

# Genome-wide association analysis in dilated cardiomyopathy reveals two new players in systolic heart failure on chromosomes 3p25.1 and 22q11.23

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## Aims

Our objective was to better understand the genetic bases of dilated cardiomyopathy (DCM), a leading cause of systolic heart failure.

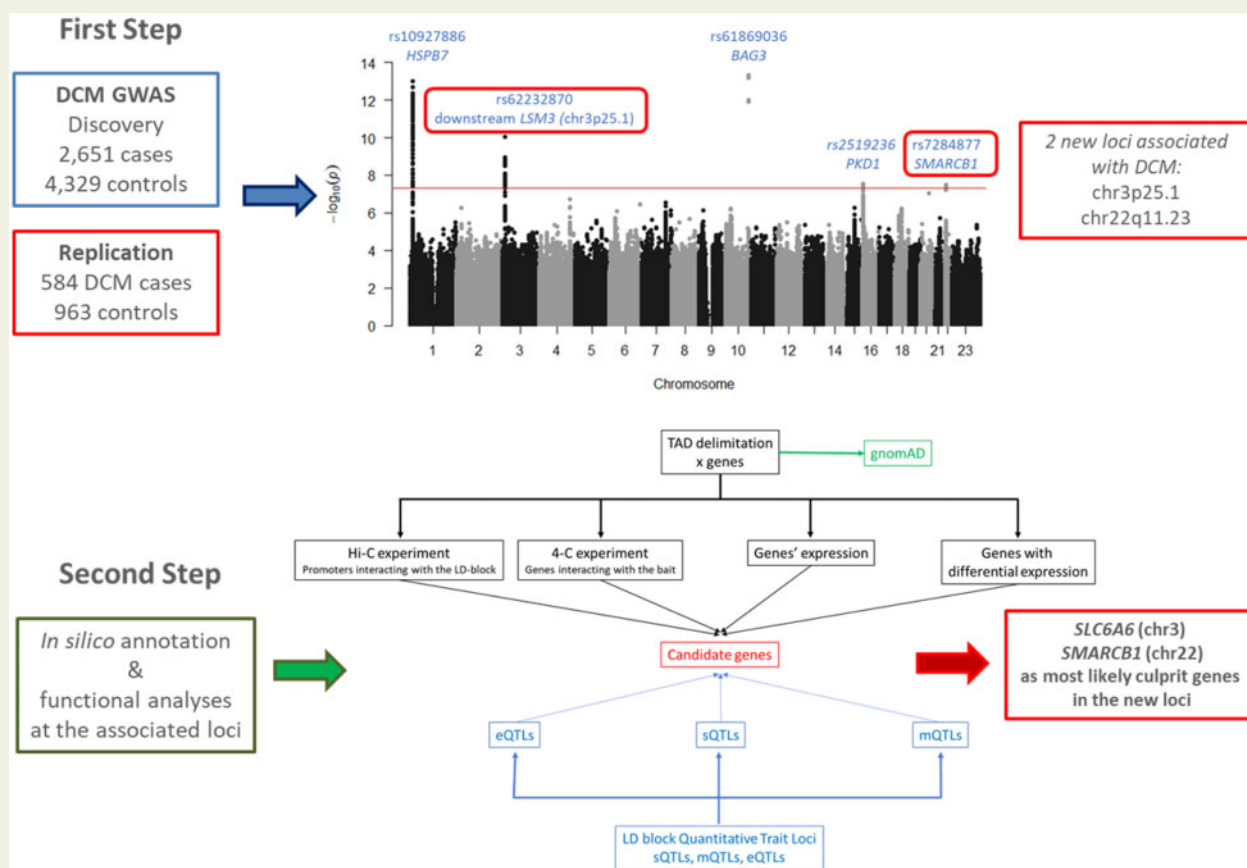
## Methods and results

We conducted the largest genome-wide association study performed so far in DCM, with 2719 cases and 4440 controls in the discovery population. We identified and replicated two new DCM-associated loci on chromosome 3p25.1 [lead single-nucleotide polymorphism (SNP) rs62232870,  $P = 8.7 \times 10^{-11}$  and  $7.7 \times 10^{-4}$  in the discovery and replication steps, respectively] and chromosome 22q11.23 (lead SNP rs7284877,  $P = 3.3 \times 10^{-8}$  and  $1.4 \times 10^{-3}$  in the discovery and replication steps, respectively), while confirming two previously identified DCM loci on chromosomes 10 and 1, *BAG3* and *HSPB7*. A genetic risk score constructed from the number of risk alleles at these four DCM loci revealed a 27% increased risk of DCM for individuals with 8 risk alleles compared to individuals with 5 risk alleles (median of the referral population). *In silico* annotation and functional 4C-sequencing analyses on iPSC-derived cardiomyocytes identify *SLC6A6* as the most likely DCM gene at the 3p25.1 locus. This gene encodes a taurine transporter whose involvement in myocardial dysfunction and DCM is supported by numerous observations in humans and animals. At the 22q11.23 locus, *in silico* and data mining annotations, and to a lesser extent functional analysis, strongly suggest *SMARCB1* as the candidate culprit gene.

## Conclusion

This study provides a better understanding of the genetic architecture of DCM and sheds light on novel biological pathways underlying heart failure.

## Graphical Abstract



Step 1: Through the largest genome-wide association study performed so far in dilated cardiomyopathy, we identified and replicated two new loci on chromosome 3p25.1 and 22q11.23. Step 2: Combined in silico and functional analyses at the associated loci revealed the best culprit gene at each locus: SLC6A6 (chromosome 3) and SMARCB1 (chromosome 22). The discovery of these two new players shed light on novel biological pathways and putative new therapeutic targets.

