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Reassessment of genes associated with dilated and hypertrophic cardiomyopathy in a Chinese Han population

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Abstract

Introduction: More than 100 genes are reportedly associated with dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM). However, the situation that many genes lack of reassessment in a large population hinders the interpretations of these genes in genetic diagnostic testing. Moreover, limited genetic data for cardiomyopathy in Chinese patients was reported.

Aim: Therefore, here we reassessed an estimated 500 putative genes in the Chinese Han population by whole exome sequencing (WES) to describe the landscape of variants in these genes and to confirm their genetic contribution to DCM and HCM.

Methods and Results: WES was performed in 1059 DCM patients, 1175 HCM patients and 514 controls. Approximately 500 candidate genes were selected for evaluation. Truncating variants of *TTN* and *MYBPC3* were the most burdensome for both groups. Gene-based association tests identified 35 and 35 genes associated with DCM and HCM, respectively. Except for the known genes of cardiomyopathy, the top three genes associated with DCM were *MUC16*, *KMT2C*, and *FBN1*, while the top three genes associated with HCM were *KMT2C, RYR2*, and *SCN5A*. After filtering for pathogenicity, *FBN1* is still significantly associated with DCM and *SCN5A* and *RYR2* remains significantly enriched in HCM patients. However, after adjustment, only *TTN* with DCM and *MYBPC3* and *MYH7 with* HCM remains significant.

Conclusion: We described the genetic landscape of Chinese patients with DCM and HCM and developed a website (www.cardioexome.cn) to enable open access to this information. Furthermore, the gene-based association test confirmed the contribution of *TTN* to DCM and *MYBPC3* and *MYH7* to HCM in Chinese Han. In addition, the website, www.cardioexome.cn, was developed to store these sequencing results.

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

INTRODUCTION

Dilated and hypertrophic cardiomyopathies (DCM and HCM, respectively) are common genetic disorders that can cause heart failure. In addition to the 16 definitive genes (*ACTC1*, *BAG3*, *DES*, *FLNC*, *FXN*, *LAMP2*, *LMNA*, *MYBPC3*, *MYH7*, *MYL2*, *MYL3*, *RBM20*, *SCN5A*, *TNNC1*, *TNNT2*, and *TTN*) by ClinGen experts, more than 100 genes identified through the candidate gene strategy in case studies have been implicated in DCM[1] and HCM[2]. Due to a lack of background population variation, the interpretations of these genes in genetic diagnostic testing were ambiguous.

To evaluate the contribution of these genes, Mazzarotto *et al.* and Walsh *et al.* analyzed the burden of rare variants in 56 putative DCM genes and 31 putative HCM genes, respectively[3,4]. Unfortunately, many genes implicated in DCM and HCM were excluded in the TruSight Cardio panel. In fact, limited genetic data for Chinese cardiomyopathy patients were reported.

Therefore, we aimed to describe the landscape of variants in known genes associated with DCM and HCM and reassess newly identified genes in the Chinese Han population using whole exome sequencing. Furthermore, we develop a website, available from: www.cardioexome.cn, to enable open access to these population data and facilitate their genetic interpretation.

METHODS

Population

The study included 1059 patients with primary DCM, 1175 patients with HCM, and 514 controls from Tongji Hospital, Wuhan, China, between July 2007 and December 2020. According to European Society of Cardiology guidelines, primary DCM was defined as left ventricular or biventricular dilatation (left ventricular end-diastolic diameter > 33 mm/m^2 [men] or > 32 mm/m^2 [women]) and systolic dysfunction (left ventricular ejection fraction < 50%) in the absence of secondary causes such as abnormal loading conditions or coronary artery disease[5]. In contrast, HCM was defined as a maximal LV wall thickness

≥15 mm without abnormal loading conditions or another cardiac or systemic disease causing hypertrophy, such as congenital heart disease or aortic stenosis[6,7].

The study conformed to the Declaration of Helsinki, and it was approved by the institutional review board of Tongji Hospital. All participants provided written informed consent. Baseline characteristic data were either extracted from a centralized hospital database or collected from a review of community health care centers. All the subjects were of Han Chinese origin based on their family name or self-report.

