

# A common polymorphism in the *SCN5A* gene is associated with dilated cardiomyopathy

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**Aims** *SCN5A* is a disease-causing gene associated with familial dilated cardiomyopathy (FDC). We examined the possible association between a common polymorphism in the *SCN5A* gene (c.1673A>G-p.H558R; rs1805124) and the risk of dilated cardiomyopathy (DCM) occurrence.

**Methods** We genotyped 185 DCM cases (familial DCM, idiopathic DCM and postischemic DCM) and 251 controls for the p.H558R polymorphism in the *SCN5A* gene, to test the association of the molecular epidemiology of the individuals with the presence/absence of various types of DCM.

**Results** Our results showed that the rs1805124 polymorphism was significantly associated with DCM, and the association was more significant in patients with FDC; furthermore, in these individuals, the less frequent GG genotype was associated with a 7.39-fold increased risk of disease [95% confidence interval (95% CI) = 2.88–18.96;  $P < 0.0001$ ] compared with the AA genotype. Moreover, logistic regression analysis showed that GG carriers had a higher risk of DCM than AA + AG carriers (odds ratio = 5.45, 95% CI = 2.23–13.35;  $P < 0.001$ ). No association was

observed between the rs1805124 and DCM risk in postischemic DCM patients.

**Conclusion** Our study demonstrates an association between familial DCM and the rs1805124 polymorphism in the *SCN5A* gene, which may unravel additional genetic predisposition to the development of a multifactorial disease as DCM.

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**Keywords:** association study, dilated cardiomyopathy, genetics, molecular epidemiology, polymorphism in *SCN5A* gene

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## Introduction

Dilated cardiomyopathy (DCM; OMIM 115200) is the most common cardiomyopathy worldwide, with a prevalence of 40/100 000,<sup>1</sup> and men are affected more frequently than women.<sup>2</sup> However, population-based studies suggest that this prevalence may be underestimated.<sup>3,4</sup> The most common DCM cause is ischemic heart disease, secondary to coronary artery disease (up to 70% of DCM cases).<sup>4–7</sup> The remaining cases are usually nonischemic DCM, most of which (50–70%) are idiopathic DCM (IDC) due to an incomplete knowledge about the etiopathogenetic background.<sup>4,7–9</sup> Features secondary to thyroid disease, iron overload, exposure to cardiotoxic drugs, chest radiation, inflammatory arthritis, infections (viral, bacterial, parasitic and fungal forms) or including structural heart disease (congenital or valvular) are less frequent causes of nonischemic DCM.<sup>1,9–11</sup> Clinical and echocardiographic screening of first-degree relatives indicates that the 30–50% of IDC can be classified as familial DCM (FDC).<sup>1,6,8,9</sup> The percentage

of the diverse forms of DCM is variable and different estimates have been reported between 2011 and 2017.<sup>1,4,7–9,11–13</sup> This reflects advances in the differential diagnosis, particularly after the implementation of DNA sequencing technology, mostly high throughput next-generation sequencing. Notably, the term 'idiopathic DCM' refers to a clinical diagnosis in which all identifiable causes were excluded, and was coined before genetic data became available.<sup>3</sup> Thanks to advances in genetic diagnosis techniques, such as next-generation sequencing and whole exome sequencing, it is now known that an increasing portion of IDC cases are genetically based and the genetic effect is known in 30–40% of cases.<sup>9</sup> However, this may be an underestimate because the FDC may be largely underdiagnosed because physicians and investigators may not know the family history of patients.

Genetic screening including all the genes known to be associated with DCM has a low sensitivity because of the very large genetic heterogeneity. To date, about 100 genes have been associated with DCM,<sup>12</sup> most of which are autosomal and encode such structural components of

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the heart muscle such as the sarcomeric and cardiac Z-disk genes.<sup>10,12,14,15</sup> Mutations in *TTN*, *MYH7*, *LMNA* and *SCN5A* genes, which appear to be pathogenic, are present in variable percentage, with Titin (*TTN*) being the most prevalent one.<sup>1,11,15,16</sup> On the contrary, there is evidence that the natural history of DCM is different depending on the various causes.<sup>17</sup> Moreover, mutations in the Sodium Voltage-Gated Channel Alpha Subunit five (*SCN5A*) gene have been implicated in DCM. The *SCN5A* gene, which encodes the human cardiac sodium channel  $\alpha$ -subunit, is responsible for the fast depolarization upstroke of the cardiac action potential. *SCN5A* gene mutations are associated with various cardiac disorders, including arrhythmogenic syndromes, namely, long QT syndrome (LQTS), Brugada syndrome (BrS), familial atrial fibrillation, sick sinus syndrome (SSS), paroxysmal familial ventricular fibrillation and progressive familial heart block type IA (PFHB1A), as well as with the structural cardiomyopathies such as DCM.<sup>18,19</sup>