## Keywords

Dilated cardiomyopathy • Heart failure • GWAS • Imputation • 4C-sequencing • Genetic risk score

## Introduction

Dilated cardiomyopathy (DCM) is a heart muscle disease characterized by left ventricular dilatation and systolic dysfunction in the absence of abnormal loading conditions or coronary artery disease.<sup>1,2</sup> It is a major cause of systolic heart failure, the leading indication for heart transplantation, and therefore a major public health problem due to the important cardiovascular morbidity and mortality.<sup>1,2</sup> Understanding of the genetic basis of DCM has improved in recent years with a role for both rare and common variants resulting in a complex genetic architecture of the disease.<sup>3,4</sup> More than 50 genes<sup>5</sup> with rare pathogenic mutations have been reported as causing DCM, mainly inherited as dominant with variable penetrance. Several large-scale association studies in sporadic cases have been performed to identify common DCM-associated alleles including several genome-wide association studies (GWAS).<sup>3,6,7</sup> Altogether, these genetic investigations have so far robustly identified two loci presenting common susceptibility alleles: a locus on chromosome 1, encompassing

multiple candidate genes in high linkage disequilibrium (LD), including ZBZTB17/MIZ-1 and HSPB7<sup>7,8</sup>; and a second on chromosome 10 whose culprit gene, BAG3, is also involved in familial forms of DCM.<sup>7,9</sup> An exome-wide association study also suggested the existence of six potential additional DCM loci.<sup>7</sup> Here, we report the results of an imputed GWAS for sporadic DCM with main findings replication in two independent case-control cohorts. *In silico* annotation and functional analyses were performed to identify the best candidate culprit genes at identified loci.

## Methods

### Population and sample collection

A full description of the studied populations is reported in [Supplementary material online](#), Cohort description; [Table S1](#). Briefly, 2719 sporadic DCM patients and 4440 controls from five populations of European ancestry (France, Germany, USA, Italy, and UK) were included

in the discovery GWAS. Two European replication cohorts totalling 584 DCM cases and 963 controls were also available. Sporadic DCM was diagnosed according to standard criteria<sup>2,4</sup> by reduced ejection fraction and enlarged left ventricular end-diastolic volume/diameter in the absence of any obvious pathology. The study protocol was approved by local ethics committees, complied with the Declaration of Helsinki, and all patients signed informed consent.

## Genotyping, genotype calling, and imputation

Descriptions of genotyping arrays, QC filtering, and imputation methods are available in [Supplementary material online, Supplementary Methods; Table S2](#).

## Association analysis

Detailed procedure is given in [Supplementary material online, Methods](#). To summarize, association of imputed single-nucleotide polymorphisms (SNPs) with DCM was investigated using a logistic regression model adjusted for sex and genome-wide genotype-derived principal components under the assumption of additive allele effects. A statistical threshold of  $5 \times 10^{-8}$  was used to declare genome-wide significance. To reveal potential multiple independent hits at the discovered loci, a conditional analysis was performed. When more than one significant SNP was found, subsequent haplotype analyses were conducted.

Replication of the findings was assessed with the same statistical methodologies in both replication cohorts, adopting one-tailed hypothesis and applying a Bonferroni correction procedure. After checking for the heterogeneity across studies, the replication cohorts' results were meta-analysed, alone, and combined with the discovery results. Sensitivity analyses were performed to assess the robustness of the main findings according to several factors including sex and clinical characteristics of patients ([Supplementary material online, Methods](#)).

At each replicated associated locus, a regional association plot was performed using LocusZoom (<http://locuszoom.sph.umich.edu/>).

## Genetic risk score analysis

The genetic risk score (GRS) was built upon SNPs associated with DCM and replicated in the current study. Association of the GRS with DCM risk was tested using logistic regression analysis ([Supplementary material online, Methods](#)).

## Genetic heritability

The LD score regression approach<sup>10</sup> was used to estimate the genome-wide genetic heritability underlying DCM and to calculate the genetic correlation between DCM and several cardiovascular and other traits capitalizing on the GWAS results available at the LD Hub (<http://ldsc.broadinstitute.org/ldhub/>).

## Candidate culprit gene selection strategy

For each identified and replicated locus, a fine-mapping strategy (fully described in [Supplementary material online, Methods](#)) was deployed using *in silico* and experimental data to select the best candidates ([Supplementary material online, Figure S1](#)).

## Cis-regulation features at associated single-nucleotide polymorphisms

DCM-associated SNPs [ $P$ -value  $\leq 5 \times 10^{-8}$  and/or in high LD ( $r^2 > 0.7$ ) with the lead SNP] defined the associated 'LD block'. Overlaps of LD blocks with DNA regulatory elements were checked by visualizing on the UCSC Genome Browser, human assembly hg19 (<http://genome.ucsc.edu/>;

last accessed date: december 2020), the ENCODE3 DNase hypersensitivity sites (HS) and transcription factor (TF) chromatin immunoprecipitation sequencing (ChIP-seq) tracks produced on 125 and 130 cell lines, respectively. To detect left ventricle (LV)-specific putative regulatory regions, we enriched those tracks with H3K27ac, H3K4me1, and H3K4me3 histone marks of ENCODE LV samples (GSM908951, GSM910575, GSM910580), looked at ORegAnno predicted regulatory elements and checked sequence conservation in several vertebrates.

## Topologically associating domains and intra-topologically associating domain chromatin interactions

Using LV topologically associating domains (TADs)<sup>11</sup> and preferential chromatin interaction measured via promoter chromatin Hi-C (PCHI-C) on iPSC-derived cardiomyocytes (iPSC-CM),<sup>12</sup> we identified the candidate genes encompassed in TAD overlapping LD blocks. TAD boundaries were confirmed by in-house circular chromatin conformation capture (4C)-sequencing data ([Supplementary material online, Figure S2, Table S17, Methods](#)).

## Biological insights into candidate genes

Cardiac expression level of each candidate was evaluated from RNA-seq data of the Genotype-Tissue Expression (GTEx) project database<sup>22</sup> (<https://www.gtexportal.org/home>; last accessed date: december 2020) and LV DCM explants produced by Heinig *et al.*<sup>13</sup> The latter study also provided differential expression data between 97 DCM patients and 108 healthy donors. Genes displaying interesting expression features were scrutinized in publicly available resources for gene annotation and functions.

