RESEARCH ARTICLE

NOTCH4 is a possible novel susceptibility gene for dilated cardiomyopathy in the Chinese population: A case-control study

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Background: The incidence of dilated cardiomyopathy (DCM) has increased in recent years, and many studies have sought to further improve the general understanding of this condition. Previous studies have demonstrated that some single nucleotide polymorphisms (SNPs) associated with systemic lupus erythematosus also affect susceptibility to DCM, suggesting that immune-related diseases may share similar genetic susceptibility. Recent large-scale and genome-wide association studies have identified *NCR3*, *NOTCH4*, *CYP1A2*, *ITGA1*, *OPRM1*, *ST8SIA2*, and *LINC00704* as genetic risk factors associated with cardiac manifestations of neonatal lupus. Here, we aimed to determine whether these SNPs conferred susceptibility to DCM in the Chinese Han population.

Methods: We investigated the relationship between these polymorphisms and DCM risk in 273 patients with DCM and 548 healthy controls. Genotyping was performed using MassArray iPLEX system.

Results: Logistic regression analysis indicated that the T allele of rs3134942 in *NOTCH4* gene increased the risk of DCM by 61% compared with the G allele ($P_a = 6.57 \times 10^{-3}$). The SNP rs3134942 was also significantly associated with increased DCM risk in the additive ($P_a = 6.57 \times 10^{-3}$) and dominant models ($P_a = 1.01 \times 10^{-2}$). Additionally, rs2472299 in *CYP1A2* gene showed suggestive association with reduced risk of DCM in the dominant model ($P_a = 4.24 \times 10^{-2}$) and was correlated with smoking status in patients with DCM ($P_a = 1.56 \times 10^{-2}$).

Conclusions: Our findings suggested that rs3134942 in *NOTCH4* may be involved in DCM risk. Further, studies in larger and ethnically diverse populations are required to confirm the results reported in this study.

KEYWORDS

CYP1A2, dilated cardiomyopathy, gene polymorphism, *NOTCH4*, susceptibility

1 | INTRODUCTION

Dilated cardiomyopathy (DCM), the most common type of cardiomyopathy worldwide, is characterized by ventricular enlargement and systolic dysfunction with normal left ventricular wall thickness.¹ Generally, DCM diagnosis is performed by two-dimensional echocardiography.² DCM leads to developmental heart failure, decreased left ventricular contractile function, arrhythmias, conduction system abnormalities, thromboembolism, and sudden or heart failure-related death.³ Moreover, DCM is the third most frequent cause of heart failure and the most common condition requiring heart transplantation.⁴ This disease also leads to an increased risk of morbidity and mortality. DCM is a general and mostly irreversible form of heart muscle disease, with an estimated prevalence of up to 1:2500; its predicted incidence is 19 in 100 000 in China⁵ and 1 in 400 in the United States.¹ In addition, DCM occurs more frequently in men than in women (2.5:1), and the distribution of DCM seems to have a population bias, occurring more frequently in African Americans than in Caucasians (2.5:1).^{6,7}

The infection-immune theory has long been hypothesized to explain the pathogenesis of etiologically unrecognized DCM.⁸ Recent evidences had suggested that immune mediators trigger the onset and progression of DCM.^{9,10} Myocarditis or myocardial fibrosis due to the infiltration of inflammatory cells is the second most common cardiac complication of systemic lupus erythematosus (SLE).¹¹ Inflammation could either resolve or continue and result in cardiac remodeling, ventricular dilation with normal or reduced left ventricular wall thickness, and systolic dysfunction, which in turn results in DCM.⁸ It has been reported that DCM is one of the most severe complications involving organs in SLE¹² and is also one of the cardiac manifestations of neonatal lupus (cardiac-NL) syndrome.¹³ Furthermore, single nucleotide polymorphisms (SNPs), such as *MCP-1* -2518 A/G (rs1024611),^{14,15} *TNF- α* -308G/A (rs1800629),^{16,17} *CTLA-4*+49 A/G (rs231775),^{18,19} and *IL-6* -174 G/C (rs1800795),^{20,21} have also been found to be associated with the risk of DCM and SLE, supporting a genetic role for their shared susceptibility that predisposes individuals to these two immune-related diseases. Therefore, we assumed that both diseases (DCM and cardiac-NL) may share certain susceptibility polymorphic loci. To date, several susceptibility loci have been shown to be associated with cardiac-NL by genome-wide association study (GWAS)²² and large-scale investigation.²³ These include *NCR3* (rs2857595), *NOTCH4* (rs3134942), *CYP1A2* (rs2472299), *ITGA1* (rs2432143), *OPRM1* (rs2272381), *ST8SIA2* (rs1487982), and *LINC00704* (rs1391511).

