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Impact of *LDB3* gene polymorphisms on clinical presentation and implantable cardioverter defibrillator (ICD) implantation in Chinese patients with idiopathic dilated cardiomyopathy*#

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Abstract: Objective: Mutations in LIM domain binding 3 (*LDB3*) gene cause idiopathic dilated cardiomyopathy (IDCM), a structural heart disease with a complicated genetic background. However, the association of polymorphisms in the *LDB3* gene with susceptibility to IDCM in Chinese populations remains unexplored as dose the impact on clinical presentation. Methods: We sequenced all exons and the adjacent part of introns of the *LDB3* gene in 159 Chinese Han IDCM patients and 247 healthy controls. Then we detected the distribution of polymorphisms in the *LDB3* gene in all participants and assessed their associations with risk of IDCM. Additionally, we conducted a stratified genotype–phenotype correlation analysis. Results: The A allele of rs4468255 was significantly associated with IDCM (P<0.01). The rs4468255, rs11812601, rs56165849, and rs3740346 were also associated with diastolic blood pressure (DBP) and left ventricular ejection fraction (LVEF) (P<0.05). Notably, a higher frequency of rs4468255 polymorphism was observed in implantable cardioverter defibrillator (ICD) recipients under a recessive model (P<0.01), whereas the significant association disappeared after adjusting for potential confounders. However, in the dominant model, notable correlations could only be observed after adjusting for multi parameters. Conclusions: The rs4468255 was significantly correlated with IDCM of Chinese Han population. A allele of rs4468255 is higher in IDCM patients with ICD implantation, suggesting the influence of genetic background in the generation of this response. In addition, rs11812601, rs56165849, and rs3740346 in *LDB3* show association with brain natriuretic peptide, DBP, and LVEF levels in patients with IDCM but did not show any association with IDCM susceptibility.

Key words: Idiopathic dilated cardiomyopathy; Implantable cardioverter defibrillator (ICD); LIM domain binding 3 (*LDB3*); Polymorphism; Han Chinese
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1 Introduction

Dilated cardiomyopathy (DCM), the most common type of cardiomyopathy worldwide with an estimated prevalence of 1:2500 in the general population (Weintraub et al., 2017), is characterized by reduced left ventricular systolic function and left ventricular dilatation (Japp et al., 2016). Males are more sensitive to DCM (2.5:1) and the distribution of DCM seems to have a population bias (Towbin and Bowles, 2002). DCM is the third most frequent cause of heart failure and the most common condition requiring heart transplantation (Halliday et al., 2017). DCM also accounts for a substantial proportion of sudden cardiac death (SCD), especially amongst people of working age (Losurdo et al., 2016). Implantable cardioverter defibrillators (ICDs) have the ability to promptly recognize and treat ventricular arrhythmias and thus form the cornerstone of SCD prevention (Priori et al., 2015). However, despite years of study, the etiology of DCM remains unclear. Most DCM is sporadic and nonfamilial (idiopathic dilated cardiomyopathy, IDCM) with multifactorial causes linked to genetic susceptibility (Garfinkel et al., 2018). To date, mutations and polymorphisms in more than 50 genes are associated with the development of IDCM (Hershberger et al., 2013). Genes encoding myocardial skeleton, nuclear membrane, sarcomere, mitochondrial proteins, and the calcium homeostasis regulating proteins are the principal genes involved (Zheng et al., 2010). Specifically, mutations in LIM domain binding 3 (*LDB3*) have been implicated in the development of cardiomyopathies, including DCM (Levitas et al., 2016). The *LDB3* gene encodes for Cypher (mouse)/ZASP (human) overlapping with a DCM locus.

Cypher/ZASP is a cytoskeletal protein, which is highly expressed in the heart and is a crucial component of the sarcomeric Z-disks in binding critical proteins including α-actinin-2, protein kinase C, and myozenin family proteins (Sheikh et al., 2007). These multiprotein complexes at the Z-line have a key role in maintaining the structure and function of the adult heart. Previous research showed that germline and cardiomyocyte-restricted ablation of *LDB3* in mice results in severe DCM and premature death due to heart failure and arrhythmic SCD (Zheng et al., 2009). Notably, the gene is highly conserved in mice and humans (Faulkner et al., 1999). However, no published studies have shown that *LDB3* gene polymorphisms are associated with IDCM susceptibility in a Chinese Han population.