## Annotation of associated single-nucleotide polymorphisms

LD block-associated SNPs were annotated using Annovar software and bioinformatics prediction of effects.<sup>14,15</sup> Various *in silico* resources were interrogated to identify potential regulatory SNPs by checking their association with expression and splicing level [e and s quantitative trait loci (QTL)] in cardiac and skeletal muscle tissues (GTEx) and with blood DNA methylation levels (mQTL).<sup>16</sup>

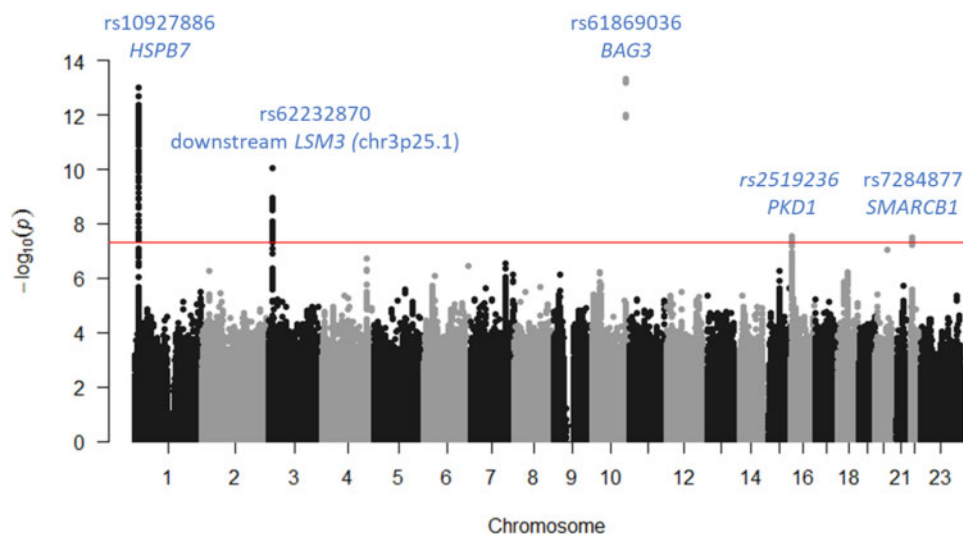
## GnomAD mutation tolerance score

The observed/expected (o/e) metric of GnomAD (<https://gnomad.broadinstitute.org/>; last accessed date: december 2020) was used to evaluate the tolerance of candidate genes to loss of function and missense mutations. An o/e confidence interval score upper limit  $< 0.35$  for LoF and a Z-score of  $> 3$  for missense were indicative of a strong intolerance, as indicated at GnomAD.

# Results

## Main statistical findings

A total of 9 152 885 SNPs (8 945 131 autosomal and 207 754 on X chromosome) were tested for association with DCM in 2651 cases and 4329 controls. Results of the discovery GWAS are summarized in [Figure 1, Supplementary material online, Figure S3, and Table 1](#). Five loci reached genome-wide significance. Two were already known, *BAG3* ( $P = 4.7 \times 10^{-14}$ , rs61869036) and *HSPB7* ( $P = 2.12 \times 10^{-13}$ , rs10927886). *BAG3* rs61869036 was in complete LD with the nonsynonymous rs2234962 reported to associate with DCM<sup>7</sup> and that was used thereafter as *BAG3* lead SNP ( $P = 5.6 \times 10^{-14}$ ). Three new loci



**Figure 1** Manhattan plot summarizing the results of the discovery genome-wide association study.

were identified on chr3p25.1 (rs62232870,  $P=8.7 \times 10^{-11}$ ) downstream *LSM3*, chr16p13.3 (*PKD1* rs2519236,  $P=3.0 \times 10^{-8}$ ) and chr22q11.23 (*SMARCB1* rs7284877,  $P=3.3 \times 10^{-8}$ ). Regional association plots are shown in [Supplementary material online, Figures S4–S8](#). Conditional GWAS adjusted for the five lead SNPs did not reveal any new genome-wide association signal ([Supplementary material online, Figures S9 and S10](#)).

At chr3p25.1, a second SNP, rs4684185, in negative LD with rs62232870 ( $r^2 = 0.12$ ,  $D' = -0.95$ ), showed a high statistical association ( $P=8.4 \times 10^{-9}$ ). After adjustment on the lead SNP, a residual signal remained ( $P=5 \times 10^{-4}$ ) suggesting a more complex association pattern ([Supplementary material online, Results; Supplementary material online, Table S3](#)).

Replication analyses did not confirm *PKD1* rs148248535 ( $P=0.11$ ) but confirmed the associations observed at chr3p25.1 ( $P=7.70 \times 10^{-4}$  and  $P=6.0 \times 10^{-3}$  for rs62232870 and rs4684185, respectively) and at chr22q11.23 ( $P=1.40 \times 10^{-3}$  for rs7284877) ([Table 1](#)).

In a combined meta-analysis of the discovery and replication findings, the resulting odds ratios for DCM were 1.36 [1.25–1.48] ( $P=5.3 \times 10^{-13}$ ) and 1.27 [1.18–1.37] ( $P=4.8 \times 10^{-10}$ ) for chr3p25.1 rs62232870 and rs4684185, respectively, and 1.33 [1.22–1.46] ( $P=5.0 \times 10^{-10}$ ) for chr22q11.23 *SMARCB1* rs7284877, with no evidence for heterogeneity across studies ([Table 1](#)). The results were also robustly confirmed by stratified analyses on phenotypic and population subgroups ([Supplementary material online, Tables S4–S6](#)). GWASs stratified by sex did not reveal any new additional signal ([Supplementary material online, Results; Supplementary material online, Figures S11 and S12](#)).

## Genetic risk score analysis

Unweighted and weighted GRS, summarized in [Figure 2](#) and [Supplementary material online, Table S7](#), presented similar results. Briefly, the unweighted GRS showed a 27% increased risk of DCM for subjects with eight risk alleles (1.27 [1.14–1.42]) and a 21%

decreased for those having only one risk allele (0.79 [0.66–0.95]) as compared with individuals with five risk alleles (median of the referral population) ([Figure 2A](#) and [Supplementary material online, Table S7A](#)). Weighted GRS (continuous scale, [Figure 2B](#) and [Supplementary material online, Table S7B](#); quintile distribution, [Supplementary material online, Figure S13](#)) presents similar results. A similar pattern was observed in the replication cohort ([Supplementary material online, Results; Supplementary material online, Table S7](#)). A significant association of the score was also detected in the subgroup of patients with left ventricular end-diastolic diameter ( $n=2187$ ; odds ratio 1.53 [1.05–2.23]) and a borderline one with prognosis (cardiac death/heart transplant) during follow-up ( $n=503$ ; odds ratio 1.23 [0.98–1.56]).