Cardiac-NL and DCM are immune-related diseases caused by inflammation; both result in the development of myocardial fibrosis. Additionally, considering the genetic overlap in immune-related diseases and the associations between these SNPs and cardiac-NL, we hypothesized that the rs2857595, rs3134942, rs2472299, rs2432143, rs2272381, rs1487982, and rs1391511 polymorphisms may constitute a genetic background that contributed to the development of DCM in the Chinese Han population. Therefore, in this

study, we analyzed SNPs in these seven susceptibility loci for cardiac-NL to determine whether they conferred susceptibility to DCM in the Chinese Han population.

2 | MATERIALS AND METHODS

2.1 | Participants

Two hundred and seventy-three patients with DCM were recruited at the Department of Clinical Laboratory of The First Affiliated Hospital of Zhengzhou University (Zhengzhou, China) between June 2015 and May 2017 for this case-control study. Subjects were recruited if they met the following criteria: 1) left ventricular end-diastolic dimension >55/50 mm (men/women); 2) left ventricle ejection fraction <45%; and 3) left ventricular shortening fraction <25% or ventricular end-diastolic diameter index >27 mm/m². Exclusion criteria applied in this study included the following: angiographically assessed evidence of coronary heart disease and ischemic cardiomyopathy, significant valvular heart disease (grade 2, such as mitral or aortic regurgitation), congenital heart disease, hypertensive cardiomyopathy, myocarditis (by endomyocardial biopsy, when available), and other secondary forms of heart failure.²⁴ Patients with a positive family history were also excluded. In addition, 548 healthy and unrelated controls were matched by sex and age to individual patients with DCM during the same period. Each control was a healthy volunteer, defined as an asymptomatic individual without any diagnosed illness who was normal based on detailed physical examination. All cases and controls were of Han ethnicity from the same region in Zhengzhou, China. This study was approved by the ethics committee of The First Affiliated Hospital of Zhengzhou University (2017-LW-61). Written informed consent was obtained from all participants.

2.2 | DCM subgroups

To test the relationships between the SNPs and various clinical characteristics of DCM, cases were stratified based on 11 characteristics of patients with DCM (Table 1). Patient characteristics at diagnosis, including sex, smoking status, alcohol consumption status, presence of other diseases, presence of hypertension, classification under the New York Heart Association (NYHA class), red blood cell distribution width (RDW), N-terminal pro-brain natriuretic peptide (NT-pro-BNP), percentage of eosinophil granulocytes (E %), neutrophil-to-lymphocyte ratio (NLR), and pulmonary artery pressure, were collected. Details of patients' clinical features are presented in Table 1.

2.3 | Genotyping of selected SNPs

The following SNPs were selected and genotyped: rs2857595, rs3134942, rs2472299, rs2432143, rs2272381, rs1487982, and rs1391511 (Table 2). Candidate SNPs were selected based on

TABLE 1 Clinical characteristics of patients with dilated cardiomyopathy enrolled in this study and standards for stratification of cases in the analysis of subgroups