The rs1805124 (c.1673A>G; p.H558R) polymorphism in exon 12 of the *SCN5A* gene (minor allele frequency: 23%) is relevant for the phenotypic expression of several co-existing mutations,<sup>20–23</sup> and for the biophysical behaviour of the sodium channel.<sup>24</sup> Although rs1805124 has been associated with various cardiac disorders,<sup>25–27</sup> no studies have evaluated the prevalence of this polymorphism in DCM patients. This study demonstrates an association between rs1805124 and DCM particularly in patients with the familial form of the disease.

## Materials and methods

### Study participants

One hundred and eighty-five unrelated patients (78.9% male) with DCM were enrolled since June 2009 and followed on average for 1.5 years at the Department of Cardiomyopathy and Inherited Heart Disease Clinic, UOC Cardiology, University of Campania ‘Luigi Vanvitelli’ of Naples and at Department of Translational Medicine, University Federico II of Naples. The study population was drawn from index patients referred for clinical DCM diagnosis. DCM was defined as dilated, hypokinetic left ventricle: left ventricular end-diastolic diameter (LVEDd) more than 117%, predicted value corrected for age and body surface; left ventricular systolic dysfunction (LVSD) defined by left ventricular ejection fraction (LVEF) less than 45% and/or fractional shortening less than 25%.<sup>9</sup> In presence of at least two DCM-affected family members and/or premature sudden cardiac death(s) (i.e. ‘familial DCM’), a cut off of LVEDd more than 112% and LVEF less than 50% or fractional shortening less than 28% were considered.<sup>9</sup> DCM patients with systemic involvement due to neuromuscular or mitochondrial disorders were excluded. A detailed family history screening of DCM patients revealed 56 patients affected by FDC. Fifty postischemic patients (pi-DCM) with evidence of coronary artery

disease and/or myocardial infarction with normal coronary arteries were enrolled. This group underwent invasive and noninvasive coronary evaluation to determine whether they were affected by postischemic DCM, using coronary imaging test, echocardiography and cardiac magnetic resonance (CMR). Finally, 79 patients with IDC were identified, after excluding coronary artery disease (>50% in one or more major branches), cardiotoxic exposures, systemic disease known to cause DCM, viral infections, alcohol abuse and severe hypertension (>160/100 mmHg). Because a genetic cause is identified in almost all patients with FDC and in almost all IDC, we combined these two cohorts in a single subgroup designated no-ischemic dilative cardiomyopathy (ni-DC). A total of 251 age, sex and ethnicity-matched healthy control individuals, unrelated to each other, were recruited in the same period at the same centres. Controls had no personal or family history of cardiovascular disease, no cardiovascular risk factors (i.e. diabetes, hypertension or hyperlipidaemia) and had unremarkable ECG and/or echocardiographic reports. All cases and controls were of European-Caucasian origin. Informed consent to perform genetic analysis was obtained from patients and controls, according to the second Helsinki Declaration.<sup>28</sup>

### Genetic analysis

Four millilitres of whole blood in vacuum collection tubes with EDTA-K2 were collected from patients and healthy control individuals. DNA was extracted by standard method<sup>29</sup> and stored at  $-80^{\circ}\text{C}$ . We genotyped both DCM patients and healthy control individuals by conventional dideoxy-chain-termination methodology,<sup>29</sup> for the c.1673A>G (p.H558R) polymorphism (rs1805124) in the *SCN5A* gene. To facilitate the sequencing analysis, a sequence-priming universal from the fago  $\lambda$ M13 (M13 forward TGTAACACGACGGCCAGT; M13 reverse CAGGAAACAGCTATGACC) was included at 5’ of forward and reverse primers.<sup>30</sup> Codon Code software (CodonCode Aligner version 2.5) was used to compare the sequences of patients and controls with the *SCN5A* Reference Sequences (Ref Seq NM\_198056.2). Variant call rate was successful in all individuals and genotyping was done blind to case–control status.