Thus, the aim of this study was to test the hypothesis that polymorphisms in the *LDB3* gene may affect the susceptibility of patients with IDCM. We also investigated the further impact of polymorphisms on the clinical presentation of Chinese patients with IDCM.

2 Methods

2.1 Study design

This study was based on a Chinese Han population living in Zhejiang Province, southeastern China. The present study was approved by the Medical Ethics Committee of the First Affiliated Hospital, School of Medicine, Zhejiang University and all subjects gave informed written consent. One hundred and fifty-nine unrelated patients with IDCM and 247 healthy subjects from the medical examination center were enrolled from 2011 to 2017. The diagnosis of IDCM was based on the current criteria as follows: (1) patient's left ventricular end diastolic dimension (LVEDD) greater than 50 and 55 mm for women and men, respectively; (2) left ventricular ejection fraction (LVEF) less than 45%. Echocardiography was performed by independent ultrasound staff who were blinded to the polymorphism analysis results with standard methods (Lang et al., 2015). Exclusion criteria used in this study included the following: a history of heavy drinking, nutritional deficiency, hypocalcemia, hypo- and hyper-thyroidism, diabetes mellitus, auto-immune disease, hypertension, coronary artery disease, inflammatory DCM, or familial DCM. Each patient underwent extensive clinical and laboratory evaluation at baseline. Details of patients' clinical features were shown in Table 1. The study strictly adhered to the current version of the Declaration of Helsinki.

2.2 DNA sequencing

Two milliliters of venous blood samples were obtained from each subject and were stored in tubes containing ethylenediaminetetraacetic acid (EDTA) at −80 °C in a refrigerator. Genomic DNA was

Table 1 Clinical characteristics of IDCM patients categorized by gender

Group	Age (year)	History						Admission vital signs		
		Diabetes mellitus	Atrial fibrillation	LBBB	Smoker	Drinker	NYHA classes III and IV	SBP (mmHg)	DBP (mmHg)	Heart rate (beat/min)
Control (*n*=247)	37.8±12.9						0			72.7±11.5
IDCM										
Male (*n*=104)	60.0±13.2	17 (16.3%)	11 (10.6%)	8 (7.7%)	65 (62.5%)	53 (51.0%)	62 (59.6%)	119.9±17.7	74.8±13.2	74.0±20.0
Female (*n*=42)	61.6±11.6	7 (16.7%)	7 (16.7%)	7 (16.7%)	1 (2.4%)	4 (9.5%)	29 (69.0%)	116.9±17.6	68.1±9.9	73.5±18.0
P-value	0.480	0.969	0.412	0.246	0.000	0.000	0.287	0.352	0.004	0.396

Group	Laboratory values at admission					Electrocardiogram data, QRS duration (ms)	Echocardiography data		Treatment during admission, ICD
	BNP	Na^+ (mmol/L)	K^+ (mmol/L)	Creatinine (μmol/L)	BUN (mmol/L)		LVEDD (cm)	LVEF (%)	
Control (*n*=247)	0					90.0±12.0			
IDCM									
Male (*n*=104)	624.0±1483.8	140.0±4.0	4.2±0.6	92.5±33.3	7.0±2.9	102.0±39.5	6.9±0.9	32.7±8.9	31 (29.8%)
Female (*n*=42)	734.0±1531.3	139.0±6.3	4.4±0.6	73.5±37.5	7.2±4.1	104.0±52.0	6.2±1.2	33.5±8.4	10 (23.8%)
P-value	0.872	0.135	0.083	0.000	0.750	0.385	0.001	0.620	0.466

IDCM: idiopathic dilated cardiomyopathy; LBBB: left bundle brunch block; NYHA: New York Heart Association; SBP: systolic blood pressure; DBP: diastolic blood pressure; BNP: brain natriuretic peptide; BUN: blood urea nitrogen; LVEDD: left ventricular end-diastolic dimension; LVEF: left ventricular ejection fraction; ICD: cardioverter defibrillator. Values were expressed as mean±standard deviation (SD) or number (percentage). 1 mmHg=133.3 Pa

extracted from whole blood using the TIANamp Blood DNA Kit (Tiangen, Beijing, China). Fourteen exons in the *LDB3* gene were amplified by polymerase chain reaction (PCR) using primers synthesized by TSINKE biological technology company (Beijing, China) (primer sequences available in Table S1). An ABI 3730 DNA sequencer (Applied Biosystems, Foster City, CA, USA) was used to sequence the PCR products. The DNAMAN software (http://www.lynnon.com) was adopted to produce alignments of the sequencing results and the templates. The individual who analyzed the genotype results was blinded to the clinical data.