Characteristics	Cases		Controls
	Stratified	Number of patients [n (%)]	Number of controls [n (%)]
Age, years (mean $\pm$ SD)		47.18 $\pm$ 15.66	46.24 $\pm$ 13.57
Sex			
Males	N	197 (72.16%)	396 (72.26%)
Females	P	76 (27.84)	152 (27.74%)
Smoking status			
Ever	P	91 (33.33%)	-
Never	N	182 (66.67%)	-
Drinking status			
Ever	P	84 (30.77%)	-
Never	N	189 (69.23%)	-
Combined with other diseases			
Yes	P	184 (67.40%)	-
No	N	89 (32.60%)	-
Combined with hypertension			
Yes	P	71 (26.01%)	-
No	N	202 (73.99%)	-
NYHA Class			
1-3	N	112 (41.02%)	-
4	P	111 (40.66%)	-
NA		50 (18.32%)	-
Biochemical parameter (median; min-max)			
RDW-CV (%)		14.70 (11.9-31.3)	-
>14.5	P	146 (53.48%)	-
$\leq$ 14.5	N	127 (46.52%)	-
E %		1.50 (0-15.3)	-
$\leq$ 0.4	N	52 (19.05%)	-
>0.4	P	221 (80.95%)	-
NLR		2.35 (0.57-23.67)	-
$\geq$ 3.75	P	68 (24.91%)	-
<3.75	N	205 (75.09%)	-
NT-pro-BNP (pg/mL)		2869.00 (29.80->25 000)	-
>1500	P	184 (67.40%)	-
$\leq$ 1500	N	77 (28.21%)	-
NA		12 (4.40%)	-
Pulmonary artery pressure (mm Hg)		39 (4-88)	-
>30	P	123 (45.05%)	-
$\leq$ 30	N	73 (26.74%)	-
NA		77 (28.21%)	-

E %, percentage of eosinophil granulocytes; N, patients negative for a certain characteristic; NA, not applicable; NLR, neutrophil-to-lymphocyte ratio; NYHA Class, classification of the New York Heart Association; P, patients positive for a certain characteristic; NT-pro-BNP, N-terminal pro-brain natriuretic peptide; RDW, red blood cell distribution width.

previously demonstrated strong associations with cardiac-NL. We focused on functional SNPs with a minor allele frequency of more than 5% in the Chinese Han population.

Peripheral blood samples (2 mL) were collected from all participants. DNA from each patient and control was extracted from peripheral white blood cells using a commercial genomic DNA kit (Tiangen, Beijing, China) and stored at -80°C , in accordance with the manufacturer's instructions. DNA from all participants was genotyped using MassArray iPLEX system (San Diego, CA, USA) according to the manufacturer's protocol. We used MassArray Assay Design 4.0 software to design primers for multiplex polymerase chain reaction (PCR) and locus-specific single-base extension (Table S1). PCR was conducted in a 384-well plate, and the products were subjected to locus-specific single-base extension reactions. The final products were then desalted and transferred to a 384-element SpectroCHIP array (Agena, CA, USA). Genotyping was performed via matrix-assisted laser desorption ionization time-of-flight mass spectrometry using MassArray Typer software.

2.4 | Association analysis of the genotyped SNPs

Seven SNPs were tested using chi-square (χ^2) tests in controls; any SNPs that deviated from the Hardy-Weinberg equilibrium (HWE; $P < .05$ in the control groups) were removed from subsequent analysis (Table S2). The association between each SNP and susceptibility to DCM was evaluated by logistic regression analysis, conditioned using sex and age as covariates as the incidence of DCM is both sex- and age-related. For the testing of the genetic model (additive, dominant, and recessive models), genotype frequencies were analyzed using a logistic regression model adjusted for sex and age. Data analysis was performed using PLINK 1.07.²⁵ The odds ratios (ORs) and 95% confidence intervals (95% CIs) were computed, and P values (corrected for multiple testing by permutation adjustment) of less than .05 (two-tailed) were considered to represent statistical significance.

3 | RESULTS

3.1 | Characteristics of participants

A total of 821 participants, including 273 DCM cases and 548 controls, were recruited from the Chinese Han population. The clinical and demographic characteristics of patients with DCM are illustrated in Table 1. There are no significant discrepancies between cases and controls in terms of age ($P_{\text{age}} = .40$, t test). Also, the ratios of male to female are well matched (2.59:1 for the case group and 2.61:1 for the control group) and were consistent with previously reported data (2.5:1).^{6,7}

The seven SNPs did not deviate from the HWE in healthy controls (all P values $>.05$), and the call rate was more than 95% (Table S2). The genotyping success rates for rs2857595, rs3134942, rs2472299, rs2432143, rs2272381, rs1487982, and rs1391511 were 98.54%, 99.15%, 98.42%, 99.03%, 99.03%, 95.37%, and 98.4%, respectively.