## Heritability

The estimated genome-wide DCM heritability was  $31 \pm 8.4\%$ . Genetic correlations between DCM and various cardiometabolic and lipid phenotypes were tested but did not reveal striking correlations ([Supplementary material online, Table S8](#)).

## Candidate culprit gene selection strategy at chr3p25.1

As shown in [Figure 3A](#), the top SNP, rs62232870, is located at the edge of an active enhancer region, distal to *LSM3*, as evidenced by H3K27ac and H3K4me3 LV histone marks. Those marks are absent in the seven ENCODE non-cardiomyocyte cell lines suggesting a cardiac tissue-specific expression. Vertebrates' interspecies sequence conservation, predicted regulatory elements, DNaseI HS, and TF-binding sites support the regulatory activity of this region.

The rs62232870 associated LD block covers  $\sim 50$  kbp [chr3:14 257 356–14 307 016] overlapping with the partially independent rs4684185 associated LD block ([Supplementary material online, Figure S5](#) and [Supplementary material online, Table S9](#)) where ENCODE H3K27ac and H3K4me1 marks and enhancers reported

**Table 1** Main association findings of the dilated cardiomyopathy genome-wide association study results

	rs62232870 <sup>a</sup>	rs4684185 <sup>b</sup>	rs148248535 <sup>b,c</sup>	rs7284877 <sup>b</sup>
Chromosome	3	3	16	22
Position (GRCh37.p13)	14257709	14272914	2183449	24155111
Locus	LSM3	LSM3	PKD1	SMARCB1
Risk allele	A	C	T	C
Discovery				
RAF <sup>d</sup>	0.23	0.70	0.82	0.81
Imputation $r^2$	0.96	0.99	0.89	0.99
Allelic OR [95% CI]	1.36 [1.24–1.49]	1.28 [1.17–1.40]	1.35 [1.21–1.50]	1.32 [1.20–1.46]
P	$8.7 \times 10^{-11}$	$8.4 \times 10^{-9}$	$3.0 \times 10^{-8}$	$3.3 \times 10^{-8}$
Replication				
Dutch study				
RAF <sup>d</sup>	0.22	0.70	0.84	0.79
Imputation $r^2$	0.95	0.99	0.92	0.99
Allelic OR [95% CI]	1.54 [1.00–2.35]	1.45 [1.03–2.04]	1.21 [0.77–1.90]	1.75 [1.44–2.68]
P <sup>e</sup>	0.024	0.017	0.199	$4 \times 10^{-3}$
German study				
RAF <sup>d</sup>	0.22	0.71	0.84	0.82
Imputation $r^2$	NA <sup>i</sup>	NA <sup>i</sup>	NA <sup>i</sup>	NA <sup>i</sup>
Allelic OR [95% CI]	1.36 [1.08–1.71]	1.19 [0.96–1.46]	1.13 [0.88–1.46]	1.26 [0.99–1.61]
P <sup>e</sup>	$5.6 \times 10^{-3}$	0.046	0.172	0.031
Sub meta-analysis				
Allelic OR [95% CI]	1.38 [1.13–1.69]	1.26 [1.05–1.51]	1.16 [0.91–1.47]	1.39 [1.12–1.72]
P <sup>f</sup>	$7.7 \times 10^{-4}$	$6 \times 10^{-3}$	0.11	$1.4 \times 10^{-3}$
Q <sup>g</sup>	0.30	0.87	0.05	0.85
I <sup>2h</sup>	0	0	0	0
P <sub>het</sub> <sup>i</sup>	0.58	0.35	0.81	0.36
Combined discovery + replication				
Allelic OR [95% CI]	1.36 [1.25–1.48]	1.27 [1.18–1.37]	1.31 [1.19–1.45]	1.33 [1.22–1.46]
P <sup>f</sup>	$5.3 \times 10^{-13}$	$4.8 \times 10^{-10}$	$3.4 \times 10^{-8}$	$5.0 \times 10^{-10}$
Q <sup>g</sup>	0.33	0.89	1.33	1.82
I <sup>2h</sup>	0	0	0	0
P <sub>het</sub> <sup>i</sup>	0.85	0.64	0.51	0.40

CI, confidence interval; OR, odds ratio.

<sup>a</sup>The minor allele is the risk allele.

<sup>b</sup>The major allele is the risk allele.

<sup>c</sup>For German replication, association analysis was done with rs35786 serving as a proxy for rs148248535 ( $r^2 = 0.97$ ).

<sup>d</sup>Risk allele frequency.

<sup>e</sup>One-sided P-value.

<sup>f</sup>Two-sided combined P-value derived from a fixed effect meta-analysis of the discovery and replication results.

<sup>g</sup>Cochrane's Q estimates heterogeneity across studies.

<sup>h</sup>I<sup>2</sup> index describes the magnitude of the heterogeneity.

<sup>i</sup>P-value of the heterogeneity test across studies.

