0.566
E/e′	22.43 ± 12.48	9.45 ± 2.97	< 0.001	24.75 ± 16.80	22.49 ± 12.36	0.609
Aortic root diameter (mm)	29.60 ± 13.27	25.47 ± 3.65	< 0.001	27.88 ± 2.80	29.63 ± 13.36	0.711
Proximal ascending aorta diameter (mm)	32.64 ± 4.83	30.11 ± 3.31	< 0.001	34.58 ± 4.46	32.60 ± 4.83	0.159
Aortic root dilatation (%)	49 (7.4)	0 (0.0)	< 0.001	0 (0.0)	49 (7.6)	0.344
Proximal ascending aorta dilatation (%)	189 (30.1)	12 (5.6)	< 0.001	6 (42.9)	183 (29.8)	0.292
Any aorta dilatation (%)	212 (26.5)	12 (5.0)	< 0.001	6 (40.0)	206 (26.2)	0.232
Pacemaker implantation (%)	54 (5.1)	-	-	0 (0.0)	54 (5.2)	0.307
Implantable cardioverter-defibrillator (%)	19 (1.8)	-	-	2 (10.5)	17 (1.6)	0.004
Cardiotonic use (%)	507 (48.1)	-	-	10 (52.6)	497 (48.0)	0.69
Diuretic use (%)	869 (82.4)	-	-	16 (84.2)	853 (82.4)	0.838
ACE inhibitor use (%)	802 (76.0)	-	-	13 (68.4)	789 (76.2)	0.434
Beta-blocker use (%)	555 (52.7)	-	-	8 (42.1)	547 (52.9)	0.353
Aldactone use (%)	814 (77.2)	-	-	17 (89.5)	797 (77.0)	0.199

DCM: Dilated cardiomyopathy; *FBN1*$^{+}$: DCM cases carrying rare deleterious variants in *FBN1*,*FBN1*$^{-}$,DCM cases not carrying rare deleterious variants in *FBN1*; NYHA: New York Heart Association; NT-proBNP: N-terminal pro-B-type natriuretic peptide; TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Cr: creatinine; IVS: interventricular septum; LVPW: left ventricular posterior wall; LVEDD: left ventricular end-diastolic diameter; LAD: left atrial diameter; LVEF: left ventricular ejection fraction. For non-normally distributed data: values are presented as the median and interquartile range (IQR). All other numerical data, which are normally distributed, are represented as the mean ± standard deviation (SD). Categorical data are provided as counts (percentages).

according to public databases, became the focus of our study. Out of the 51 genes associated with DCM and classified by the Clinical Genome Resource (ClinGen), we excluded seven due to contentious pathogenic evidence. Thus, our investigation involved the remaining 44 genes (comprising 19 high-evidence genes and 25 low-evidence genes)[7] and the gene *FBN1*. These 44 genes were incorporated to mitigate potential confounding due to the influence of deleterious variants in known DCM-associated genes during our subsequent analyses.

In the *FBN1* gene, missense and truncating variants classified as pathogenic or likely pathogenic (P/LP) by the American College of Medical Genetics and Genomics (ACMG) guidelines were included in subsequent analyses[21]. For missense variants deemed variants of uncertain significance (VUS) by ACMG, the deleteriousness prediction was made using REVEL and VEST3[22,23], the most precise *in silico* functional prediction methods[24]. The thresholds for a deleterious prediction were a REVEL score ≥ 0.4 and a VEST3 score ≥ 0.5, based on a previous evaluation study of missense variant prediction tools[24].For cross-validation, we utilized CADD and MutationTaster, with a CADD-phred-like score > 20 and a "D" prediction from MutationTaster, indicating a deleterious prediction[25,26].

In our analysis, *FBN1* variants were deemed deleterious either when they were predicted to be so by *in silico* tools or were classified as P/LP by the ACMG. These rare deleterious *FBN1* variants were included in the gene-based association test [Figure 1].