2.3 Statistical analysis

Statistical analyses were performed with SPSS 19.0.0 (SPSS Inc., Chicago, IL, USA) and a *P*-value less than 0.05 was considered statistically significant. Continuous variables were described as mean± standard deviation (SD) or as median with interquartile range (IQR). Categorical variables were represented by numbers and percentages. According to the distribution of continuous variables, we used the Student's *t*-test or the Welch's *t*-test. As for categorical variables, we used the Pearson's Chi-square when appropriate or the Fisher's exact test. Logistic regression analysis was used to assess the risk of IDCM associated with the polymorphisms. Odds ratio (OR) and respective 95% confidence interval (95% CI) were used to assess the impact of any difference between alleles and genotypes. Univariate and multivariate logistic regressions were performed to assess the association of genetic and clinical variables. SHEsis online software platform (http://analysis.bio-x.cn/myAnalysis.php) was used either to perform Hardy-Weinberg equilibrium (HWE) testing to analyze the allele and genotype frequencies or haplotype analysis. Standardized *D*' was used to measure linkage disequilibrium.

3 Results

3.1 Clinical characteristics of IDCM patients and controls

The study included 406 subjects, consisting of 159 patients and 247 controls (13 IDCM patients lack

the basic information). In the control group, 56.7% were males with an average age of (37.8±12.9) years. One hundred and forty-six IDCM patients were divided into male and female subgroups, with an average age of (60.0±13.2) years and (61.6±11.6) years, respectively. Baseline clinical and demographic characteristics of patients with IDCM and healthy controls are listed in Table 1. Higher heart rate, brain natriuretic peptide (BNP), longer QRS duration, and a more severe New York Heart Association (NYHA) functional class were observed in IDCM patients than in the controls in the present study. The clinical and demographic characteristics were similar in the two subgroups among IDCM patients, except for smoking status, drinking status, diastolic blood pressure (DBP), creatinine, and LVEDD. Tobacco smokers and drinkers in patients were predominantly male. In addition, remarkably higher values could be found in male patients in terms of creatinine (92.5 versus 73.5 μmol/L; $P<0.01$), DBP (74.8 versus 68.1 mmHg; $P<0.01$), and LVEDD (6.9 versus 6.2 cm; $P<0.01$) than in the female group at entry. Finally, 31 (29.8%) males and 10 (23.8%) females had undergone ICD implantation.

3.2 Distribution of allele frequencies and genotype between IDCM patients and controls

The genotypic distribution of the identified nine single nucleotide polymorphisms (SNPs), except for rs3740342, rs2248643, rs55815121, rs1578895, and rs3740347 (all $P<0.05$), did not deviate from HWE ($P>0.05$) among the case or control population (Table S2). Therefore, rs3740342, rs2248643, rs55815121, rs1578895, and rs3740347 were excluded from further tests. The allele frequency distributions of the nine SNPs are shown in Table 2. Further analyses were performed based on two genetic models (dominant and recessive models), and the results are summarized in Table 3. The total numbers of some allele and genotypes were fewer than 406 owing to failed sequencing. There were no significant differences between cases and controls in the allele and genotype frequencies of rs4256897, rs2675692, rs2803555, rs11812601, rs2803558, rs3740343, rs56165849, or rs3740346. The A allele of rs4468255 was less frequent in the IDCM patients (OR, 0.15; 95% CI, 0.11–0.20; $P<0.01$). Furthermore, at logistic regression analysis, the rs4468255 showed a significant difference between patients and control under both the dominant and recessive models ($P<0.01$). The difference remained the same after adjusting for gender and age.

3.3 Relationship between clinical features and genotype in patients with IDCM

We next examined the association between the SNPs and various characteristics of IDCM, including

Table 2 Allele and genotype distributions of *LDB3* genetic polymorphisms in IDCM patients and controls