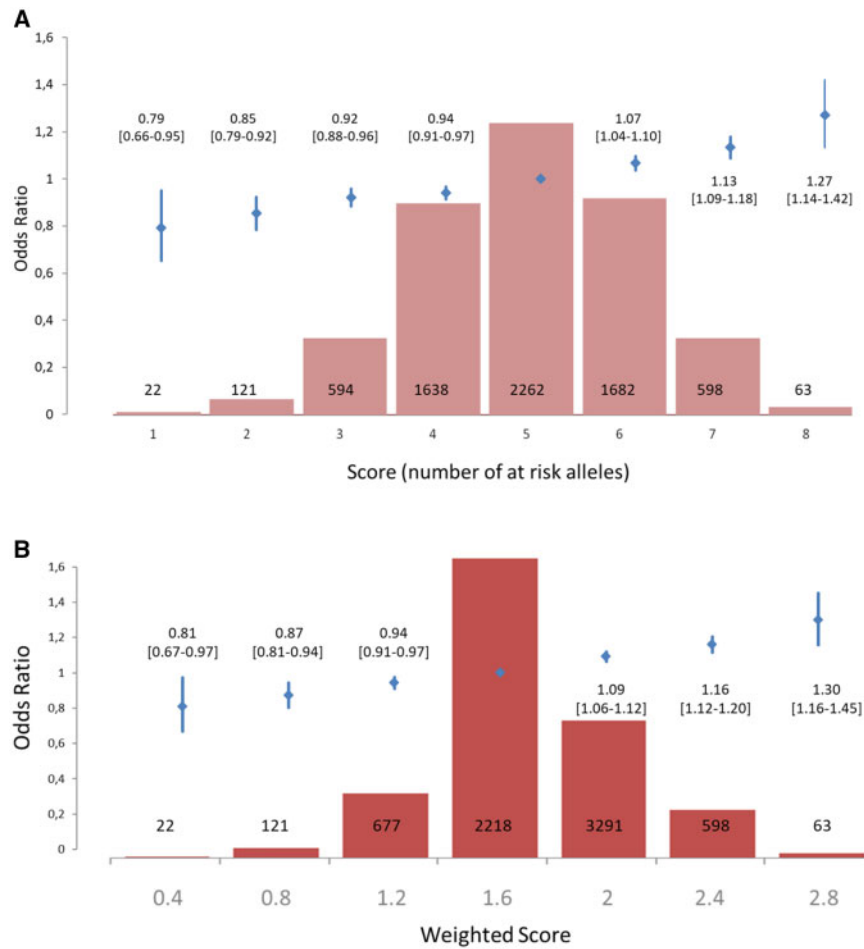
<sup>j</sup>Not applicable.

by Leung et al.<sup>11</sup> are predicted (Figure 3). It is located in a predicted TAD spanning [chr3:14 160 000–14 680 000] (Figure 4A) that encompasses six genes (*CHCHD4*, *TMEM43*, *XPC*, *LSM3*, *SLC6A6*, and *GRIP2*) (Supplementary material online, Table S10). Using PCHI-C in iPSC-CM, H3K27ac/H3K4me1 enhancer marks inside the LD block specifically interact with the *SLC6A6* and *GRIP2* promoters (Figure 4A).

The in-house 4C-seq results show significant interactions ( $P < 10^{-8}$ ) between the associated region bait and intra-TAD regional promoters/enhancers, confirming TAD boundaries. The highest

interaction signals localized on the *SLC6A6* promoter and intragenic enhancer and on the *XPC/LSM3* promoter region (Figure 4A,  $P < 10^{-50}$ ; Supplementary material online, Table S11).

Each positional candidate gene (Supplementary material online, Table S10) is expressed in the LV and atrial appendage: *TMEM43*, *CHCHD4*, *LSM3*, *SLC6A6*, *XPC*, and *GRIP2* (from the most to the least expressed). Moreover, *XPC* ( $P = 8.3 \times 10^{-15}$ ) and *SLC6A6* ( $P = 6.9 \times 10^{-6}$ ) LV expressions were significantly increased in DCM patients compared to healthy donors (Supplementary material online, Table



**Figure 2** Bar plot of the unweighted (A) and weighted (B) scores.

S12A), while *LSM3* expression was significantly decreased ( $P = 7.6 \times 10^{-8}$ ).

The rs62232870-associated LD block was screened for eQTL, sQTL, and mQTL. rs62232870 is not an eQTL for nearby genes but other SNPs in the LD block were significantly associated with *SLC6A6* expression in atrial appendage (highest signal, rs62231957,  $P = 1.9 \times 10^{-5}$ ) (Supplementary material online, Table S13 and Supplementary material online, Figure S14). No sQTL was present, but all the SNPs strongly associate with the methylation level of *SLC6A6* CpGs (cg08926287,  $P < 10^{-28}$ ) and less significantly in three other genes (*TMEM43*, *CHCHD4*, *XPC*;  $10^{-23} < P < 10^{-8}$ ). Interestingly, the partially independent rs4684185-associated LD block correlates even more strongly with the same mQTLs (cg08926287,  $P < 10^{-72}$  for *SLC6A6*) (Supplementary material online, Table S14).