Among the 43 selected DCM-associated genes, variants were deemed deleterious if they were classified as P/LP by ACMG. As for *TTN*, we specifically focused on truncating variants (*TTN*tv) with a "percentage spliced in" (PSI) greater than 90%, given that *TTN*tv are the major genetic cause of DCM and *TTN*tv with PSI > 90% pose an above-average risk for DCM[27-29]. The PSI data were sourced from cardiodb.org[30]. Classification of all variants adhered to the ACMG guidelines, executed using InterVar[31].

Gene-based association test

Utilizing the optimal sequence kernel association test (SKAT-O)-a nuanced synthesis of unidirectional and variance-component tests-we evaluated the association of rare deleterious *FBN1* variants with DCM risk[32]. A $P < 0.05$ was set as the threshold for statistical significance.

Follow-up and clinical outcomes

Clinical endpoint events were adjudicated by two independent clinicians who were blinded to imaging and genetic data. Primary endpoints were defined as cardiac mortality (verified by official death certificates or relative interviews) or heart transplantation (validated via medical records). Secondary endpoints included all-cause mortality or heart failure recurrence, either in-hospital or post-discharge.

Statistical analysis

Baseline characteristics were represented through suitable descriptive statistics. For normally distributed continuous variables, we reported the mean ± standard deviation (SD), while for non-normally distributed variables, we reported the median with interquartile range [IQR]. Categorical variables were expressed as counts (percentages). Depending on the data distribution, we used independent samples t-tests or Wilcoxon's tests for continuous variables, and chi-squared or Fisher's exact tests for categorical ones.

We estimated survival rates via Kaplan-Meier methods, comparing groups with the log-rank test. Cox proportional hazards models assessed the influence of various clinical characteristics on outcome likelihood. All tests were two-tailed with a P less than 0.05 indicating statistical significance. All analyses and data visualizations were performed using R (version 3.6.2).

RESULTS

Profile of the rare variants in the FBN1 gene

In our WES analysis of 1,059 DCM cases and 514 controls, 47 rare *FBN1* variants were identified in 53 cases and 9 controls [Supplementary Table 1]. Upon applying the ACMG guidelines, we excluded two likely benign variants, leaving us with 44 VUS missense variants and one likely pathogenic frameshift insertion (p.S932fs). After assessing deleteriousness, we classified 17 of these missense variants as deleterious [Supplementary Table 2], yielding a final count of 18 rare deleterious *FBN1* variants. These variants were detected in 19 out of 1,059 DCM patients, with none found in the control group [Supplementary Table 3]. All variants were heterozygous, distributed across the *FBN1* gene without any specific hotspots [Figure 2].

Gene-based association test

To mitigate confounding effects, we excluded individuals carrying pathogenic or likely pathogenic variants, known as P/LP variants, in the 44 known DCM-associated genes. The details of these genes are provided in [Supplementary Table 4]. Among 215 P/LP variants identified in these genes, 197 were in 208 DCM patients and 21 in 22 controls. [Supplementary Table 5]. Within the 208 DCM patients, one also carried a deleterious