Whole exome sequencing

DNA samples were extracted from peripheral blood leukocytes using the TIANamp Blood DNA Kit (Tiangen, Beijing, China) and quantified with the Nanodrop 2000 (Life Technology, USA). Qualified samples were exome sequenced by Berry Genomics Co. Ltd. (Beijing, China) and WuXiAppTec (Shanghai, China) with a SureSelect Human All Exon library kit (Agilent, USA) and Novaseq 6000 or Hiseq Xten platform in paired-end mode of 150 base pairs (Illumina, California, USA). The whole exome sequencing (WES) data were processed in accordance with the best practice of The Genome Analysis Toolkit[8]. Adapters and low-quality reads were trimmed by Trimmomatic[9], and the filtered reads were aligned to the human reference genome GRCH37 (hg19) using the Burrows-Wheeler Alignment Tool. After marking duplicates, sorting bam and recalibration, HaplotypeCaller called variants of each sample, followed by consolidation across multiple samples with the GenomicsDBImport and GenotypeGVCFs. Finally, the variant call set was recalibrated by the VariantRecalibrator and ApplyRecalibration.

Bioinformatics

Variants with a read depth of < 20 were defined as missing and then removed when the missing rate was greater than 20% across the whole cohort. The resulting variants were annotated using ANNOVAR[10]. Rare variants were defined as those with a minor allele frequency of < 0.001 in East Asian databases from The 1000 Genomes Project, and gnomAD. Deleterious variants were defined as loss-of-function variants (nonsense variants, frameshift variants, and canonical splicing-disrupting variants) and missense variants predicted to be deleterious by REVEL and M-CAP[11,12].

Selection of cardiomyopathy-relevant genes

Genes associated with DCM and HCM were first selected from the DCM and HCM expert panel from ClinGen[13] for the evaluation of pathogenicity in the Chinese Han population. [Supplementary Table 1] The selection of additional candidate genes for DCM and HCM was conducted by database searching and literature screening. First, the keywords "dilated cardiomyopathy" and "hypertrophic cardiomyopathy" were used to search against the OMIM database and the gene bank database on the National Center for Biotechnology Information website. In addition to genes included in the HCM and DCM expert panels of ClinGen, 260 genes associated with HCM and 446 genes related to DCM were included in the downstream analysis. (Search date: 15 December 2021)

Gene-based association test

Candidate genes in DCM and HCM were evaluated separately by comparison of rare variants between DCM and control or between HCM and control. Genes with less than two variants were filtered out, which left 530 and 533 genes for downstream DCM and HCM evaluation, respectively. The optimal sequence kernel association test (SKAT-O) was used to assess associations of candidate genes and risks of DCM and HCM. Moreover, rare deleterious variants were filtered for further SKAT-O tests. Considering the multiple comparisons, we adopted Benjamini-Hochberg to adjust the *P* values.

Statistical analysis

Continuous variables are presented as mean ± SD and categorical variables are described as percentages. Fisher exact test or Pearson chi-square test of association was used to compare the population frequencies across the groups. Two-tailed *P* values < 0.05 were considered statistically significant.

RESULTS

Overall, 1059, 1175, and 514 individuals were enrolled in the DCM, HCM, and control groups, respectively. The baseline characteristics are shown in Table 1. The mean age of DCM cohort is 54.97 years, while the mean age of HCM cohort is 53.18 years. To make sure the control cohort is really healthy and not that they are not of the age at which symptoms appear, the mean age of control cohort is significantly older than the cardiomyopathy cohort. According to principle component analysis of variants with minor allele frequency of > 0.05, all participants are East Asian. [Supplementary Figure 1]

Rare variants of DCM- and HCM-curated genes

First, we evaluated the well-established genes of DCM and HCM and compared the frequency of rare truncating variants detected in 49 DCM genes and 25 HCM genes by comparing HCM cases with controls and DCM cases with controls. Overall, 200 (18.9%) patients with DCM, 133 (11.3%) patients with HCM, and 31 (6.0%) control patients carried at least one truncating variant of all cardiomyopathy genes ($P < 0.001$, Supplementary Figure 2).