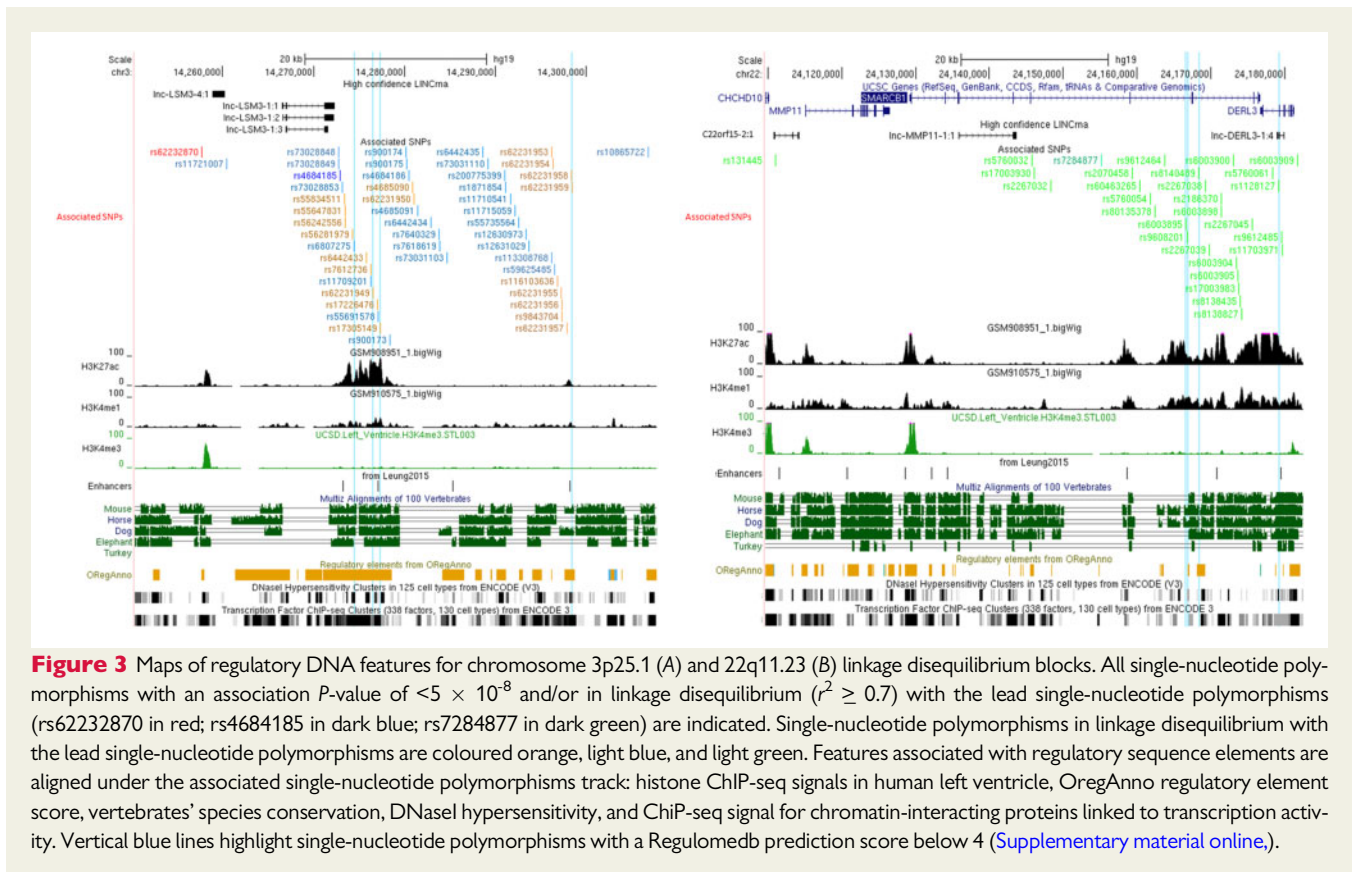
In addition, gene tolerance to mutation based on GnomAD metrics only pinpoints *SLC6A6* as a strongly evolutionarily constrained gene upon the candidates (Supplementary material online, Table S15).

Combining all the data available (Supplementary material online, Table S14 and Supplementary material online, Figure S16), *SLC6A6* appeared as the strongest culprit gene at this locus.

### Candidate culprit gene selection strategy at chr22q11.23 locus

The LD block extends over 70 kbp from the 5' region of *MMP11* and *CHCHD10* to the 5' region of *DERL3* including *SMARCB1* where the lead SNP maps to [chr22:24 110 180–24 182 174] (Supplementary material online, Figure S8 and Supplementary material online, Table S9). This region contained H3K27ac, H3K4me1, and H3K4me3 LV marks witnessing the presence of cardiac active promoters and enhancers and numerous other features (interspecies conservation, regulatory elements, DNaseI HS, and TF-binding sites) support its regulatory role (Figure 3B).

The LD block is located at the edge of two cardiomyocyte-predicted TADs covering 1.2 Mb [chr22:23 480 001–24 680 000] (Figure 4B) and the 21 genes covered by those TADs were considered as positional candidates (Supplementary material online, Table S10). Published PCHi-C showed a dense pattern of chromatin interaction linking the LD block with promoters inside the TAD: *ZNF70*, *CHCHD10*, *MMP11*, *SMARCB1*, *DERL3*, and *SLC2A11* confirming the regulatory role of the region. In-house CM 4C-seq confirmed strong



**Figure 3** Maps of regulatory DNA features for chromosome 3p25.1 (A) and 22q11.23 (B) linkage disequilibrium blocks. All single-nucleotide polymorphisms with an association  $P$ -value of  $< 5 \times 10^{-8}$  and/or in linkage disequilibrium ( $r^2 \geq 0.7$ ) with the lead single-nucleotide polymorphisms (rs62232870 in red; rs4684185 in dark blue; rs7284877 in dark green) are indicated. Single-nucleotide polymorphisms in linkage disequilibrium with the lead single-nucleotide polymorphisms are coloured orange, light blue, and light green. Features associated with regulatory sequence elements are aligned under the associated single-nucleotide polymorphisms track: histone ChIP-seq signals in human left ventricle, OregAnno regulatory element score, vertebrates' species conservation, DNaseI hypersensitivity, and ChIP-seq signal for chromatin-interacting proteins linked to transcription activity. Vertical blue lines highlight single-nucleotide polymorphisms with a Regulomedb prediction score below 4 (Supplementary material online).

interactions with enhancer elements located close by (Figure 4B), especially with *SMARCB1* and *DERL3* (Supplementary material online, Table S16;  $P < 10^{-50}$ ).

The most highly expressed gene was *CHCHD10*, followed by *GSTT1*, *DDT*, *SMARCB1*, *CABIN1*, and *SLC2A11*, the other 15 genes being very weakly or not expressed. Differential expression was observed for *CHCHD10* and, to a lesser extent, for *DDT* and *SMARCB1* (Supplementary material online, Table S12B).

Supplementary material online, Table S13 presents the significant eSNPs in cardiac and skeletal muscle tissues. Among the six cardiac-expressed genes, only *SMARCB1* expression was influenced by SNPs within the LD block (Supplementary material online, Figure S15). No sQTL was present, but all SNPs in the LD block associated with methylation level variation (mQTL) of nearby genes (Supplementary material online, Table S14) (strongest signals, *SMARCB1*-cg08219923 and *DERL3*-cg25907215,  $P < 10^{-200}$ ).

Finally, GnomAD mutation tolerance score only suggested *SMARCB1* and *BCR* as genes under evolutionary constraints (Supplementary material online, Table S15).

Combining all the data available (Supplementary material online, Table S14 and Supplementary material online, Figure S17), *SMARCB1* appears to be the strongest candidate at chr22q11.23 locus.