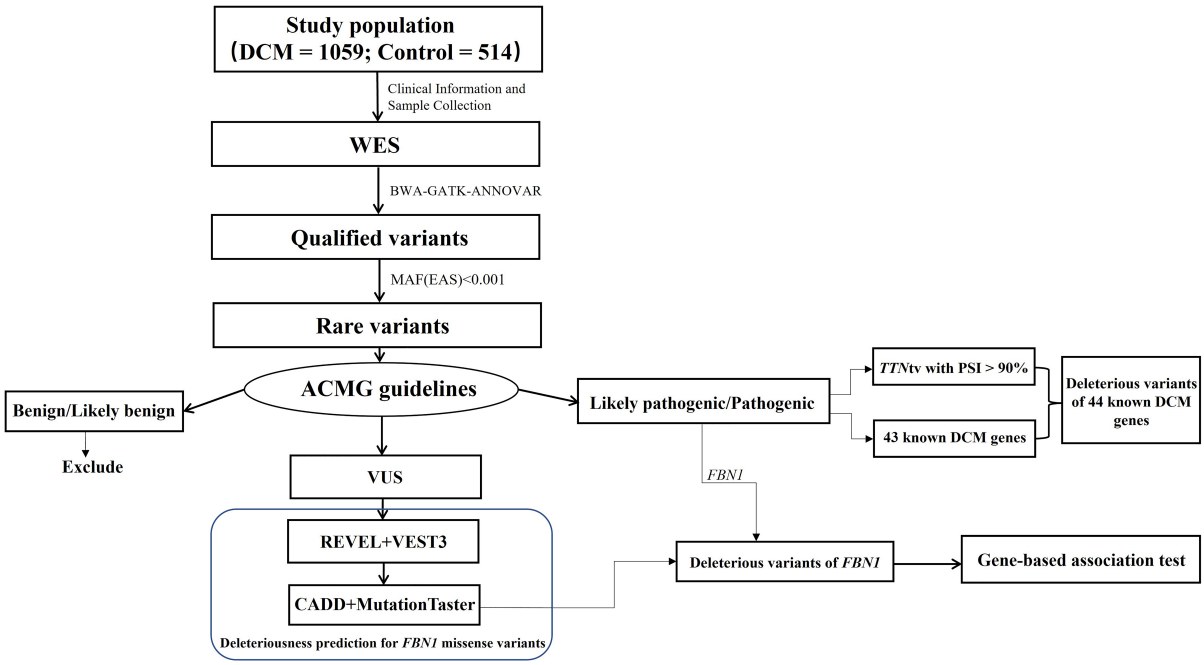

Figure 1. Flowchart illustrating the screening criteria for identifying rare deleterious variants in *FBN1* and other known DCM-associated genes. DCM: Dilated cardiomyopathy; WES: whole-exome sequencing; BWA: burrows-Wheeler algorithm; GATK: genome analysis toolkit; MAF: minor allele frequency; EAS: East Asian; ACMG: American College of Medical Genetics and Genomics; VUS: variants of uncertain significance; REVEL: rare exome variant ensemble learner; VEST3: variant effect scoring tool version 3; CADD: combined annotation dependent depletion; *TTN*tv: *TTN* truncating variants; PSI: percentage spliced in.

FBN1 variant and was thus excluded from the subsequent association tests. This exclusion strategy ultimately led to a cohort consisting of 851 DCM patients (including 18 carrying rare deleterious *FBN1* variants) and 492 controls for the *FBN1* gene-based association test. Using the sequence kernel association test-optimal (SKAT-O), we found a significant enrichment of deleterious *FBN1* variant carriers in the DCM cohort (18 of 851 *vs.* 0 of 492, P_{SKAT-O} = 7.49E-04), even when we adjusted for sex and age at enrollment.

Comparative analysis of clinical characteristics in DCM and controls

To better understand the associations between the *FBN1* genotype and DCM phenotype, as well as the differences between DCM patients and controls, we conducted a comprehensive analysis of various clinical characteristics [Table 1]. This analysis not only compared DCM patients to controls but also differentiated DCM patients based on the presence or absence of rare deleterious *FBN1* variants.

When comparing the overall DCM group to the control group, we noted several significant differences in clinical features. DCM patients were primarily male (73.3% *vs.* 48.2%, $P < 0.001$) and were younger at the time of enrollment (54.97 ± 14.14 *vs.* 60.53 ± 14.18 years, $P < 0.001$). Moreover, these patients exhibited a significantly higher proportion of NYHA Class III/IV status (68.6% *vs.* 0%, $P < 0.001$), indicating severe impairment of cardiac function, which was further supported by echocardiographic data. Specifically, DCM patients had larger left ventricular end-diastolic diameters (LVEDD: 66.64 ± 8.26 *vs.* 45.02 ± 3.76 mm, $P < 0.001$) and lower left ventricular ejection fractions (LVEF: 31.41 ± 10.00 *vs.* 65.75 ± 4.96 %, $P < 0.001$). Additionally, biochemical parameters and disease history including hypertension and diabetes were significantly different between the two groups.