Specifically, truncating variants carried by DCM cases were detected in 22 DCM genes, while at least one truncating variant was found in eight HCM genes in the HCM group [Figure 1]. In the DCM arm, 128 (12.1%) DCM patients carried at least one truncating variant of *TTN* (TTNtv), which was the most frequently mutated gene in DCM-curated genes, accounting for 64.0% among all the 22 mutated genes [Figure 1A and Supplementary Table 2]. As the TTNtv variants with a percentage of spliced in (PSI) > 90% are considered more likely to be pathogenic, we further stratified the truncating variants and assessed their population frequencies among the three study groups. In total, there are 111 variants with a PSI > 90%, accounting for 120 DCM patients, one control and zero HCM patients, where 85 variants in A band, 15 variants in I band, six variants in M band, three in Z disk and two near Z disk [Figure 2]. Similarly, *MYBPC3* ranked first among the nine HCM-relevant genes according to the frequency of individuals carrying truncating variants, wherein 55 (41.4%) HCM cases were identified in HCM cohort [Figure 1 and Supplementary Table 2]. In addition, four genes - *MYBPC3*, *DSP*, *MYH6*, and *ANKRD1* - manifested both DCM and HCM phenotypes. As for *MYBPC3*, three truncating variants were identified in six patients with DCM, which consists of one nonsense variant in one DCM patient, one frameshift variant from three patients with DCM, and one variant disrupting splicing in two patients in DCM cohort. On the other hand, in HCM cohort, the most burdened variant is a frameshift variant, that is, NM_000256:exon31:c.3624delC:p.P1208fs, which accounted for 16 HCM patients. In the current study, all truncating variants of *DSP* were from the DCM cohort, where there are six frameshift variants, four nonsense variants and one canonical splicing-disrupting variant. In *MYH6*, there are two DCM patients carrying two splicing-disrupting variants, five HCM patients carrying one frameshift variant and three nonsense variants, as well as one control carrying one nonsense variant. When it comes to ANKRD1, it seems not significantly enriched in the cardiomyopathy group compared to the control, where one frameshift variant is identified in two DCM patients and two controls and another frameshift variant in one HCM patient. In summary, variants of *MYBPC3* were significantly enriched in the HCM cohort, while *DSP* variants were more common in the DCM cohort. Compared to the control group, *TTN*, *DSP*, and *FLNC* were more frequently mutated in the DCM cohort ($P < 0.001$, $P = 0.046$, and $P = 0.061$, respectively; Supplementary Table 2), while *MYBPC3* was more often mutated in the HCM cohort ($P < 0.001$; Supplementary Table 2).

Table 1. Baseline characteristics of the study population

Characteristics	Control	DCM	HCM	*P*values
N	514	1059	1175	
Age, years [95%CI]	60.53 ± 14.18 [59.30, 61.76]	54.97 ± 14.14 [54.12, 55.82]	53.18 ± 13.50 [52.41, 53.95]	< 0.001
Male, *n* (%)	248 (48.2)	776 (73.3)	835 (71.1)	< 0.001
Hypertension, *n* (%)	21 (4.1)	526 (49.7)	543 (46.2)	< 0.001
Diabetes, *n* (%)	1 (0.2)	176 (16.6)	193 (16.4)	< 0.001
LVEDD, mm [95%CI]	-	66.64 ± 8.26 [66.14, 67.14]	48.52 ± 7.97 [48.06, 48.98]	< 0.001
LAEDD, mm [95%CI]	-	45.66 ± 7.90 [45.18, 46.14]	42.05 ± 7.78 [41.61, 42.49]	< 0.001
IVS, mm [95%CI]	-	9.56 ± 1.42 [9.47, 9.65]	17.18 ± 4.16 [16.94, 17.41]	< 0.001
LVPW, mm [95%CI]	-	9.55 ± 1.34 [9.47, 9.63]	12.17 ± 2.92 [12.00, 12.34]	< 0.001
E/e' [95%CI]	-	22.43 ± 12.48 [21.68, 23.18]	20.59 ± 13.11 [19.84, 21.34]	0.45
E/A [95%CI]	-	1.74 ± 1.54 [1.65, 1.83]	1.21 ± 0.69 [1.17, 1.25]	0.051
LVEF, % [95%CI]	-	31.41 ± 10.00 [30.81, 32.01]	58.55 ± 12.92 [57.81, 59.29]	< 0.001

DCM: Dilated cardiomyopathy; HBP: hypertension; HCM: hypertrophic cardiomyopathy; IVS: interventricular septum; LVEDD: left ventricular end-diastolic diameter; LAEDD: left atrial end-diastolic diameter; LVEF: left ventricular ejection fraction.