### Statistical analysis

Statistical analysis was performed using the SPSS software, version 22.0 (SPSS Inc., Chicago, Illinois, USA). The Hardy–Weinberg equilibrium in all patients and controls was verified by the  $\chi^2$  test. The distribution of the continuous variables was assessed using the Kolmogorov–Smirnov test. Median (2.5th–97.5th percentiles) was estimated for nonparametric continuous variables. Categorical variables were expressed as percentages. Clinical, anamnestic and demographic data from ni-DC and pi-DC patients were compared using Mann–Whitney test for continuous variables and Pearson Chi-square test for categorical variables. Pearson Chi-square

**Table 1 Clinical, anamnestic and demographic characteristics of dilated cardiomyopathy patients grouped by sex**

Parameter/Characteristic	DCM <i>n</i> = 185		<i>P</i>
	Male ( <i>n</i> = 146)	Female ( <i>n</i> = 39)	
Age at diagnosis (years)	49.0 (38.0–57.0)	40.0 (30.2–54.7)	<b>0.02</b>
Hypertension	47.7	46.2	1.00
Atrial fibrillation	17.3	17.4	1.00
Ventricular tachycardia	23.9 <sup>a</sup>	25.7 <sup>b</sup>	0.83
LVEF (%) (r.v. >50%)	30.0 (25.0–37.0)	30.0 (25.0–47.0)	0.07
Palpitations	11.7	23.8	0.16
Lipothymia	2.9	0.0	1.00
Syncope	5.8	19.0	0.06
Dyspnoea	71.2	90.5	0.09
NYHA I	24.2	12.1	0.16
NYHA II	37.1	36.4	1.00
NYHA III	31.1	48.5	0.06
NYHA IV	7.6	3.0	0.69
Heart transplantation	5.7	9.5	0.78
ICD	44.8	42.9	1.00
Family history of sudden death	4.8	5.1	1.00

The two continuous variables (age at diagnosis and LVEF) are expressed as median (25th–75th percentile); all the other categorical variables are expressed as percentages. DCM, dilated cardiomyopathy; LVEF, left ventricular ejection fraction; NYHA New York Heart Association; I to IV indicates degree of severity according to cardiac resynchronization therapy. <sup>a</sup> 12.6% nonsustained ventricular tachycardia, 11.3% sustained ventricular tachycardia. <sup>b</sup> 25.7% nonsustained ventricular tachycardia, 0.0% sustained ventricular tachycardia; ICD, implantable cardioverter-defibrillator; Pearson Chi-square and Mann–Whitney tests for categorical and continuous variables respectively; Significant *P* values (<0.05) are shown in bold.

test was used to identify statistically significant differences in allele and genotype frequencies of the rs1805124 polymorphism, in the whole DCM population, as well as in FDC, ischemic DCM, ni-DC and pi-DC patients *versus* healthy control individuals. Logistic regression analysis was carried out to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs) of c.1673A>G homozygous and heterozygous genotype *versus* wild-type c.1673A>G (reference group) to calculate the DCM risk in the whole DCM cohort and in the FDC; ischemic DCM, ni-DC and pi-DC patients. To test the association with the predisposition to DCM risk, we constructed dominant and recessive models for the rare allele of the rs1805124 polymorphism and calculated the univariate OR with 95% CI.

## Results

### Clinical characteristics of dilated cardiomyopathy patients and controls

A total of 185 DCM patients [median age at diagnosis 48 years (25<sup>th</sup>–75<sup>th</sup> percentiles = 36–56); male 78.9%] and 251 age and sex-matched controls [median age 58.0 years (25<sup>th</sup>–75<sup>th</sup> percentiles = 31.0–70.0); Male 74.0%; LVEF (%): median 60.0 (25th–75th percentiles = 58.0–66.0)] of the same regional area (Southern Italy) were enrolled in the study. The clinical, anamnestic and demographic characteristics of the two groups are reported in Table 1. Female patients were diagnosed at an earlier age than male (Table 1). Table 2 summarizes the data of