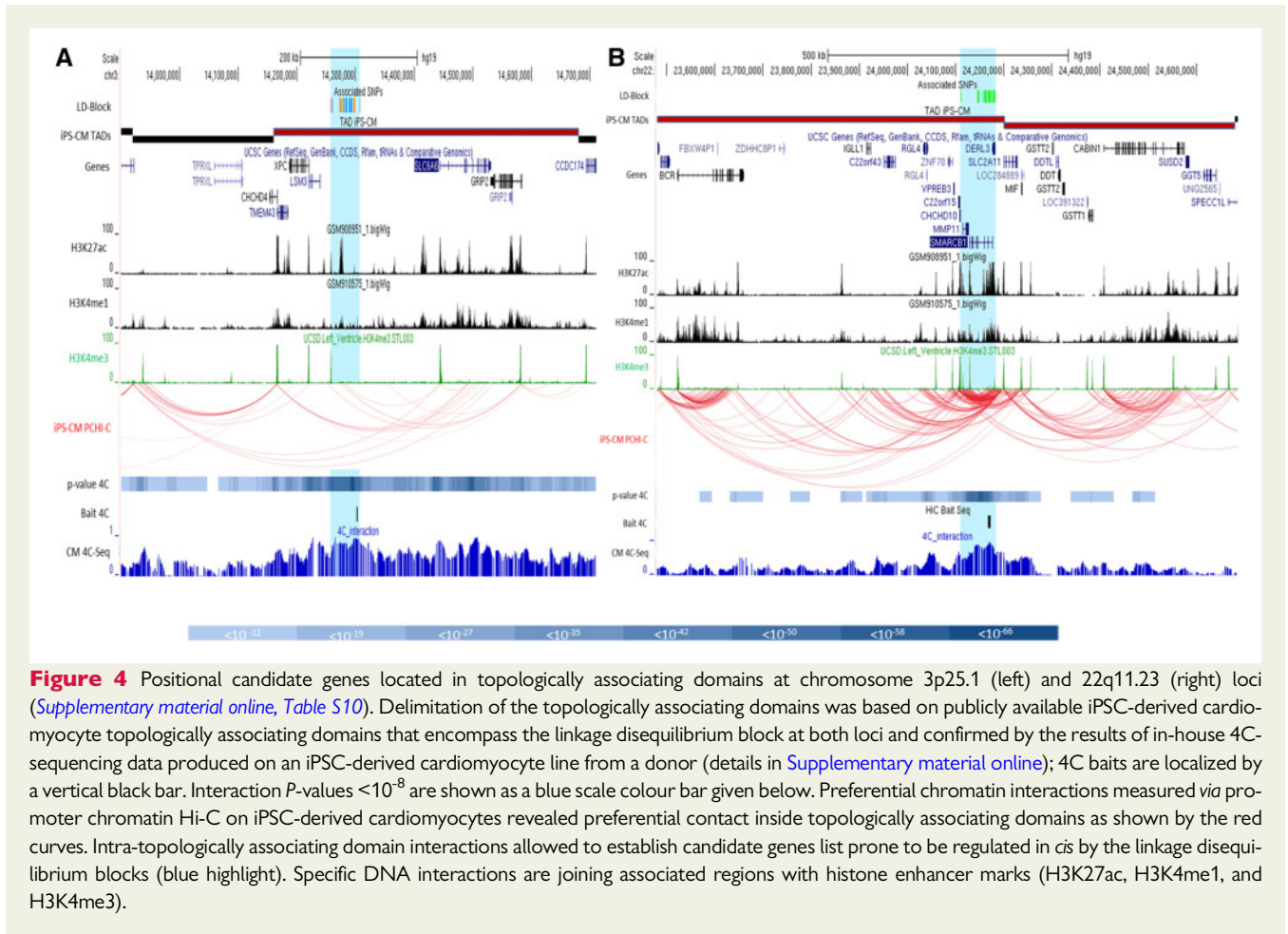
## Discussion

By adopting a GWAS strategy performed in the largest DCM population assembled so far, we identified and replicated two new

susceptibility loci while confirming two previously reported ones, *HSPB7* and *BAG3*. Interestingly, some SNPs in the two new loci we identified as associated with DCM were recently associated with cardiac structure and function in the general population (with a normal average ejection fraction) (UK Biobank study).<sup>17</sup> These authors also constructed polygenic risk scores and observed that some of these scores were associated with incident DCM cases ( $n = 388$ ). The association with incident DCM was based on polygenic scores as a whole, therefore providing no association between single SNP/loci and DCM in this study.<sup>17</sup>

The first novel locus maps to chr3p25.1. The LD block extends over six genes two of which, *TMEM43* and *SLC6A6*, are expressed in the heart and have been suspected to be involved in human cardiac disorders. Two SNPs at that locus, rs73028849 and rs11710541, were associated with left ventricular imaging in a general population (not in heart failure/DCM).<sup>17</sup> Rare pathogenic variants in *TMEM43* have been reported in arrhythmogenic right ventricular cardiomyopathy<sup>18</sup> and a homozygous missense mutation in *SLC6A6* was described in a family with hypokinetic cardiomyopathy and retinal degeneration.<sup>19</sup> Several evidences pinpointed *SLC6A6* as the culprit gene (Supplementary material online, Figure S16). DCM-associated SNPs in this LD block were significantly associated with *SLC6A6* expression in atrial appendage and methylation. They also specifically interact with *SLC6A6* regulatory elements through chromatin interaction analysis. Remarkably, the GnomAD mutation tolerance score also suggests that *SLC6A6* is the best candidate among the genes of the locus. *SLC6A6* encodes a taurine transporter whose expression





**Figure 4** Positional candidate genes located in topologically associating domains at chromosome 3p25.1 (left) and 22q11.23 (right) loci ([Supplementary material online, Table S10](#)). Delimitation of the topologically associating domains was based on publicly available iPSC-derived cardiomyocyte topologically associating domains that encompass the linkage disequilibrium block at both loci and confirmed by the results of in-house 4C-sequencing data produced on an iPSC-derived cardiomyocyte line from a donor (details in [Supplementary material online](#)); 4C baits are localized by a vertical black bar. Interaction  $P$ -values  $<10^{-8}$  are shown as a blue scale colour bar given below. Preferential chromatin interactions measured via promoter chromatin Hi-C on iPSC-derived cardiomyocytes revealed preferential contact inside topologically associating domains as shown by the red curves. Intra-topologically associating domain interactions allowed to establish candidate genes list prone to be regulated in cis by the linkage disequilibrium blocks (blue highlight). Specific DNA interactions are joining associated regions with histone enhancer marks (H3K27ac, H3K4me1, and H3K4me3).