For missense variants, the overall mutation frequency of DCM- and HCM-associated genes was much higher than in truncating variants. Variants of DCM-associated genes were found in 930 (88.6%) patients with DCM versus 438 (85.2%) controls, while variants of HCM-associated genes were identified in 501 (42.6%) patients with HCM versus 164 (31.9%) controls. Similarly, the most frequently mutated genes were *TTN* and *MYBPC3* in the DCM and HCM cohorts, respectively. Additionally, the mutation burden of *TTN* (59.9% in DCM patients *vs.* 53.9% in controls, $P = 0.028$), *TPM1* (1.0% in DCM patients *vs.* 0.0% in controls, $P = 0.046$), *LDB3* (3.1% in DCM patients *vs.* 0.8% in controls, $P = 0.007$) and *LMNA* (2.6% in DCM patients *vs.* 1.0% in controls, $P = 0.048$) were significantly higher against controls in DCM-associated genes. For HCM-associated genes, variants of *MYBPC3* (9.7% in HCM patients *vs.* 2.5% in controls, $P < 0.001$) and *MYH7* (6.9% in patients with HCM *vs.* 1.4% in controls, $P < 0.001$) were more frequent relative to the controls [Figure 1].

Reassessment of DCM and HCM candidate genes

To evaluate the candidate genes based on the population-wise strategy, SKAT-O identified 35 and 35 genes associated with DCM and HCM, respectively [Supplementary Tables 3 and 4]. *TTN* and *MYBPC3* ranked first according to the number of carriers for DCM and HCM, respectively [Figure 3]. In addition to the genes from the expert panel, the top three genes associated with DCM were *MUC16*, *KMT2C,* and *FBN1,* and 303 (28.6%), 76 (7.2%), and 59 (5.6%) carried at least one variant relative to 187 (36.4%), 21 (4.1%), and 24 (4.7%) of the controls. The most burdened variants of *MUC16, KMT2C* and *FBN1* are *MUC16*:NM_024690:c.C5771T:p.T1924I, *KMT2C*:NM_170606:c.T2603C:p.I868T and *FBN1*:NM_000138:c.T1217A:p.L406H, respectively. Among patients with HCM, the top three genes were *KMT2C, RYR2*, and *SCN5A*, affecting 87 (7.4%), 85 (7.2%), and 74 (6.3%) of the patients with HCM and 21 (4.1%), 19 (3.7%), and 22 (4.3%) of the controls, respectively. The most burdened variants of *KMT2C*, *RYR2*, and *SCN5A* are *KMT2C*:NM_170606:c.C71T:p.A24V, *RYR2*:NM_001035:c.A6484C:p.M2162L and *SCN5A*:NM_001099405:c.C5485T:p.R1829C. *KMT2C* was associated with both DCM and HCM [Supplementary Figure 3]. Considering that multiple comparisons may lead to false positive results, we

