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Research Article

Genetic architecture of hypertrophic cardiomyopathy in individuals of Chinese and United Kingdom ancestry

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Abstract

Background: No studies have explored the genetic differences between the Chinese and other ethnic hypertrophic cardiomyopathy (HCM) populations.

Methods: This cross-sectional study included Chinese patients ($n = 593$) with HCM and controls ($n = 491$) who underwent whole-exome sequencing. Rare variants in 16 validated HCM genes were assessed and compared with a United Kingdom HCM cohort ($n = 1\,232$) and controls ($n = 344\,745$).

Results: Chinese HCM patients have a higher proportion of rare variants (52.8% vs 13.6%, $P < 0.001$) but have a similar proportion of pathogenic (P) or likely pathogenic (LP) variants compared to the UK cohort. In addition, the Chinese cohort had additional associations with the combined thin filament genes ($P = 1.29E{-}9$) and myosin light chain genes ($P = 4.43E{-}3$). The United Kingdom cohort was significantly associated with *MYBPC3* non-truncating variants ($P = 2.99E{-}7$). By classifying variants using the tool genebe, the variants of uncertain significance were minimized to 46.8% compared to other tools (63.3% by Intervar; 91.3% by CardioClassifier). Furthermore, we report that c.3624del in *MYBPC3* and c.300C > G in *TNNT2* account for 2.9% and 1.5% of all Chinese HCM cases, respectively.

Conclusion: Our findings suggested that patients of Chinese ancestry with HCM have a higher proportion of rare variants but are less likely to be classified as P/LP variants in HCM genes than those of European origin. The variants of c.3624del in *MYBPC3* and c.300C > G in *TNNT2* were specific to Chinese individuals and provide important insights into the ethnic differences of HCM genetic architecture.

Keywords: hypertrophic cardiomyopathy; United Kingdom (UK) Biobank; whole exome sequencing (WES); pathogenicity

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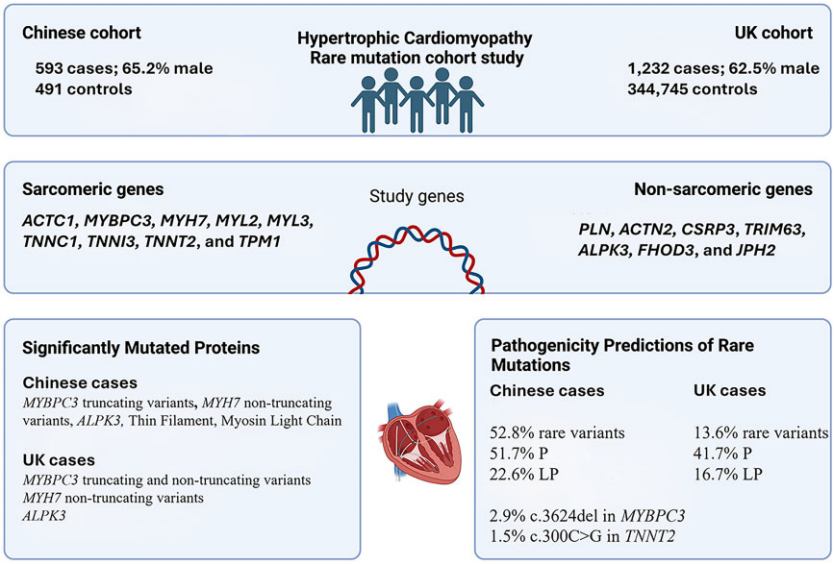
Graphical Abstract



In this cross-sectional study including 1 825 patients with HCM, Chinese patients have a higher proportion of rare variants but have a similar proportion of likely pathogenic or pathogenic variants compared with Europeans. In addition, c.3624del in MYBPC3 and c.300C>G in TNNT2 are predominantly present in patients of East Asian ancestry. In this study, ethnic disparities of HCM genetic architecture were observed, and a lack of prior research in Chinese populations limits variant interpretation in Chinese ancestry with HCM.