SNP	Group	Genotype			Allele frequency			Allelic model	
		11	12	22	1	2	MAF/dbSNP	OR (95% CI)	*P*-value
rs4256897G>A	Case	8	56	79	72	214	0.25	0.87 (0.62–1.21)	0.398
	Control	17	97	120	131	337	0.28/0.24	Ref	
rs2675692A>T	Case	80	56	7	216	70	0.25	1.11 (0.79–1.57)	0.552
	Control	143	84	12	370	108	0.23/0.17	Ref	
rs2803555T>C	Case	81	56	6	218	68	0.24	0.92 (0.65–1.31)	0.658
	Control	144	83	12	371	107	0.22/0.16	Ref	
rs11812601G>T	Case	26	81	36	133	153	0.47	0.95 (0.71–1.27)	0.716
	Control	52	110	75	214	260	0.45/0.36	Ref	
rs2803558T>C	Case	85	54	4	224	62	0.22	1.03 (0.72–1.47)	0.872
	Control	145	82	12	372	106	0.22/0.25	Ref	
rs4468255G>A	Case	10	66	83	86	232	0.27	0.15 (0.11–0.20)	0.000
	Control	126	101	20	353	141	0.29/0.24	Ref	
rs3740343G>A	Case	0	24	135	24	294	0.08	0.86 (0.51–1.44)	0.559
	Control	3	37	207	43	451	0.09/0.02	Ref	
rs56165849A>G	Case	68	78	12	214	102	0.32	1.24 (0.91–1.68)	0.178
	Control	129	97	20	355	137	0.28/0.44	Ref	
rs3740346G>C	Case	64	77	18	205	113	0.36	1.07 (0.80–1.43)	0.663
	Control	95	121	31	311	183	0.37/0.18	Ref	

Genotype: 11, homozygote mutant; 12, heterozygote mutant; 22, wild genotype. IDCM: idiopathic dilated cardiomyopathy; SNP: single nucleotide polymorphism; MAF: minor allele frequency; OR: odds ratio; CI: confidence interval; Ref: reference group. dbSNP: http://www.ncbi.nlm.nih.gov/snp

Table 3 Distribution of SNPs in *LDB3* among cases and controls and their association with IDCM risk based on two genetic models

SNP	Dominant model				Recessive model			
	OR (95% CI)	*P*	AOR[a] (95% CI)	*P*[a]	OR (95% CI)	*P*	AOR[a] (95% CI)	*P*[a]
rs4256897	0.85 (0.56–1.30)	0.46	0.99 (0.56–1.75)	0.97	0.76 (0.32–1.80)	0.53	0.68 (0.19–2.49)	0.56
rs2675692	1.03 (0.40–2.67)	0.96	0.68 (0.20–2.28)	0.53	0.85 (0.56–1.30)	0.46	0.87 (0.49–1.53)	0.62
rs2803555	1.21 (0.44–3.29)	0.71	0.75 (0.21–2.64)	0.65	0.86 (0.57–1.31)	0.49	0.87 (0.49–1.53)	0.63
rs11812601	1.38 (0.86–2.19)	0.18	1.11 (0.60–2.09)	0.74	0.79 (0.47–1.34)	0.38	0.75 (0.36–1.60)	0.46
rs2803558	1.84 (0.58–5.81)	0.30	1.03 (0.27–3.95)	0.97	0.95 (0.62–1.45)	0.81	0.95 (0.54–1.67)	0.85
rs4468255	0.08 (0.05–0.14)	0.00	0.07 (0.03–0.16)	0.00	0.06 (0.03–0.13)	0.00	0.04 (0.02–0.11)	0.00
rs3740343	0.92 (0.53–1.60)	0.77	1.23 (0.59–2.58)	0.58		1.00		1.00
rs56165849	1.08 (0.51–2.27)	0.85	1.08 (0.41–2.83)	0.88	0.69 (0.46–1.03)	0.07	0.67 (0.39–1.18)	0.17
rs3740346	1.12 (0.61–2.09)	0.71	2.16 (0.91–5.12)	0.08	1.08 (0.72–1.62)	0.72	1.35 (0.76–2.38)	0.30

[a] Adjusted for gender and age. SNP: single nucleotide polymorphism; IDCM: idiopathic dilated cardiomyopathy; OR: odds ratio; CI: confidence interval; AOR: adjusted odds ratio

age, sex, presence of other diseases, NYHA class, systolic blood pressure (SBP), DBP, BNP, LVEF, and LVEDD. SNPs rs11812601 and rs56165849 were shown to correlate with BNP level in patients with IDCM under the recessive model (P<0.05 for both; Table 4). As for SNP rs4468255, a lower level of DBP and LVEF in IDCM patients was observed under the dominant model (P<0.05 for both). In addition, the rs3740346 polymorphism was significantly associated with lower LVEF under a dominant but not under a recessive model in the whole IDCM population. However, we found no correlation between rs4256897, rs2675692, rs2803555, rs2803558, rs3740343 and any of the examined clinical features except for gender (data not shown).