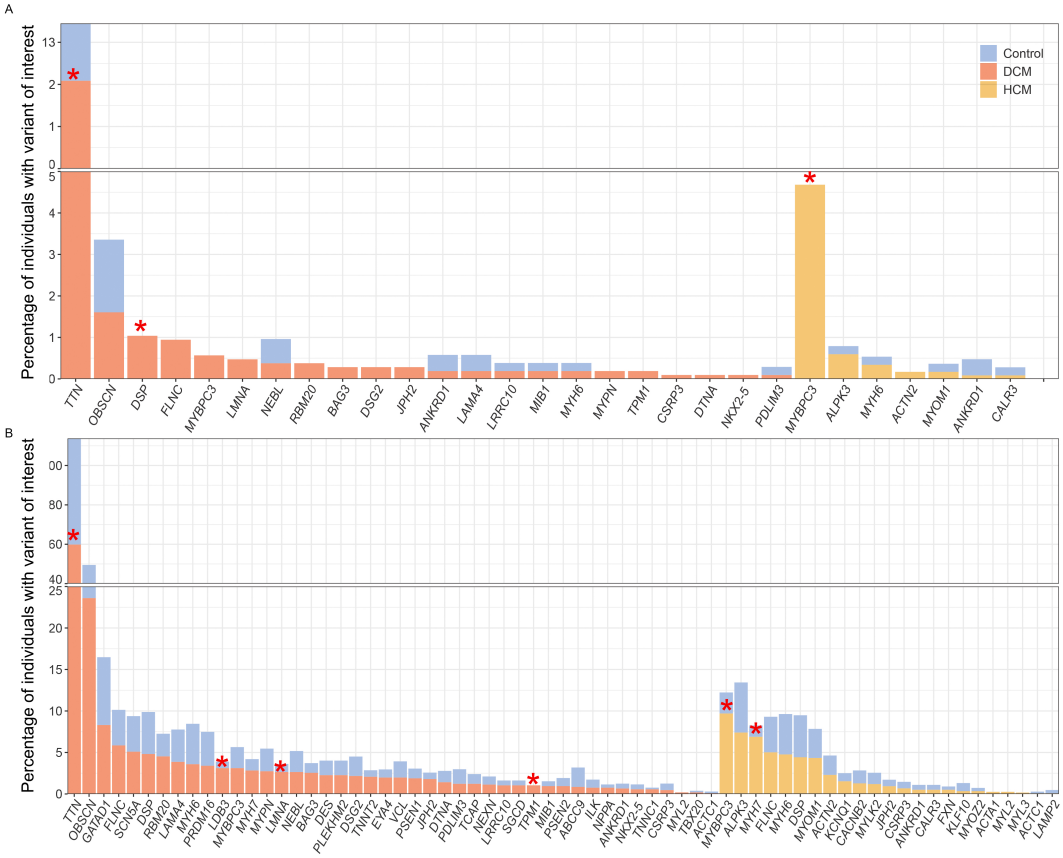

Figure 1. Proportion of individuals with rare variants in dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) compared with control cohort. Variants are shown separately for rare truncating (A) and missense variants (B). Red stars indicate the genes that have statistically significant distributions of the truncating and missense variants compared to controls.

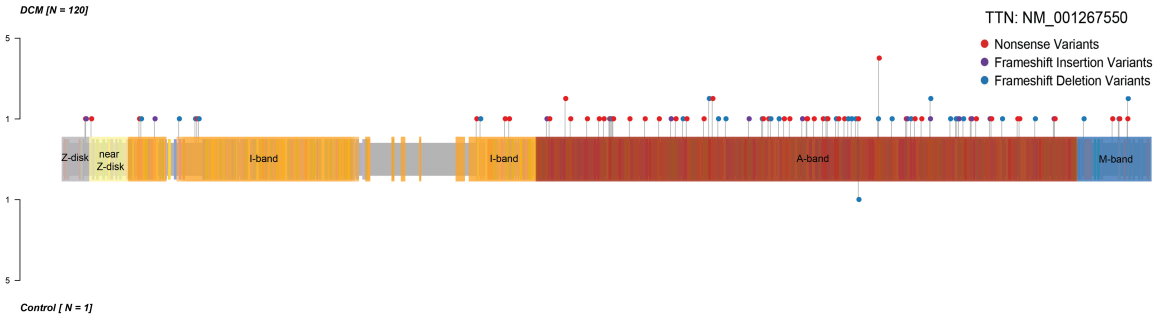

Figure 2. The distributions of the *TTN* truncating variants across the TTN protein in each study population. The schematic architecture of TTN protein was graphed using "maftools" and the bands of TTN were drawn according to *TTN* Transcript & Exon Structure (available from: https://www.cardiodb.org/titin/titin_transcripts.php). The variants affecting splicing were not shown in the plot. The X axis represents the length of TTN protein and the Y axis represents the number of individuals carrying certain variants.

adjusted the *P* values using Benjamini-Hochberg. After adjustment, only the associations of *MYH7* (adjusted $P = 0.006$) and *MYBPC3* (adjusted $P = 0.006$) with HCM remained significant.

Furthermore, rare deleterious variants defined as loss-of-function and missense variants predicted to be pathogenic by REVEL and M-CAP were filtered. Then we performed another SKAT-O test based on these