Introduction

Hypertrophic cardiomyopathy (HCM) is mainly caused by pathogenic variants in genes encoding sarcomere-related proteins or manifested by cardiac hypertrophy of unknown etiology, with a prevalence of 1 : 200 to 1 : 500 [1, 2, 3]. Left ventricular wall hypertrophy, potential left ventricular outflow tract obstruction in hypertrophic obstructive cardiomyopathy, and increased risk of sudden cardiac death are the main hallmarks of HCM after excluding other cardiovascular diseases with increased afterload and systemic or metabolic diseases [1]. While pathogenic variants in sarcomere-related genes, including the cardiac myosin-binding protein C (*MYBPC3*) and myosin heavy chain 7 (*MYH7*), account for 30%–60% of cases [4], substantial interindividual variability in penetrance and phenotypic expression persists, influenced by genetic background, environmental factors, and ethnicity [5]. Therefore, it is important to study the genetic characteristics of HCM in different populations with different ethnic backgrounds to assess individualized evaluation of disease phenotypes, enhance variant interpretation among diverse ancestral populations, and contribute novel mechanistic perspectives into the disease.

Critically, the genetic architecture of HCM differs across ancestries. Egyptian patients exhibit higher rates of homozygous variants in *MYL2*, *MYL3*, and *CSRP3*, and biallelic *TRIM63* mutations compared to Europeans [6]. Similarly, Black Americans show reduced sarcomeric variant prevalence vs White patients, and rare variants in underrepresented populations are often misclassified as benign (B) variants [7]. Genetic architecture could be similarly different in Chinese HCM populations.

Despite China bearing a significant HCM burden, genetic studies remain limited [8, 9], and no research has directly compared Chinese and European cohorts. This gap impedes variant interpretation, obscures population-specific mechanisms, and hinders personalized management.

Thus, our study aims to explore the genetic architecture of HCM in a Chinese HCM population and compare it with a United Kingdom (UK) cohort to identify unique genetic characteristics in order to enhance the understanding of genetic variation in HCM across different populations.

Materials and methods

Study design and population

Chinese cohort

This prospective cohort study was approved by the Ethical Committee of West China Hospital, Sichuan University, and was registered in the Chinese Clinical Trial Registry (URL: https://www.chictr.org.cn/searchprojEN.html; Registry number: ChiCTR1900022965). Written informed consent was obtained from each participant.

Consecutive adult participants diagnosed with HCM and referred for cardiovascular magnetic resonance imaging from September 2013 to December 2021 at West China Hospital of Sichuan University were enrolled. The HCM diagnostic criteria were: (i) maximal end-diastolic wall thickness of any segment in the left ventricle ≥ 15 mm (or ≥13 mm for the first-degree relative of a patient with confirmed HCM); and (ii) absence of other causes (cardiac, systemic, or metabolic disease) of myocardial hypertrophy assessed by echocardiography and CMR [10].

Whole-exome sequencing genetic testing was performed for all recruited patients with confirmed HCM. Methods for genetic sequencing and *in silico* analysis were consistent with a previous report [11]. Exon-enriched DNA was sequenced using a HiSeq2000 Sequencing System (Illumina, San Diego, CA, USA). After sequenc-

ing, the raw data were saved as FASTQ. Illumina sequencing adapters and low-quality reads (<80 bp) were filtered by cutadapt [12]. Following quality control, the clean reads were mapped to the University of California, Santa Cruz hg19 human reference genome using Burrows–Wheeler alignment [13]. Duplicated reads were removed using the Picard tools (http://broadinstitute.github.io/picard), and mapping reads were used for variation detection. Variants, including single-nucleotide variants and small insertions or deletions (indels), were identified using both the VarScan 2.2.7 [14] software package and the variant quality score recalibration protocol in the Genome Analysis Toolkit [15].