Genes	SNPs	Positions	Major/minor	MAF	Regions
<i>NCR3</i>	rs2857595	6p21.33	G/A	0.49	Intergenic
<i>NOTCH4</i>	rs3134942	6p21.32	G/T	0.08	Synonymous
<i>CYP1A2</i>	rs2472299	15q24.1	G/A	0.40	Intergenic
<i>ITGA1</i>	rs2432143	5q11.2	T/C	0.16	Intronic
<i>OPRM1</i>	rs2272381	6q25.2	A/G	0.21	Intron
<i>ST8SIA2</i>	rs1487982	15q26.1	A/G	0.10	Intron
<i>LINC00704</i>	rs1391511	10p15.1	A/G	0.48	Intron

MAF, minor allele frequency; SNP, single nucleotide polymorphism.

TABLE 2 Genomic characteristics of the single nucleotide polymorphisms (SNPs)

TABLE 3 Genetic association analysis of single nucleotide polymorphisms (SNPs) in patients with dilated cardiomyopathy and controls by logistic regression analysis

Genes	SNPs	Alleles	Cases (%)	Controls (%)	Allelic test		
					<i>P</i>	<i>P_a</i>	OR (95% CI)
<i>NCR3</i>	rs2857595	A	225 (42.13)	475 (43.82)	.52	.62	0.93 (0.76-1.15)
		G	309 (57.87)	609 (56.18)			
<i>NOTCH4</i>	rs3134942	T	66 (12.22)	87 (8.00)	6.07×10^{-3}	6.57×10^{-3}	1.61 (1.15-2.27)
		G	474 (87.78)	1001 (92.00)			
<i>CYP1A2</i>	rs2472299	A	200 (37.31)	444 (41.11)	.15	.26	0.86 (0.70-1.06)
		G	336 (62.69)	636 (58.89)			
<i>ITGA1</i>	rs2432143	C	79 (14.63)	159 (14.64)	.98	1.00	1.00 (0.75-1.33)
		T	461 (85.37)	927 (85.36)			
<i>OPRM1</i>	rs2272381	G	114 (21.11)	245 (22.56)	.45	.39	0.91 (0.70-1.17)
		A	426 (78.89)	841 (77.44)			
<i>ST8SIA2</i>	rs1487982	G	45 (8.72)	109 (10.38)	.30	.56	0.83 (0.58-1.19)
		A	471 (91.28)	941 (89.62)			
<i>LINC00704</i>	rs1391511	G	250 (47.17)	505 (46.50)	.78	.86	1.03 (0.84-1.26)
		A	280 (52.83)	581 (53.50)			

CI, confidence interval; OR, odds ratio; *P_a*, *P*-value corrected by permutation (1 000 000 times); SNP, single nucleotide polymorphism.

3.2 | *NOTCH4* was significantly associated with DCM

The allele frequency distributions of the seven SNPs are shown in Table 3. For the non-HLA class region, rs2472299, rs2432143, rs2272381, rs1487982, and rs1391511 did not show significant differences between cases and controls in terms of allele frequencies (all, *P_a* > .05). For the HLA class region, the T allele frequency of rs3134942 in *NOTCH4* was strongly associated with DCM (OR = 1.61, 95% CI: 1.15-2.27, *P_a* = 6.57×10^{-3}). This indicated that the T allele of rs3134942 was associated with increased DCM risk by 61% compared with the G allele. However, for the other SNPs (rs2857595 in *NCR3*) in the HLA class region, no significant association was observed (*P_a* > .05).