**Table 2 Clinical, anamnestic and demographic characteristics of ni-DC and pi-DC patients**

Parameter/Characteristic	ni-DC <i>n</i> = 135	pi-DC <i>n</i> = 50	<i>P</i>
Sex			
Male	73.3	94.0	<b>0.002</b>
Female	26.7	6.0	
Age at diagnosis (years)	44.0 (34.0–55.0)	54.0 (44.5–60.0)	<b>&lt;0.0001</b>
Hypertension	46.5	50.0	0.72
Atrial fibrillation	17.5	16.7	0.88
Ventricular tachycardia	27.3 <sup>a</sup>	16.3 <sup>b</sup>	0.13
LVEF (%) (r.v.>50%)	30.0 (25.0–40.0)	25.0 (20.0–30.0)	<b>0.009</b>
Palpitations	14.6	11.4	0.64
Lipothymia	3.4	0.0	0.27
Syncope	9.0	5.7	0.54
Dyspnoea	71.9	80.6	0.22
NYHA I	23.1	18.8	0.68
NYHA II	36.8	37.5	1.00
NYHA III	33.3	37.5	0.72
NYHA IV	6.8	6.3	1.00
Heart transplantation	6.7	5.6	0.81
ICD	40.0	55.6	0.12
Familial history of sudden death	5.20	4.0	1.00

The two continuous variables (age at diagnosis and LVEF) are expressed as median (25th–75th percentile); all the other categorical variables are expressed as percentages of the total number of patients of each type. Significant *P* values (<0.05) are shown in boldface type. LVEF, left ventricular ejection fraction; ni-DC, nonischemic DCM; NYHA, New York Heart Association; I to IV indicates degree of severity according to cardiac resynchronization therapy; pi-DC, post ischemic DCM. <sup>a</sup> 20.3% nonsustained ventricular tachycardia, 7.0% sustained ventricular tachycardia. <sup>b</sup> 2.0% nonsustained ventricular tachycardia, 14.3% sustained ventricular tachycardia; ICD, implantable cardioverter-defibrillator; Pearson Chi-square and Mann–Whitney tests for categorical and continuous variables, respectively.

nonischemic patients (i.e. those with FDC and those with IDC) designated 'ni-DCM' and those of postischemic (pi-DC) patients. The clinical, anamnestic and demographic characteristics were similar in the two groups, except for sex, age at diagnosis and left ventricular ejection fraction. Particularly, ni-DC patients showed an early age at diagnosis compared with pi-DC (44.0 versus 54.0; *P* < 0.0001).

### Screening for c.1673A>G polymorphism (rs1805124) in SCN5A gene

The Hardy–Weinberg equilibrium of the c.1673A>G polymorphism was positive (*P* > 0.05 for all the case cohorts and for the control group). Allele and genotype distribution did not differ significantly between men and women in either the patient or control group. Then, allele and genotype frequencies of c.1673A>G polymorphism in *SCN5A* gene (rs1805124) were compared between the whole DCM groups as well as the ni-DC and pi-DC versus controls (Table 3).

The genotype frequencies of the c.1673A>G polymorphism differed significantly between DCM patients and controls (*P* = 0.006). Moreover, allele and genotype frequencies of the c.1673A>G polymorphism differed significantly between ni-DC and controls (*P* = 0.04; *P* = 0.001, respectively). Conversely, no significant differences were found between pi-DC patients and controls. Furthermore, the whole DCM cohort and ni-DC patients with the GG genotype had a higher disease risk

**Table 3 Allele and genotype frequencies of the rs1805124 polymorphism in SCN5A gene, in dilated cardiomyopathy, ni-DC and pi-DC patients versus controls and their association with dilated cardiomyopathy risk**

Individual groups	Allele frequencies			Genotype frequencies			OR (95% CI)	P		
	N	%	P*	N	%	P*				
DCM (n = 185)	A	267	72	0.129	AA	100	54.1	<b>0.006</b>	1.0 <sup>a</sup>	
	G	103	28		AG	67	36.2		<b>1.63 (1.07–2.47)<sup>b</sup></b>	<b>0.022<sup>b</sup></b>
ni-DC (n = 135)	A	187	69	<b>0.04</b>	AA	69	51.1	<b>0.001</b>	1.0 <sup>a</sup>	
	G	83	31		AG	49	36.3		<b>1.73 (1.09–2.73)<sup>b</sup></b>	<b>0.020<sup>b</sup></b>
pi-DC (n = 50)	A	80	81	1.00	GG	17	12.6		<b>2.73 (1.70–8.55)<sup>c</sup></b>	<b>0.001<sup>c</sup></b>
	G	20	19		AA	31	62.0	0.420	1.0 <sup>a</sup>	
Controls (n = 251)	A	410	82		AG	18	36.0		1.41 (0.74–2.68) <sup>b</sup>	0.300 <sup>b</sup>
					GG	1	2.0		0.50 (0.06–4.00) <sup>c</sup>	0.510 <sup>c</sup>
	G	92	18		AA	170	67.7			
				AG	70	27.9				
				GG	11	4.4				