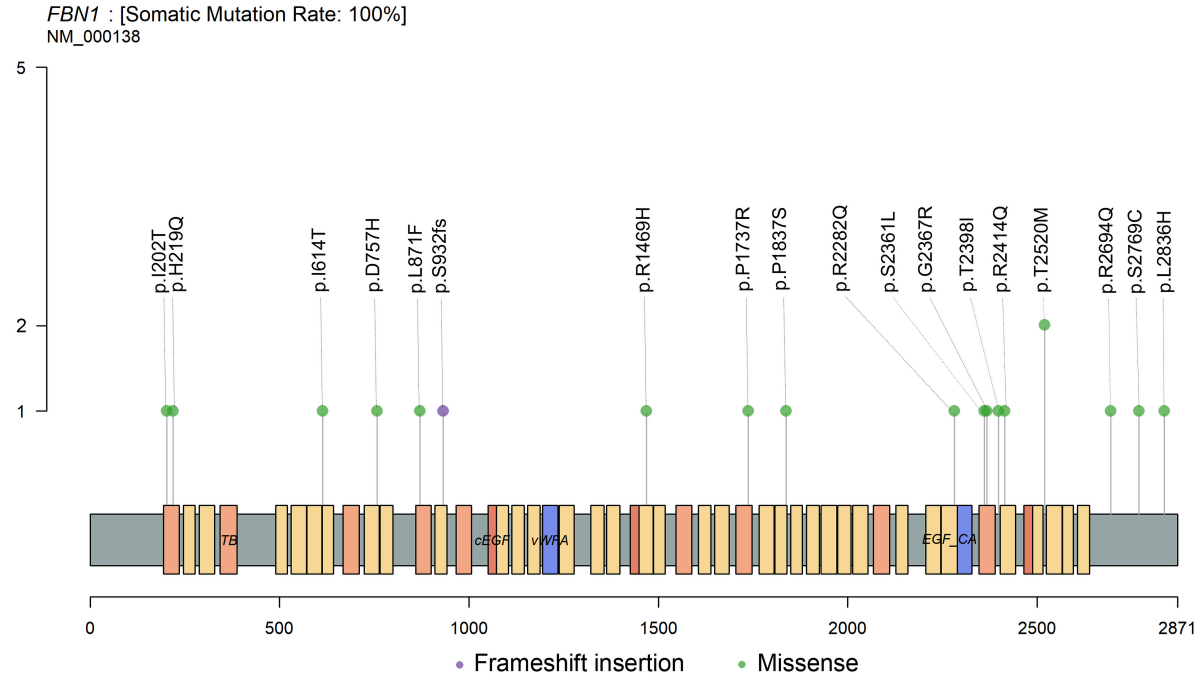

Figure 2. Distribution of rare deleterious variants in *FBN1*. Schematic representation of *FBN1* protein domains and the identified variant sites in patients. Frameshift insertion variants are shown in purple, and missense variants are shown in green. Domains include TB (TB domain), cEGF (Complement Clr-like EGF-like domain), vWFA (von Willebrand factor type A domain), and EGF_CA (Calcium-binding EGF domain). The X-axis represents the length of the *FBN1* protein, and the Y-axis represents the number of individuals carrying specific variants.

Within the DCM group, no significant differences were observed in enrollment age, onset age, or sex between *FBN1* variant carriers (*FBN1*$^+$) and non-carriers (*FBN1*$^-$). However, the occurrence of atrial fibrillation was significantly higher in *FBN1*$^+$ patients (42.1% *vs.* 22.0%, $P = 0.037$). This group also had a higher rate of implantable cardioverter-defibrillator (ICD) implantation (10.5% *vs.* 1.6%, $P = 0.004$), possibly due to their increased susceptibility to atrial fibrillation. The incidence of "any arrhythmia," which is a collective measure of atrial fibrillation, non-sustained ventricular tachycardia (NSVT), and left bundle branch block (LBBB), did not differ significantly between the two groups (63.2% *vs.* 42.1%, $P = 0.066$). Moreover, renal insufficiency was more prevalent in *FBN1*$^+$ patients (33.3% *vs.* 8.5%, $P < 0.001$), suggesting a potential link between *FBN1* variants and renal impairment, a hypothesis that would require more robust evidence to validate.