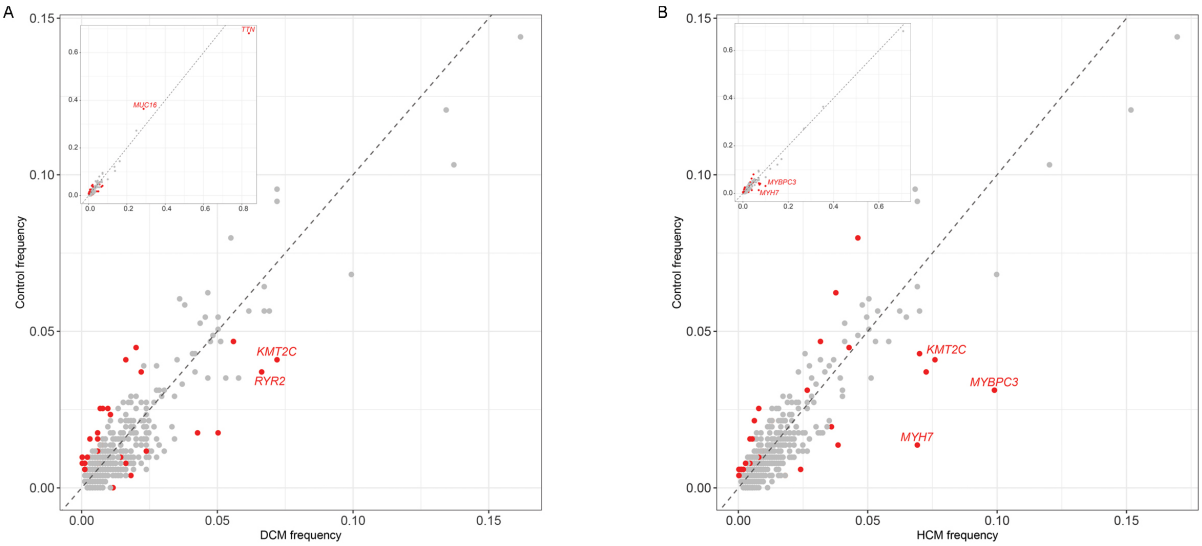

Figure 3. The prevalence of rare variation in DCM, HCM and control cohorts. Genes with a statistically significant association with disease are highlighted in red. Results are shown separately for DCM (A) and HCM (B) cohorts. *KMT2C* is significantly associated with both DCM and HCM. DCM: Dilated cardiomyopathy; HBP: hypertension; HCM: hypertrophic cardiomyopathy.

rare deleterious variants and identified 11 and five genes significantly enriched in DCM and HCM, respectively [Supplementary Tables 5 and 6]. After adjustment for multiple comparisons, the associations of *TTN* (adjusted $P = 0.003$) with DCM and *MYH7* (adjusted $P = 0.003$) and *MYBPC3* (adjusted $P = 0.003$) with HCM remained significant.

DISCUSSION

Here we described the genetic profile of curated genes for DCM and HCM in Chinese patients. We also compared the burden of rare variants in DCM- and HCM-associated genes in patients with DCM and HCM to the background variation in controls. More importantly, we uploaded the annotated variants to our website: www.cardioexome.cn. The CardioExomes database provided allelic frequencies of all variants identified in the DCM, HCM, and control cohorts. All variants were annotated using ANNOVAR, including ClinVar (31 May 2021), gnomAD, The 1000 Genomes Project, InterVar, and dbNSFP4.1a. All information was made available by supplying a simple query term that supports various formats: genomic coordinate, dbSNP ID, and HGVS format.

In accordance with previous studies[14], our study found that *TTN* and *MYBPC3* were the most frequently mutated DCM- and HCM-associated genes from the ClinGen expert panels, respectively. Truncating variants of *TTN* and *MYBPC3* accounted for ~12% and ~5% of the DCM and HCM cohorts, respectively. To evaluate the contribution of genes reportedly implicated in DCM and HCM, we included over 500 additional genes in the assessment. The gene-based association test confirmed the enrichment of *TTN, ABCC1,* and *TPM1* in DCM and *MYBPC3, MYH7,* and *MYLK2* in HCM in the Chinese cohort. In addition to these genes, 32 and 32 candidate genes were also significantly associated with DCM and HCM, respectively. The top genes were *MUC16, KMT2C,* and *FBN1* for DCM and *KMT2C, RYR2*, and *SCN5A* for HCM.

The results of this study were supported by strong evidence. The simultaneous inclusion of DCM, HCM, and controls for comparison gave us the opportunity to examine the real excess of variation burden in

cardiomyopathies by considering the Chinese population background. Furthermore, the use of the WES strategy enabled us to comprehensively analyze the genes associated with cardiomyopathies. Another strength is the cross-comparison of variants in genes associated with both DCM and HCM. Moreover, we performed a bidirectional gene-based association test SKAT-O, to explore the association of candidate genes with DCM and HCM. SKAT-O enabled us to identify positive, irrelevant, or negative variants by considering all rare variants.