3.4 Logistic regression analysis of ICD implantation in patients with IDCM for primary prevention of SCD

Univariate analysis demonstrated that rs4468255 in the present study was associated with the ICD implantation status of IDCM patients (Table 5). Notably, a higher frequency of rs4468255 polymorphism was observed in ICD recipients under the recessive model (OR, 8.83; 95% CI, 1.70–45.77; P=0.009), whereas the significant association disappeared after adjusting for age, sex, SBP, BNP, LVEDD, LVEF, and NYHA class (P=0.999). However, notable correlations could only be observed in the dominant model after adjusting for multi parameters (adjusted odds ratio (AOR), 3.18; 95% CI, 1.24–8.16; P=0.016).

3.5 Linkage disequilibrium and haplotype analysis

Haplotype analysis is a powerful strategy for resolving the controversy regarding association studies based on individual polymorphisms and determining whether the SNPs would have greater predictive value when analyzed together. As displayed in the linkage disequilibrium map (Fig. 1), the SNP pairs rs4256897-rs2675692-rs2803555-rs11812601-rs2803558-rs3740343-rs56165849 in *LDB3* exhibited strong linkage disequilibrium (D'>0.7). The haplotypes consisting of these seven SNPs were investigated in the current study using SHEsis software. No statistically significant difference was found between patients and controls in the haplotype analysis (partial data shown in Table 6).

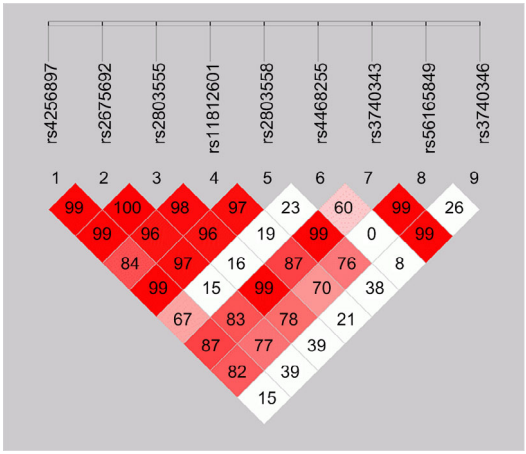

Fig. 1 Pairwise SNP linkage disequilibrium map
The D' values are shown inside each diamond. The map was drawn based on the genotype data of all case and control samples using SHEsis

4 Discussion

Recent molecular genetic studies have confirmed the increasingly obvious primary role of genetic factors

Table 4 Association of *LDB3* polymorphisms with clinical characteristics of IDCM patients

SNP	Model	Genotype	BNP		DBP		LVEF	
			Median±IQR	*P*-value	Median±IQR	*P*-value	Median±IQR	*P*-value
rs4256897	Dominant	AA+AG	760±1670	0.542	70±15	0.226	32±12	0.622
		GG	710±1842		73±16		33±12	
	Recessive	AA	1582±4770	0.303	72±10	0.898	29±11	0.311
		GA+GG	747±1774		71±17		32±11	
rs2675692	Dominant	TT+AT	751±1814	0.548	71±15	0.131	32±10	0.871
		AA	815±1302		64±19		33±20	
	Recessive	TT	714±1289	0.568	70±17	0.681	32±12	0.363
		AT+AA	850±2141		71±16		33±12	
rs2803555	Dominant	CC+CT	754±1791	0.292	71±16	0.345	32±11	0.815
		TT			65±19		39±23	
	Recessive	CC	714±1285	0.566	70±17	0.790	32±13	0.519
		CT+TT	946±2240		71±16		33±11	
rs11812601	Dominant	TT+TG	759±2205	0.553	72±17	0.153	32±12	0.717
		GG	578±1349		70±10		32±12	
	Recessive	TT	446±792	0.032	75±16	0.557	34±14	0.105
		TG+GG	828±2227		71±16		32±12	
rs2803558	Dominant	CC+CT	754±1791	0.292	71±15	0.232	32±11	0.273
		TT			64±20		40±17	
	Recessive	CC	714±1289	0.627	70±17	0.600	32±13	0.430
		CT+TT	850±2141		71±17		33±10	
rs4468255	Dominant	AA+AG	587±1238	0.112	69±16	0.015	31±11	0.037
		GG	760±1774		74±18		34±11	
	Recessive	AA	1080±4514	0.307	69±12	0.904	26±12	0.033
		GA+GG	641±1382		71±16		32±12	
rs3740343	Dominant	AA+AG	436±995	0.308	69±18	0.437	30±10	0.100
		GG	685±1721		71±16		33±12	
	Recessive	AA						
		GA+GG						
rs56165849	Dominant	GG+GA	642±1451	0.726	71±16	0.480	32±13	0.653
		AA	1429±1619		70±16		31±9	
	Recessive	GG	818±1844	0.041	71±15	0.998	32±11	0.784
		GA+AA	641±1440		71±16		32±12	
rs3740346	Dominant	CC+CG	614±1346	0.241	71±16	0.368	32±11	0.012
		GG	1396±1485		70±18		38±14	
	Recessive	CC	753±2021	0.201	71±15	0.828	33±11	0.096
		CG+GG	520±1075		71±18		33±13	