Interestingly, no significant differences in echocardiographic parameters, including LVEF, LVEDD, and left atrial diameter (LAD), were found between *FBN1*$^+$ and *FBN1*$^-$ DCM patients. This indicates that *FBN1* variants may not significantly influence these specific cardiac structural and functional parameters. Detailed statistics and additional findings can be found in [Table 1].

Continuing our exploration into the influence of the *FBN1* genotype on DCM phenotype, we conducted a more granular stratification of DCM patients into three distinct groups for a detailed baseline comparison: (1) those carrying deleterious variants in *FBN1* (*FBN1*$^+$); (2) those without *FBN1* variants but carrying variants in other known DCM-associated genes (*FBN1*$^-$/DCMGenes$^+$); and (3) those without deleterious variants in either *FBN1* or any other known DCM-associated genes (*FBN1*$^-$/DCMGenes$^-$). Relative to the *FBN1*$^-$/DCMGenes$^+$ group, the *FBN1*$^+$ group demonstrated a heightened prevalence of atrial fibrillation

(42.1% *vs.* 20.5%, $P = 0.03$), left bundle branch block (21.1% *vs.* 5.9%, $P = 0.014$), ICD implantation (10.5% *vs.* 2.0%, $P = 0.027$), and renal dysfunction (33.3% *vs.* 4.4%, $P < 0.001$). Similarly, the *FBN1*$^+$ group had increased rates of atrial fibrillation, ICD implantation, and renal dysfunction compared to the *FBN1*$^-$/DCMGenes$^-$ group. These findings suggest potential specific impacts of *FBN1* variants on cardiac electrophysiology and renal function [Supplementary Table 6].

FBN1 is known to be associated with MFS, which primarily manifests as dilation of the ascending aorta. We further examined the aortic diameters in DCM patients carrying *FBN1* variants. After adjusting for enrollment age and body surface area, the aortic root diameter and proximal ascending aorta diameter showed no significant disparities between *FBN1* variant carriers and non-carriers ($P = 0.711$ and $P = 0.159$, respectively). Moreover, rates of the aortic root and proximal ascending aorta dilatations, conditions defined by exceeding the upper reference limits (adjusted for age and body surface area), did not differ significantly ($P = 0.344$ and $P = 0.292$, respectively). The term "Any aorta dilatation" signifies the presence of either or both of these conditions, *i.e.,* dilatation of the aortic root and/or proximal ascending aorta [Table 1].

Remarkably, none of the DCM patients with rare deleterious *FBN1* variants in our cohort were diagnosed with MFS during our follow-up. Instead, cardiac dilatation served as their primary cardiovascular phenotype, deviating from the severe aortic dilatation typical of MFS. Considering patients' medical histories, it appears that *FBN1* rare deleterious variants in our DCM cohort may not result in typical MFS phenotypes, underscoring the need for more research on *FBN1* genotype-phenotype correlations in cardiovascular diseases.

Clinical outcomes

We sought to discern the potential impact of *FBN1* variants on the prognosis of DCM patients, utilizing sophisticated statistical methodologies including Kaplan-Meier survival analysis and Cox proportional hazards model analysis. A total of 1,056 (99.7%) participants accepted the final evaluation. The mean follow-up time was 47.84 ± 24.73 months. Upon comparing DCM patients with and without deleterious *FBN1* variants [Figure 3], we found no significant difference in the risk of primary endpoints (Hazard Ratio - HR: 1.38 [0.71, 2.68], $P = 0.25$). However, the risk of secondary endpoints was significantly higher in the *FBN1*$^+$ group (HR: 1.78 [1.03, 3.10], $P = 0.019$).