Significant P values are shown in boldface type. CI, confidence interval; DCM, dilated cardiomyopathy; ni-DC, nonischemic dilated cardiomyopathy; OR, odd ratio; pi-DC, postischemic dilated cardiomyopathy; P\*, Pearson Chi-square test between patients versus controls; P, Logistic regression analysis. <sup>a</sup> AA genotype as Reference group. <sup>b</sup> AG genotype versus Reference group. <sup>c</sup> GG genotype versus Reference group.

than controls (OR = 2.78, 95% CI = 1.26–6.13, P = 0.011; OR = 2.73, 95% CI = 1.70–8.55, P = 0.001, respectively). Subsequently, the same comparisons were performed between the FDC and IDC patients versus controls (Table 4).

The allele and genotype frequencies of the c.1673A>G polymorphism differed significantly between patients with FDC (P = 0.0005; P ≤ 0.0001, respectively) and controls, but not between patients with IDC and controls. Furthermore, at regression analysis, the GG genotype

conferred a significantly higher risk of disease (P < 0.0001) in patients with FDC (OR 7.39, 95% CI 2.88–18.96) than in controls.

At univariate analysis of the SCN5A rs1805124 polymorphism performed using two genetic models of inheritance (dominant and recessive model), the rs1805124 polymorphism was significantly associated with predisposition to DCM under a recessive but not under a dominant model in the whole DCM population and particularly in ni-DC and FDC groups (see OR values in Table 5).

**Table 4 Allele and genotype frequencies of rs1805124 polymorphism in SCN5A gene, in FDC, and IDC patients versus controls and their association with dilated cardiomyopathy risk**

Individual groups	Allele frequencies			Genotype frequencies			OR (95% CI)	P		
	N	%	P*	N	%	P*				
FDC (n = 56)	A	68	60.7	<b>0.0005</b>	AA	23	41.1	<b>&lt;0.0001</b>	1.0 <sup>a</sup>	
	G	44	39.3		AG	22	39.3		<b>2.22 (1.15–4.26)<sup>b</sup></b>	<b>0.017<sup>b</sup></b>
IDC (n = 79)	A	119	75.3	0.15	GG	11	19.6	0.076	<b>7.39 (2.88–18.96)<sup>c</sup></b>	<b>&lt;0.0001<sup>c</sup></b>
	G	39	24.7		AA	46	58.2		1.0 <sup>a</sup>	
Controls (n = 251)	A	410	82		AG	27	34.2		1.48 (0.86–2.55) <sup>b</sup>	0.161 <sup>b</sup>
					GG	6	7.6		2.01 (0.71–5.74) <sup>c</sup>	0.189 <sup>c</sup>
	G	92	18		AA	170	67.7			
				AG	70	27.9				
				GG	11	4.4				

Significant P values are shown in *italic bold*. CI, confidence interval; DCM, dilated cardiomyopathy; FDC, familial dilated cardiomyopathy; IDC, idiopathic dilated cardiomyopathy; OR, odd ratio; P\*, Pearson Chi-square test between patients versus controls; P, Logistic regression analysis. <sup>a</sup> AA genotype as Reference group. <sup>b</sup> AG genotype versus Reference group. <sup>c</sup> GG genotype versus Reference group.

**Table 5 Risk (as odds ratio and P) of dilated cardiomyopathy between the groups of patients according to dominant and recessive genetic models, respectively (GG+AG versus AA; AA+AG versus GG)**

Individual groups	Dominant model				Recessive model			
	GG+AG %	AA %	OR (95% CI)	P	AA+AG %	GG %	OR (95% CI)	P
DCM (n = 185)	46.0	54.0	0.96 (0.65–1.40)	0.85	90.0	10.0	<b>2.21 (1.0–4.83)</b>	<b>0.05</b>
ni-DC (n = 135)	49.0	51.0	1.08 (0.70–1.63)	0.749	87.4	12.6	<b>3.14 (1.43–6.92)</b>	<b>0.006</b>
pi-DC (n = 50)	38.0	62.0	0.69 (0.37–1.28)	0.278	98.0	2.0	0.95 (0.93–0.98)	0.70
FDC (n = 56)	58.2	41.8	1.57 (0.87–2.83)	0.140	80.0	20.0	<b>5.45 (2.23–13.35)</b>	<b>&lt;0.001</b>
IDC (n = 79)	42.5	57.5	0.83 (0.50–1.38)	0.52	92.5	7.5	1.77 (0.63–4.94)	0.26
Controls (n = 251)	47.0	53.0			95.6	4.4		