IDCM: idiopathic dilated cardiomyopathy; SNP: single nucleotide polymorphism; BNP: brain natriuretic peptide; DBP: diastolic blood pressure; LVEF: left ventricular ejection fraction; IQR: interquartile range

in the pathogenesis of IDCM. SNPs are the most common type of genetic variation in the human genome, and many SNPs have been identified in patients with IDCM (Jiang et al., 2016). Abnormalities in cytoskeletons, connecting the sarcomere and sarcolemma, are the main cause of the pathogenesis of DCM. To our knowledge, the data presented in this study provided the first evidence that the SNP rs4468255 in *LDB3* was associated with IDCM in a Chinese Han population.

In the present study, we found rs4468255 was significantly associated with IDCM instead of the other eight identified SNPs. The A allele of rs4468255 might be associated with IDCM (OR, 0.15; $P<0.01$). This result was also confirmed by univariate analysis under a recessive model, indicating that the AA genotype was significantly associated with IDCM. In haploid analysis, however, the haplotype consisting of seven highly linkage SNPs showed no significant association with IDCM.

Because of the complicated genetic architecture of IDCM, the etiology of IDCM may involve multiple genes, and the effect of each individual risk allele is likely to be small. Moreover, the factors underlying

Table 5 Genetic basis of *LDB3* gene polymorphisms in ICD recipients

SNP	Model	OR (95% CI)	*P*-value	AOR[a] (95% CI)	*P*-value[a]
rs4256897	Dominant	1.21 (0.57–2.57)	0.617	1.97 (0.76–5.09)	0.161
	Recessive	5.09 (0.89–29.03)	0.067	9.29 (0.81–106.71)	0.074
rs2675692	Dominant	0.58 (0.15–4.85)	0.855	0.92 (0.06–14.68)	0.955
	Recessive	1.57 (0.73–3.36)	0.251	0.19 (0.06–0.57)	0.109
rs2803555	Dominant	1.75 (0.19–16.15)	0.623	2.25 (0.84–6.05)	0.999
	Recessive	1.50 (0.70–3.22)	0.301	2.21 (0.81–6.00)	0.121
rs11812601	Dominant	0.76 (0.33–1.79)	0.535	0.42 (0.14–1.27)	0.124
	Recessive	1.42 (0.54–3.72)	0.476	1.85 (0.55–6.23)	0.321
rs2803558	Dominant	1.30 (0.13–12.86)	0.825		0.999
	Recessive	1.53 (0.71–3.32)	0.281	2.10 (0.77–5.70)	0.147
rs4468255	Dominant	2.00 (0.96–4.19)	0.064	3.18 (1.24–8.16)	0.016
	Recessive	8.83 (1.70–45.77)	0.009		0.999
rs3740343	Dominant	1.69 (0.67–4.23)	0.265	2.26 (0.69–7.38)	0.177
	Recessive			1.59 (0.50–5.04)	0.432
rs56165849	Dominant		0.999		0.999
	Recessive	1.50 (0.72–3.12)	0.278	1.70 (0.67–4.30)	0.263
rs3740346	Dominant	3.25 (0.71–14.90)	0.129	2.35 (0.46–12.13)	0.307
	Recessive	1.61 (0.78–3.34)	0.200	1.48 (0.61–3.58)	0.389

[a] Adjusted for age, gender, systolic blood pressure, left ventricular ejection fraction, left ventricular end-diastolic dimensions, brain natriuretic peptide, New York Heart Association class, history of diabetes mellitus, smoking, and drinking. ICD: cardioverter defibrillator; SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval

Table 6 *LDB3* haplotype distribution and effect on IDCM and controls

Haplotype	Frequency[a]		χ^2-test	OR (95% CI)	*P*-value
	IDCM	Control			
A-T-C-G-C-A-G	17.28 (6.1%)	40.88 (8.8%)	1.465	0.698 (0.39–1.25)	0.226
A-T-C-G-C-G-G	43.39 (15.3%)	76.07 (16.3%)	0.033	0.963 (0.64–1.45)	0.857
G-A-T-G-T-G-A	50.27 (17.7%)	84.82 (18.2%)	0.002	1.009 (0.68–1.49)	0.964
G-A-T-G-T-G-G	10.44 (3.7%)	13.13 (2.8%)	0.550	1.366 (0.60–3.13)	0.458
G-T-C-G-C-G-A	13.09 (4.6%)	21.66 (4.6%)	0.006	1.028 (0.51–2.08)	0.939
G-T-C-T-C-G-A	10.60 (3.7%)	15.75 (3.4%)	0.119	1.150 (0.52–2.54)	0.731
G-T-C-T-C-G-G	113.39 (39.9%)	186.91 (40.1%)	0.116	1.055 (0.77–1.44)	0.734

[a] Data are expressed as number (percentage). Loci chosen for haplotype analysis: rs4256897-rs2675692-rs2803555-rs11812601-rs2803558-rs3740343-rs56165849; IDCM: idiopathic dilated cardiomyopathy; OR: odds ratio; CI: confidence interval

inter-individual variations in the susceptibility to IDCM may also include demographic, clinical, and environmental variables. Among IDCM patients, we did find significant associations between BNP, DBP, LVEF levels and genotypes, including rs11812601, rs56165849, rs4468255, and rs3740346. However, rs11812601, rs56165849, and rs3740346 showed no significant association with IDCM. Considering that IDCM encompasses a broad and time-dependent spectrum of structural and functional abnormalities (Bondue et al., 2018), we hypothesized that these SNPs may be associated with late onset cardiomyopathy. For the above-mentioned effect of the A allele of rs4468255, A allele was also associated with lower LVEF under both the dominant and recessive models. It is well known that low LVEF identifies a group of patients with a relatively increased risk of SCD (Reibis et al., 2017). To sum up, our study provided some clues regarding the potential clinical association of the *LDB3* gene with IDCM. However, the biological mechanism explaining the association between the polymorphisms in *LDB3* and IDCM risk remains unclear.

Observational studies suggested that up to 30% of deaths in patients with DCM are sudden (Tamburro and Wilber, 1992). Current guidelines recommend the use of ICDs for the primary prevention of SCD in patients with DCM, NYHA class II–III heart failure, and LVEF <35% (Priori et al., 2015). However, four individual randomized trials together with one meta-analysis have failed to show a significant reduction in all-cause mortality with ICD therapy in patients with

DCM (Bänsch et al., 2002; Strickberger et al., 2003; Kadish et al., 2004; Køber et al., 2016; Wolff et al., 2017). In conclusion, current research demonstrates the inadequacy of a risk stratification algorithm based on LVEF and illustrates the importance of developing a more sensitive and specific approach.

As gene sequencing expands, more high-risk mutations may be discovered, which can help better clinical decision-making and in-depth understanding of disease mechanisms. Thus, the role of genetics in risk stratification is emerging. For instance, one non-missense mutation in *LMNA* commonly underpins more aggressive management that ICDs should be implanted earlier than current guidelines because of the malignant nature of the associated phenotype (Glöcklhofer et al., 2018). In addition, van der Zwaag et al. (2013) studied 403 cases carrying a specific mutation in the *PLN* gene which encodes phospholamban with an important role in calcium homeostasis and found 19% had malignant ventricular arrhythmia defined as SCD over 42 months. These studies suggested that mutations in genes controlling calcium handling may influence arrhythmic risk independent of structural changes. Meanwhile, our team showed in preliminary work that Cypher is a novel A-kinase anchoring protein regulating L-type calcium channel through a Ser/Thr phosphatase (Lin et al., 2013). Later, studies identified that Cypher/ZASP interacts with protein kinase A (PKA) and the Ser/Thr phosphatase CaN, making Cypher/ZASP-PKA-CaN a signaling center for sarcomeric proteins or channels such as the cardiac $Ca_V1.2$ channel (Yu et al., 2018). Vatta et al. (2003) found a missense mutation in *LDB3* (S196L) in a family with DCM. Interestingly, the brother of the proband died with SCD (Vatta et al., 2003). Moreover, *LDB3* S196L knockin mice exhibit a disturbance in calcium currents which also develop a DCM phenotype (Li et al., 2010). Taken together, we hypothesized that specific mutations or polymorphisms in *LDB3* may affect arrhythmic risk due to ion channel dysfunction. Based on this, we analyzed the relationship between SNPs in *LDB3* and the trend of receiving ICD therapy. However, because this study was not designed to determine the effect of ICD therapy, we cannot definitively predict the efficacy of ICD implants in the early phase. We noticed that rs4468255 tended to obtain a lower frequency in ICD recipients under the dominant model. Further studies are warranted to investigate the role of *LDB3* gene alterations leading to arrhythmias in patients with DCM.