Further comparative analysis among pre-defined groups [Supplementary Figure 1] revealed that the *FBN1*$^+$ group did not exhibit a significantly different risk for primary endpoints ($P = 0.28$). However, an elevated risk for secondary endpoints was discerned in this group ($P = 0.049$).

The above findings indicate that *FBN1* variants may not significantly affect the primary clinical endpoints in DCM patients, yet they seem to increase the risk of secondary endpoints. These observations underscore the need for further investigation into the specific influence of *FBN1* variants on the clinical trajectory and outcome of DCM.

DISCUSSION

Drawing upon existing research, our study significantly enhances our understanding of *FBN1*'s involvement in dilated cardiomyopathy (DCM). While prior research had implied a possible *FBN1*-DCM connection, our study represents the first dedicated analysis of *FBN1*'s impact on DCM susceptibility, clinical features, and prognosis in a sizable DCM cohort. We uncovered 18 rare deleterious *FBN1* variants in 19 of 1,059 DCM patients and detailed the variants' distribution and types. Notably, we observed variations in clinical

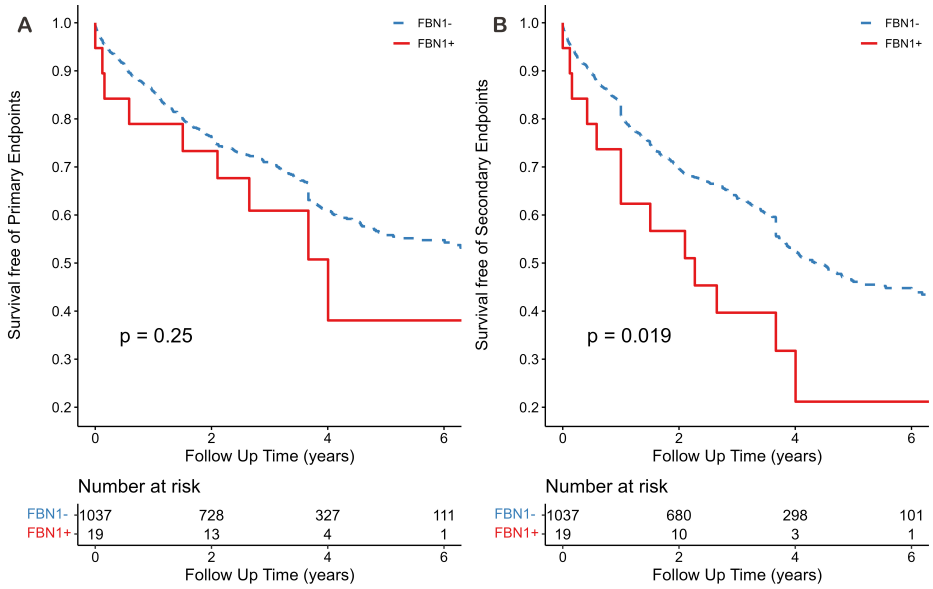

Figure 3. Clinical outcomes in DCM patients with and without rare deleterious variants in *FBN1*. (A) Kaplan-Meier curve illustrates survival free of primary endpoints, which include cardiac mortality or heart transplantation. (B) The curve represents survival free of secondary endpoints, comprising all-cause mortality or heart failure recurrence. Probability values were calculated using the log-rank test. *FBN1*$^+$: DCM cases carrying rare deleterious variants in *FBN1*; *FBN1*$^-$: DCM cases not carrying rare deleterious variants in *FBN1*.

characteristics between DCM patients with and without *FBN1* variants. *FBN1* variant carriers displayed higher rates of atrial fibrillation (42.1% *vs.* 22.0%, $P = 0.037$) and ICD implantation (10.5% *vs.* 1.6%, $P = 0.004$) than non-carriers. Using SKAT-O for gene-based association analysis, we found a significant enrichment of rare deleterious *FBN1* variants in DCM cases, further supporting the *FBN1*-DCM relationship. This highlights the relevance of *FBN1* in DCM genetics and the need for focused analyses like ours, alongside broader genetic assessments.