Further analysis was performed based on three genetic models (additive, dominant, and recessive models), and the results are summarized in Table 4. In the non-HLA region, for rs2472299 in *CYP1A2*, a suggestive association was observed in the dominant

model (OR = 0.72, 95% CI: 0.53-0.97, *P_a* = 4.24×10^{-2}), which indicated that genotype AA/AG reduced the risk of DCM by 28% compared to genotype GG. However, in the HLA region, significant associations with increased risk were noted in patients with DCM for rs3134942 in *NOTCH4* in the additive (OR = 1.61, 95% CI: 1.15-2.27, *P_a* = 6.57×10^{-3}) and dominant models (OR = 1.63, 95% CI: 1.13-2.36, *P_a* = 1.01×10^{-2}). None of the additionally tested variants alone (rs2857595, rs2432143, rs2272381, rs1487982, and rs1391511; all, *P_a* > .05, Table 4) showed any significant association with DCM in the genetic model analysis.

3.3 | *CYP1A2* was significantly associated with smoking status in patients with DCM

We next examined the associations between the SNPs and various characteristics of DCM. Cases were stratified based on 11 characteristics of DCM (Table 1), including sex, smoking status, alcohol consumption status, presence of other diseases, presence

TABLE 4 Analysis of the seven single nucleotide polymorphisms (SNPs) in dilated cardiomyopathy based on three genetic models

Genes	SNPs	Additive model			Dominant model			Recessive model		
		P	P_a	OR (95% CI)	P	P_a	OR (95% CI)	P	P_a	OR (95% CI)
NCR3	rs2857595	.52	.62	0.93 (0.76-1.15)	.43	.43	0.88 (0.64-1.21)	.84	.75	0.96 (0.66-1.41)
NOTCH4	rs3134942	6.07×10^{-3}	6.57×10^{-3}	1.16 (1.15-2.27)	8.79×10^{-3}	1.01×10^{-2}	1.63 (1.13-2.36)	.18	.13	2.83 (0.63-12.81)
CYP1A2	rs2472299	.15	.26	0.86 (0.70-1.06)	3.27×10^{-2}	4.24×10^{-2}	0.72 (0.53-0.97)	.94	1.00	1.01 (0.69-1.51)
ITGA1	rs2432143	.98	1.00	1.00 (0.75-1.33)	1.00	1.00	1.00 (0.72-1.39)	.93	1.00	0.96 (0.39-2.38)
OPRM1	rs2272381	.45	.39	0.91 (0.70-1.17)	.72	.62	0.95 (0.70-1.28)	.21	.24	0.60 (0.27-1.34)
ST8SIA2	rs1487982	.30	.56	0.83 (0.58-1.19)	.19	.20	0.76 (0.51-1.14)	.48	.50	1.61 (0.43-6.07)
LINC00704	rs1391511	.78	.86	1.03 (0.84-1.26)	.89	1.00	1.02 (0.74-1.41)	.75	.70	1.06 (0.75-1.50)

CI, confidence interval; OR, odds ratio; P_a , P-value corrected by permutation (1 000 000 times); SNP, single nucleotide polymorphism.

of hypertension, NYHA class, high RDW, high NT-pro-BNP, low E %, NLR, and pulmonary hypertension. SNP rs2472299 in CYP1A2 was shown to correlate with smoking status in patients with DCM. A total of 268 patients with DCM (5 were not successfully genotyped), including 91 patients with DCM with smoking (A/G, 54/128) and 177 patients with DCM without smoking (A/G, 146/208), were successfully genotyped in rs2472299 (OR = 1.67, 95% CI: 1.14-2.44, $P_a = 1.56 \times 10^{-2}$). No associations were found between rs2857595, rs3134942, rs2432143, rs2272381, rs1487982, and rs1391511 and any of the examined clinical features ($P_a > .05$; Table 5).

4 | DISCUSSION

In the present study, we investigated the genetic contributions of NCR3, NOTCH4, CYP1A2, ITGA1, OPRM1, ST8SIA2, and LINC00704 to DCM based on the strong associations reported for cardiac-NL and the potentially shared genetic susceptibilities of two immune-related diseases (DCM and cardiac-NL). Our research confirmed that T allele of rs3134942 in NOTCH4 was significantly associated with increased risk of DCM. In addition, associations were found between rs3134942 and DCM under the additive and dominant models, indicating that rs3134942 represented a putative susceptibility gene for DCM in Chinese Han patients. Taken together, these results supported the notion that rs3134942 may act as a possible genetic factor affecting the pathogenesis of DCM and cardiac-NL.