Significant P values are shown in *italic bold*. CI, confidence interval; DCM, dilated cardiomyopathy; FDC, familial dilated cardiomyopathy; IDC, idiopathic dilated cardiomyopathy; ni-DC, nonischemic dilated cardiomyopathy; OR, odds ratio; pi-DC, postischemic dilated cardiomyopathy; P, univariate analysis.

These results indicate that the homozygous rs1805124 genotype (GG) was significantly associated with disease risk in ni-DC (OR 3.14, 95% CI 1.43–6.92;  $P=0.006$ ), and particularly in FDC (OR 5.45, 95% CI 2.23–13.35;  $P<0.001$ ). Overall, the attributable risk of DCM in our patients indicated that GG genotype at position c.1673 of the *SCN5A* gene accounts for 21% of disease risk and for 28% when postischemic cases were excluded from the analysis.

## Discussion

This is the first study to report a significant association between the c.1673G>A polymorphism of the *SCN5A* gene and the development of genetically based DCM, in particular in patients with FDC. Mutations in the *SCN5A* gene, encoding the  $\alpha$ -subunit of voltage-gated sodium channel, responsible for the fast depolarization upstroke of the cardiac action potential, have been reported in a variety of cardiac diseases. For example, the loss-of-sodium channel function mutations result in BrS subtype-1, idiopathic ventricular fibrillation, cardiac conduction diseases and congenital sick sinus syndrome, whereas gain-of-function mutations are mainly associated with congenital LQTS type 3 and atrial fibrillation.<sup>18,19,31</sup> In addition, *SCN5A* mutations related to both loss and gain function have also been linked to DCM, which suggests that dysfunction in electrical excitability, caused by disturbance of sodium channel function, also leads to dilation remodelling.<sup>32–34</sup> It has been suggested that deranged functioning of the *SCN5A* channel may cause dysfunction of cytoskeletal protein binding partners and so result in DCM.<sup>32,35</sup> Moreover, Gosselin-Badaroudine *et al.*<sup>36</sup> demonstrated that the *SCN5A*-R219H mutation causes an inward proton current thereby producing intracellular acidification of cardiac myocytes that could cause the DCM phenotype. Furthermore, gain-of-function mutations increase intracellular sodium concentration thereby causing a secondary increase in intracellular calcium that, in turn, leads to cellular remodelling and heart failure.<sup>37,38</sup> The p.H558R polymorphism, located in the interdomain linker loop 1–2, is a polymorphism in the *SCN5A* gene reported to be relevant in modulating the effects of coexisting *SCN5A* mutations in DCM, sick sinus syndrome, BrS and other cardiac disorders.<sup>20–23</sup> It also affects the biophysical behaviour of the normal channel.<sup>24</sup> In particular, Cheng *et al.*<sup>20</sup> found that the combined variants p.R222Q/p.H558R and p.I1835T/p.H558R caused a reduction in  $I_{NA}$  peak density in two DCM families, but not in families carrying only p.R222Q and p.I1835T, which confirms the crucial role played by p.H558R in the cellular biophysical phenotype of DCM-related *SCN5A* variants. The p.H558R polymorphism restores gating and trafficking anomalies induced by mutations.<sup>23,39–41</sup> It has been reported that the R558 allele may have a reduced cardiac  $I_{NA}$  peak density in wild-type channels showing the splice variant lacking glutamine at position 1077 (Q1077del), which reaches