Previous studies have identified causal or associated genes through rigorous cosegregation and linkage analyses in extensive DCM families[33,34]. Although these conventional methods are reliable, they are limited to identifying variants with a large effect size. For low-penetrance variants, perfect cosegregation with the phenotype is improbable[35]. Hence, we performed a gene-based association analysis on DCM cases and controls, offering robust statistical measures to evaluate the impact of rare variants[36].

Our study reveals an intriguing phenomenon: while some DCM patients with *FBN1* rare deleterious variants showed MFS features, most did not present typical MFS phenotypes, such as severe aortic dilatation. Instead, their primary cardiovascular phenotype was left ventricular dilatation and systolic dysfunction, evidenced by reduced LVEF.

Our cohort included a DCM patient with an *FBN1* frameshift variant who had significant aortic dilatation. Although her condition and the pathogenic *FBN1* variant could suggest MFS, further follow-ups revealed no MFS family history or lens dislocation. However, the potential for her to develop MFS cannot be completely ruled out. On the contrary, most of our patients carrying *FBN1* variants did not have pronounced aortic dilatation, indicating that while *FBN1* variants in our study are associated with DCM, they may not lead to classic MFS phenotypes. This warrants further investigation into *FBN1* genotype-phenotype correlations in *FBN1*-associated cardiovascular diseases.

Previous studies have reported the frequency of *FBN1* variants in MFS to be between 50% and 90%[37,38]. In contrast, we found a lower detection rate of rare deleterious *FBN1* variants in our DCM cohort (1.79%). Additionally, like in MFS where missense variants are most common[8], our DCM patients with *FBN1* variants primarily had missense variants. These variations in variant prevalence underscore the complex role of *FBN1* in cardiac pathophysiology and the need for disease-specific investigations.

With over 50 implicated genes, DCM's genetic landscape is diverse[39]. Genes such as *TTN* and *LMNA* contribute significantly to DCM's clinical features, causing around 25% and 5% of autosomal dominant DCM cases, respectively[2,40-42]. In our study, we found that 1.79% of DCM patients carried deleterious *FBN1* variants, thus prompting further investigation into the *FBN1*-DCM connection.

Our findings suggest that rare deleterious *FBN1* variants may predispose individuals to DCM, highlighting the possible importance of *FBN1* in DCM's genetic structure. This lays the groundwork for future research into *FBN1*'s role in DCM pathogenesis.

Study limitations

Our study has several limitations, such as potential false positives due to focusing solely on *FBN1*, difficulty in performing pathogenicity research due to the recruitment of sporadic DCM patients without a family history, and the relatively small sample size. The identification of rare deleterious missense variants was based on computational prediction tools, introducing a possible risk of false positives. Moreover, our study is a single-center investigation conducted exclusively in an East Asian population, possibly limiting its generalizability. *FBN1* is at best expressed at low levels in the cardiac myocytes but at high levels in cardiac fibroblasts. Therefore, the deleterious effect of the rare variants in the *FBN1* gene on cardiac function could be mediated through their effects on cardiac fibroblasts and the myocardial architecture.

Conclusion

We found enrichment of rare, deleterious variants in the *FBN1* gene in patients with sporadic DCM. Our findings indicate that *FBN1* variants may increase the risk of atrial fibrillation and secondary endpoints in DCM patients. This discovery could potentially improve DCM diagnostic and therapeutic strategies. However, given the complex nature of DCM and the various factors affecting its phenotype, our findings require further validation in larger, more diverse cohorts.

DECLARATIONS

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Author's contributions

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Contribute to the enrollment and follow-up of research subjects, sample and clinical baseline data collection: Chen P, Wang H, Yu B, Wei H, Li R, Song X, Yu T
Contributed to funding, conception, revision, and final approval of the manuscript: Wang DW

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Conflict of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

This study was approved by the institutional review board of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (IRB TJ-C20181101), and was registered at ClinicalTrials.gov (ID: NCT03754101). All participants provided written informed consent.

Consent for publication

Participants provided written informed consent.

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