NOTCH4 is located at 6p21.3, at the centromeric end of the HLA class III region, and encodes a 2003-amino acid transmembrane protein involved in the Notch signaling pathway. This pathway is active early in cardiac development.²⁶ Defective Notch signaling has been shown to cause various cardiac pathologies, including left ventricular noncompaction and bicuspid aortic valve.²⁷ Mutations in Notch signaling components are implicated in coronary heart disease.²⁷ The present findings indicated that mutations in NOTCH4 may be associated with DCM risk. In situ hybridization experiments have shown that NOTCH4 transcripts are mostly restricted to endothelial cells both in embryonic and adult mammals, indicating a specific and significant role of NOTCH4 during vascular development.²⁸ Previous study showed that activated NOTCH4 inhibits angiogenesis,²⁹ and mice with mutations in genes encoding Notch system components presented major defects in vascular morphogenesis.³⁰ Moreover, Notch signaling is an evolutionarily conserved intercellular signaling pathway that regulates interactions between physically adjacent cells through the binding of Notch family receptors to their cognate ligands. These results suggested that NOTCH4 has a pivotal and important role in angiogenesis and vascular remodeling, which leads to myocardial ischemia and fibrosis, resulting in DCM. Here, our study showed that NOTCH4 (rs3134942) was related to DCM susceptibility, similar to the results of a GWAS of cardiac-NL. However, further fine mapping analysis is required to define the causal variant in NOTCH4 or nearby, and replication in other populations and in larger sample sizes is needed to confirm the observation.

TABLE 5 Association analysis of seven single nucleotide polymorphisms (SNPs) with various characteristics of dilated cardiomyopathy

Subgroups	rs2857595			rs3134942			rs2472299			rs2432143		
	P/N	P	P _a	OR (95% CI)	P	P _a	OR (95% CI)	P	P _a	OR (95% CI)	P	P _a
Sex	75/197	.53	.64	0.88 (0.60-1.30)	.50	.78	0.81 (0.45-1.48)	.81	1.00	0.95 (0.64-1.41)	.99	1.00
Smoking	91/181	.76	.78	0.94 (0.66-1.36)	.78	.86	1.08 (0.63-1.86)	8.71 × 10 ⁻³	1.56 × 10 ⁻²	1.67 (1.14-2.44)	.38	.50
Drinking	84/87	.71	.86	1.07 (0.74-1.56)	.70	.78	0.89 (0.51-1.58)	.25	.29	0.80 (0.55-1.17)	.93	1.00
Combined with other diseases	88/184	.69	.86	0.93 (0.64-1.34)	.12	.10	1.51 (0.89-2.56)	.80	.86	1.05 (0.72-1.52)	.56	.86
Combined with hypertension	71/201	.57	.86	1.12 (0.76-1.66)	.43	.55	1.25 (0.71-2.21)	.65	.78	0.91 (0.61-1.36)	.54	.52
RDW-CV (%)	145/127	.56	.48	1.11 (0.79-1.56)	.52	.64	0.84 (0.50-1.41)	.14	.28	1.31 (0.92-1.86)	.44	.86
NT-pro-BNP	184/76	.96	1.00	1.01 (0.69-1.48)	.59	.78	1.18 (0.65-2.12)	.97	1.00	1.01 (0.68-1.50)	.60	.64
E%	220/52	.96	.73	0.99 (0.64-1.54)	.79	.78	1.09 (0.57-2.09)	.22	.33	0.75 (0.47-1.19)	.52	.86
NLR	68/204	.74	.86	0.94 (0.63-1.40)	.27	.27	0.70 (0.37-1.33)	.54	.86	1.14 (0.76-1.70)	.31	.38
Pulmonary hypertension	123/72	.44	.37	1.18 (0.77-1.80)	.21	.20	0.68 (0.37-1.24)	.67	1.00	0.91 (0.59-1.40)	.91	1.00
NYHA class	112/111	.07	.10	0.70 (0.48-1.03)	.94	1.00	0.98 (0.56-1.72)	.62	.86	0.90 (0.59-1.38)	.09	.13