5 Conclusions

The polymorphism rs4468255 is associated with IDCM in a Chinese Han population and may impact on primary prevention of IDCM. This is the basis of the personalized medicine to identify at-risk individuals. In addition, rs11812601, rs56165849, and rs3740346 in *LDB3* show an association with BNP, DBP, and LVEF levels in patients with IDCM but did not show any association with IDCM susceptibility. However, further investigations are needed to better understand the molecular mechanisms and the clinical implications, particularly to assess whether IDCM patients with specific gene polymorphisms would benefit from a more aggressive medical and interventional management. Our study group was not very large, so the research should be replicated and extended to better designed studies with larger populations of different ethnic groups worldwide and well-matched controls.

Contributors

Dong-fei WANG, Jia-lan LYU, and Juan FANG prepared samples and conceived the experiment. Dong-fei WANG, Hong-qiang CHENG, and Xiao-gang GUO designed the experiment. Jian-mei JIN and Fang-hong DONG collected the data. Jia-qi HUANG and Shu-dong XIA compared the sequencing results with the templates. Dong-fei WANG, Jia-lan LYU, and Jian CHEN processed and analyzed data. Dong-fei WANG, Wan-wan CHEN, Ying-ke XU, and Xiao-gang GUO wrote the manuscript with input from all authors.

Compliance with ethics guidelines

Dong-fei WANG, Jia-lan LYU, Juan FANG, Jian CHEN, Wan-wan CHEN, Jia-qi HUANG, Shu-dong XIA, Jian-mei JIN, Fang-hong DONG, Hong-qiang CHENG, Ying-ke XU, and Xiao-gang GUO declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study.

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List of electronic supplementary materials

Table S1 Primer sequences of *LDB3* gene used in polymerase chain reaction

Table S2 Hardy-Weinberg equilibrium (HWE) testing among cases and controls

中文概要

题　目：中国汉族特发性扩张型心肌病患者 *LDB3* 基因多态性与临床表现及植入型心律转复除颤器（ICD）植入的相关性研究

目　的：探讨 *LDB3*（LIM domain binding 3）基因多态性与中国汉族特发性扩张型心肌病发病的相关性，并分析 *LDB3* 基因多态性对患者临床表现和植入型心律转复除颤器（ICD）植入的影响。

创新点：首次明确 *LDB3* 基因多态性与中国汉族特发性扩张型心肌病发病密切相关，发现了三个与脑钠肽、舒张压、左室射血分数相关的多态性位点和一个与植入心律转复除颤器相关的多态性位点。

方　法：本研究纳入我院 159 例中国汉族特发性扩张型心肌病患者和 247 例无亲缘关系健康对照并收集基线临床资料。提取外周血 DNA 后，聚合酶链式反应扩增 *LDB3* 基因的 14 个外显子，经 Sanger 一代测序，获得基因突变位点。通过关联分析，研究基因多态性与特发性扩张型心肌病发病之间的相关性。根据连锁不平衡系数和基因分布频率进行单倍体分析，研究不同单倍体分型对特发性扩张型心肌病发病的影响。

结　论：在 *LDB3* 基因多态性分析中，发现 9 个多态性位点符合哈代-温伯格平衡。其中 rs4468255 位点多态性与中国汉族特发性扩张型心肌病发病密切相关。rs11812601、rs56165849 和 rs3740346 位点多态性与患者舒张压、左室射血分数存在显著关联（$P<0.05$）。且携带 rs4468255 多态性的患者更倾向于安装植入型心律转复除颤器。

关键词：特发性扩张型心肌病；植入型心律转复除颤器；*LDB3*；基因多态性；中国汉族