65% of the *SCN5A* transcript in heart.<sup>24</sup> To date, few studies have evaluated the presence of the p.H558R alone, as a risk factor for atrial fibrillation, Purkinje-related ventricular fibrillation and Keshan disease,<sup>25–27</sup> but no studies have investigated the prevalence of this polymorphism in DCM patients. We hypothesized that the p.H558R polymorphism is a risk factor for DCM. First, clinical, anamnestic and demographic analysis of the patient data displayed that men, as compared with women, were more frequently affected by DCM as expected.<sup>42,43</sup> On the contrary, women showed a lower age at diagnosis, with respect to men, probably related to the high incidence of nonischemic DCM in the female group. In fact, when we split the whole DCM population into nonischemic and postischemic DCM patients, the comparison between these two groups showed a lower age at diagnosis and a higher ejection fraction in the nonischemic DCM patients, as the onset of disease is generally earlier in ni-DC and the clinical presentation (including haemodynamic status and cardiac function) is generally more heterogeneous than pi-DC. We observed statistically significant differences in frequencies of p.R558H genotypes, both in whole DCM cohort ( $P=0.006$ ), and in ni-DC ( $P=0.001$ ) and mostly in FDC ( $P<0.0001$ ) subgroups of patients, compared with normal controls. Particularly, our results indicate that the presence of the polymorphism is associated with the risk for DCM, primarily in the familial forms (OR: 2.22 and 7.39 for the AG and GG genotypes, respectively). Interestingly, the association was still strongly significant in the ni-DC group (OR: 1.73 in heterozygous and 2.73 in homozygous). Instead, no association, both for genotype and DCM risk, was found in the pi-DC patients. These results, also confirmed by univariate analysis, under a recessive model, indicate that the GG genotype was significantly associated with DCM risk in ni-DC and particularly in familial cases.

DCM is a multifactorial disease that develops when genetic and environmental factors together reach a threshold to disease.

Furthermore, genetic forms of DCM are also suggested by the presence of clinical-laboratory traits, sometimes referred to as diagnostic red flags.<sup>44</sup> The presence of clinical-laboratory evidence such as atrio-ventricular conduction abnormalities, improvement of markers (i.e CPK, BNP/NT-proBNP) or familial history of DCM may suggest a genetic substrate for the DCM disease.<sup>45</sup>

In the last two decades, it has been demonstrated that genetic variations in numerous genes may contribute to the pathogenesis of FDC,<sup>6,46,47</sup> or may be disease-associated polymorphisms that control the susceptibility to DCM,<sup>48</sup> although each polymorphism alone may determine the disease at a small percentage. Our results demonstrate that the GG genotype of the rs1805124 polymorphism in the *SCN5A* gene confers a risk of

disease of about 3% in the whole DCM population, which increases to more than 7% in the case of clear inheritance. The attributable risk of DCM in patients with the GG genotype was between 20 and 28%, depending on considering the whole DCM population or only ni-DC cases.

The possible mechanism underlying the association between the p.H558R polymorphism in *SCN5A* gene and DCM phenotype is difficult to establish. The presence of a sodium channel with a slight decrease of  $I_{NA}$  density may be another genetic/environmental factor that contributes to the development of DCM primarily in the presence of disease-causing variations.

### Study limitations

Our study population was not very large and limited to tertiary centres of a specific geographic area. Thus, we cannot exclude the presence of selection bias in patient enrolment. Consequently, the data should be replicated and extended to larger populations of other ethnic groups worldwide.

Although the principal finding of this study is that common variant rs1805124 is associated with DCM (in particular in patients with FDC), a common genetic background arising from different pathogenic or likely pathogen mutations modulating DCM phenotype has not been investigated in detail because of a more sophisticated sequencing approach. Moreover, further investigation needs to characterize, ideally on a prospective base, the effect of this polymorphism and other genetic and nongenetic modifiers in patients with ischemic and nonischemic DCM.

Further limitations are the lack of ECG data as well of clinical-laboratory clues previously associated with a genetic DCM substrate, which should be performed in a prospective study (see also ref. 45).

Furthermore, a better characterization of the nonischemic group and eventual differences in myocardial remodelling related to the gene mutations would definitely represent an important point, but it would be certainly possible to investigate it on a prospective base.

### Conclusion

The c.1673G>A polymorphism of the *SCN5A* gene is significantly associated with the development of genetically based DCM, particularly the familial form. To our knowledge, this is the first report about rs1805124 and DCM, and may impact on primary prevention of DCM, namely to identify at-risk individuals, which is also the basis of the personalized medicine. In our setting, this concept refers to genetic risk factors. Therefore, the presence of c.1673G>A might be considered a predictive risk factor for the development of the disease in DCM families. In the assessment of relatives at risk for the disease, special attention should be paid to asymptomatic

carriers of the p.H558R polymorphism of DCM from an early age.

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### Conflicts of interest

There are no conflicts of interest.

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