and activity regulates taurine, an amino acid with cyto-protective effects especially in the heart.<sup>20</sup> Taurine deficiency was observed in several mammalian species with DCM and in a family with hypokinetic cardiomyopathy, while its supplementation in the same models and patients was associated with left ventricular function normalization.<sup>19,21,22</sup> Accordingly, mice knockout for *SLC6A6* exhibit taurine level depletion and present DCM.<sup>23</sup> Interestingly, GTEx LV transcriptomic data show that the haplotype containing rs62232870-A risk allele is associated with the lowest *SLC6A6* expression. A link between *SLC6A6* depletion and impaired myocardial function is therefore emerging, and our finding of *SLC6A6* association with DCM is remarkable in this context. Even though the underlying pathway leading to heart failure remains to be fully studied in humans, and efficacy of taurine supplementation remains to be fully demonstrated, our results may suggest the potential for a new therapeutic perspective through taurine administration or modulation.

The second novel DCM locus maps to chr22q11.23 where six positional candidates showed significant expression in the heart, of which three also presented differential left ventricular expression between DCM and healthy heart (*CHCHD10*, *DDT*, and *SMARCB1*). *SMARCB1* (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily b member 1) is the sole gene under the influence of the lead rs7284877 in the LV. Interestingly,

rs7284877 is in complete LD with *SMARCB1*-rs5760054, *SMARCB1*-rs2070458, and *DERL3*-rs5760061, recently reported as associated in the general population with systolic left ventricular internal dimension and fractional shortening<sup>17,24</sup> and in strong LD ( $r^2 = 0.8$ ) with rs6003909, associated with left ventricular mass to end-diastolic volume ratio in a UK Biobank GWAS on heart disease.<sup>25</sup> Although *SMARCB1* function cannot be directly related to heart morphogenesis or function, its involvement in left ventricular dimension or function in a general population, *in silico* and data mining annotations, evolutionary constraints' prediction, and, to a lesser extent, functional analysis, suggest this gene as the more convincing candidate gene at the locus ([Supplementary material online, Figure S17](#)).

This GWAS also provided an innovative estimate of the genome-wide heritability of the disease in Europeans ( $31 \pm 8\%$ ), a value consistent with that ( $h^2 \sim 30\%$ ) recently reported in a population of African origin.<sup>8</sup> However, the four independent lead SNPs (*BAG3*, *HSPB7*, *SLC6A6*, and *SMARCB1*) only contribute to 2% of the heritability, suggesting the role of additional genetic factors and gene/gene and gene/environment interactions yet to be identified. Based upon those four SNPs, we developed the first GRS in DCM. This score may have practical implications by improving the management of subjects at risk for DCM or systolic dysfunction, such as patients taking drugs increasing the risk of myocardial dysfunction, or relatives in

DCM families. However, further clinical studies are warranted to validate its clinical utility.

Since some genes, such as BAG3, can be both involved in monogenic and multifactorial DCM forms, we checked whether genes known to cause monogenic DCM forms could also present common SNPs associated with sporadic DCM (Supplementary material online, Table S18). Except for FLNC and FHOD3, none of the familial form genes presents statistically suggestive association signals. We also performed the exon sequencing of *SLC6A6* and *SMARCB1* genes in a cohort of 769 index DCM patients and detected three rare missense likely pathogenic variants in *SLC6A6* (Supplementary material online, Table S19) that suggest a potential role of *SLC6A6* in monogenic DCM, although this requires further functional studies to be able to conclude.

Despite its innovative findings, this study may have some limitations. First, we robustly identified two new DCM loci and convincing candidates but were not able to definitely demonstrate which culprit variants are responsible for the observed susceptibility to the disease. Further molecular and cellular investigations are needed to fill this gap. Second, despite being the largest GWAS ever performed on DCM, with both a discovery and a replication phase, our study may have been suboptimal in identifying common susceptibility alleles due to the absence of perfectly matched healthy controls for the British and US populations. Therefore, we performed our discovery GWAS on combined individual data while handling any potential hidden population stratification through adjustment on genetically-derived principal components. The robust replication of two out of three genome-wide significant associations in two European cohorts provides strong support for the validity of that strategy. Finally, our results do apply to sporadic DCM and cannot be extrapolated at that stage to familial DCM. The replication of the reported genetic associations in non-European ancestry populations as well as the analysis of familial forms of DCM, are now needed.

In conclusion, we identified two new genetic loci associated with DCM at chr3p25.1 and chr22q11.23, in which *SLC6A6* and *SMARCB1* stand out as the most likely culprit candidate genes. A GRS was built with a potential clinical perspective for the prediction of DCM or its prognosis but additional work is required to conclude about this potential application. These findings not only provide a better understanding of the genetic architecture of DCM but also identify new players in the pathophysiology of systolic heart failure, with the potential for new therapeutic developments, especially through taurine modulation.

## Supplementary material

Supplementary material is available at *European Heart Journal* online.

## Data availability

The data that support the findings of this study are available on request from the corresponding authors (S.G and P.C).

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## Translational perspective

We present the results of the largest genome-wide association study performed so far in dilated cardiomyopathy (DCM), a leading cause of systolic heart failure. We identified two new DCM-associated loci and two strong culprit genes, *SLC6A6* and *SMARCB1*, on chromosomes 3p25.1 and 22q11.23, respectively. A polygenic risk score was constructed to better predict the risk of DCM. Furthermore, *SLC6A6* gene encodes a taurine transporter whose involvement in myocardial dysfunction is supported by numerous observations in humans and animals. This study sheds light on novel biological pathways underlying heart failure, and putative new therapeutic targets.