Subgroups	rs2272381			rs1487982			rs1391511		
	P/N	P	P _a	OR (95% CI)	P	P _a	OR (95% CI)	P	P _a
Sex	75/197	.21	.17	1.33 (0.85-2.08)	.62	.78	1.19 (0.61-2.30)	.32	.42
Smoking	91/181	.06	.07	0.64 (0.41-1.02)	.79	.78	1.09 (0.58-2.07)	.35	.36
Drinking	84/87	.16	.12	0.71 (0.45-1.14)	.34	.86	0.71 (0.35-1.44)	.53	.55
Combined with other diseases	88/184	.68	.86	1.10 (0.71-1.70)	.35	.36	1.35 (0.72-2.52)	.36	.27
Combined with hypertension	71/201	.54	.78	0.86 (0.53-1.39)	.64	.78	1.18 (0.60-2.31)	.17	.24
RDW-CV (%)	145/127	.08	.09	1.45 (0.95-2.21)	.97	1.00	1.01 (0.55-1.87)	.86	.86
NT-pro-BNP	184/76	.60	.55	0.88 (0.56-1.40)	.74	.78	0.89 (0.45-1.77)	.71	.67
E%	220/52	.14	.11	0.65 (0.36-1.15)	.69	.63	1.17 (0.54-2.52)	.70	.86
NLR	68/204	.25	.19	1.31 (0.83-2.08)	.89	.86	1.05 (0.51-2.14)	.64	.86
Pulmonary hypertension	123/72	.52	.78	1.18 (0.71-1.96)	.87	1.00	1.07 (0.48-2.38)	.40	.86
NYHA class	112/111	.81	.86	1.06 (0.67-1.68)	.56	.73	0.89 (0.61-1.31)	.45	.41

CI, confidence interval; E%, percentage of eosinophil granulocytes; N, patients negative for a certain characteristic; NA, not applicable; NLR, neutrophil to lymphocyte ratio; NT-pro-BNP, N-terminal pro-brain natriuretic peptide; NYHA Class, classification of the New York Heart Association; OR, odds ratio; P, patients positive for a certain characteristic; P_a, P-value corrected by permutation (1 000 000 times); RDW, red blood cell distribution width.

For rs2472299 in CYP1A2, an association with DCM was observed using the dominant model. However, allelic testing revealed no significant association between rs2472299 and the development of DCM. This result may be attributable to the lack of association between DCM and CYP1A2. While, in the subgroup analysis, this SNP was found to be associated with smoking status in patients with DCM. Previous studies indicated that CYP1A2 is activated in smokers and plays an important role in phase I metabolism of compounds found in tobacco smoke, such as dioxins and polycyclic aromatic hydrocarbons.^{31,32} Most importantly, mutagens, such as aromatic amines found in tobacco smoke, were identified to increase the development of cardiovascular diseases in experimental animals.^{33,34} Thus, we assumed that polymorphisms in CYP1A2, particularly rs2472299, which is located in a promoter region, may potentially increase CYP1A2 activity and catalyze the production of more aromatic amines in patients who smoked, thereby resulting in susceptible to DCM. However, our study was limited at the genetic level, and functional studies are therefore required to interpret the biological significance of these loci and to determine their role in DCM.

In this study, a limitation was the relatively small sample size, which was attributable to our strict inclusion criteria and based on an assumption of 0.19% DCM prevalence in the Chinese population.⁵ Here, only rs3134942 in NOTCH4 was identified as a susceptibility-associated SNP for DCM. However, we suggest that further fine mapping of NOTCH4 gene using larger sample sizes should be performed to gain additional insights into the relationships between NOTCH4 and DCM susceptibility.

5 | CONCLUSIONS

In conclusion, we found that the rs3134942 in NOTCH4 may be involved in DCM risk in Han Chinese population. In addition, rs2472299 in CYP1A2 shows association with smoking status in patients with DCM but did not show any association with DCM susceptibility. Further analyses in larger and ethnically diverse populations were warranted to confirm these findings.

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SUPPORTING INFORMATION

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