We also identified an interesting gene *KMT2C*, also known as *MLL3*, associated with DCM and HCM. This gene was first reported by Jiang *et al*., who observed a significant increase in *KMT2C* expression in both human DCM hearts and hypertrophic mouse hearts[15]. *KMT2C* is a member of the myeloid/lymphoid or mixed-lineage leukemia (MLL) family, which has been identified as key H3K4 mono-, di-, and tri-methyltransferases at enhancers or promoters that facilitate gene expression[16]. Our population-wise study further confirmed the associations of *KMT2C* with DCM and HCM. However, after filtration for pathogenicity of rare variants, *KMT2C* was not significantly associated with DCM or HCM, which indicates that the single variant in *KMT2C* might not be pathogenic enough to cause DCM or HCM and more studies are required to explore the contribution of *KMT2C* in cardiomyopathy.

Besides *KMT2C*, this study also confirmed the contribution of *MUC16* and *FN1* to DCM and the association of *RYR2* and *SCN5A* with HCM. *MUC16* is also known as *CA125*, whose protein product is the tumor marker carbohydrate antigen 125. Serum carbohydrate antigen 125 level is reportedly correlated with DCM prognosis and clinical severity[17,18]. *FBN1* was mostly reported as a causal gene of Marfan syndrome[19,20] while in this study, we found the association of *FBN1* and DCM, which will be reported in another work of our team. *RYR2* was mostly known as a well-established causal gene of catecholaminergic polymorphic ventricular tachycardia[21]. This gene was first reported by Alvarado *et al*. to cause cardiac hypertrophic in mice models[22]. However, the variant RyR2-P1124 reported in their study was not found in our cohorts. *SCN5A* was a definite gene of Brugada syndrome, dilated cardiomyopathy, and familial long QT syndrome, according to ClinGen[13]. Unfortunately, the association between *SCN5A* and DCM was not identified in this study. One possible explanation is the background variation of *SCN5A* in controls and that few variants cause DCM in our study, where approximately 5% of individuals carried at least one rare variant of *SCN5A*, attenuating power to identify the differences. Surprisingly, *SCN5A* was significantly associated with HCM, which requires confirmation in further studies. After pathogenicity filtration, *FBN1* is still significantly associated with DCM and *SCN5A*, *RYR2* remains significantly enriched in HCM patients. The novel genes identified in the current study added more evidence for genetic diagnosis of DCM and HCM, especially the data stored on our website, which may contribute to the assessment of clinical pathogenicity in clinical practice. However, to confirm the causal role of these associated genes, more efforts are required, of which molecular dynamics analysis helps to filter for key variants, and the construction of animal model and linkage analysis are the key strategies to confirm it.

There are several limitations to our study. The control cohort was relatively small compared to other DCM and HCM cohorts, which can attenuate the detectability of differences on the gene-based association test. In addition, the candidate genes identified here were not investigated in another population.

In conclusion, the genetic landscape of Chinese patients with DCM and HCM resembled those of studies of Caucasian populations with minimal differences. Furthermore, the gene-based association test confirmed the contribution of *TTN* to DCM and *MYBPC3* and *MYH7* to HCM. In addition, the website, available from: www.cardioexome.cn, was developed to store these sequencing results and facilitate genetic interpretation in clinical practice.

DECLARATIONS

Acknowledgments

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Author's contributions

Offered advice for developing the websites: Wang T
Offered advice and suggestions for whole exome sequencing: Liu C, Zeng B
Designed the study, drafted and revised the manuscript: Sun Y, Wang H
Contributed to analysis and interpretation of data: Huang M, Li K, Song X, Xiao L, Dai J, Wang L, Chen Y, Wu D
Designed the study and revised it critically for important intellectual content: Yu T, Li R, Ma F, Li Z, Chen P, Wang H, Wang Y, Sun Z, Jin L
Contributed to funding, conception, revision and final approval of the manuscript: Wang DW, Chen G, Wang DW

Availability of data and materials

All data were available in Supplementary Materials.

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Conflict of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

This study was approved by the institutional review board of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (IRB TJ-C2018001), and was registered at ClinicalTrials.gov (ID: NCT03754101). All participants provided written informed consent.

Consent for publication

Participants provided written informed consent.

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