UK Biobank

UK Biobank is a repository of half a million individuals registered with the UK National Health Service and recruited at ages between 40 and 69 years old. Detailed demographic information was collected and linked to the healthcare system, providing updated diagnoses throughout their lives [16]. Whole-exome sequencing for 454 787 of these individuals was carried out by Regeneron using the Illumina NovaSeq 6000 platform [17]. The study genes were extracted from the Research Analysis Platform, provided by UK Biobank and DNA Nexus, using the Swiss Army Knife utility (v4.10.0) and the supplied version of BCFTools (v1.15.1) [18].

For this study, the cohort was made up of individuals flagged as 'Caucasian' for genetic ethnic grouping and having been diagnosed with HCM via International Classification of Diseases (ICD) 10 codes I42.1 or I42.2 (including from GP records as converted from read codes), an ICD9 code of 425.1, or a self-reported illness code of 1588, resulting in 1 238 cases. This left 392 618 individuals identified as Caucasian as controls. Cases and controls were checked for related individuals using pre-computed relatedness information from the UK Biobank, and for any who were related as a third-degree relative or closer, one of the pair was removed randomly. This left 1 232 cases and 344 745 controls.

Quality control and formatting

All HCM and control samples were processed by the same metrics following the workflow in Fig. 1. Steps were carried out using BCFTools, with each cohort processed individually. VCF files were filtered based on (i) minor allele count ≥ 1; (ii) using the set GT plugin, loci with a format depth ≤ 8 or genotype quality ≤ 10 for a sample were set as missing; (iii) with the 'norm' function, variants were aligned to the hg38 reference and multiallelic sites were split to biallelic. The 'annotate' function was used to standardize chromosome names to remove the leading 'chr', and the ID field was set to chr_bp_a1_a2. The 'query' function was used to convert to a tsv showing site information in rows and genotype per sample in columns. Genotypes were converted from diploid to counts using a series of sed commands, resulting in a matrix per group containing minor rows of variants and a column per sample containing the minor allele count.

Relatedness and ancestral background

Samples from the UK Biobank were already filtered by ancestry using their ethnicity filter and precomputed relatedness estimates up to third-degree relations. For each related pair, one was randomly removed using 'sample' in R. This led to the removal of 7 cases and 53 611 controls.

This process was mimicked for the Chinese samples, following a similar process to that used in the UK Biobank, including carrying out principal component analysis alongside 1000 Genomes [19] data (29/11/2018). First, shared SNPs were found with the 1000 Genomes data. All files were then converted into PLINK [20] format, filtering for shared SNPs. Next, SNPs were filtered for linkage disequilibrium, using the PLINK (v1.9) flag '—indep-pairwise 50 10 0.1'. From this pruned dataset, principal component analysis was carried out, as well as estimating relatedness using KING [21] up to third-degree relations. It was observed that some highly related individuals existed in the case data, so these were removed first, with any that appeared equally often chosen by sampling. For the controls, this was not the case, so random sampling was used between pairs. This yielded 50 cases to remove and 9 controls. All samples were positioned in the same region as the three Chinese groups from 1000 Genomes (supplementary Fig. S1, see online supplementary material).

Annotations and filtering

The annotation of variants was carried out using several sources. VEP (v110.1) [22] was used to collect Human Genome Variation Society [23] nomenclature for DNA and protein changes, as well as consequences, in the Sequence Ontology [24] format. Filtering allele frequencies and unformatted allele frequencies were obtained from gnomAD [25] v4.0.0 VCF files. If an allele was not found in gnomAD, it was assigned an allele frequency of 0. Variants were then filtered to keep those with a filtering allele frequency at 95% confidence of <4E−5; these are calculated to be rare enough to potentially be monogenic causal mutations [25].

The American College of Medical Genetics and Genomics (ACMG) pathogenicity predictions [26] were sourced using genebe [27] via their Python client (v0.0.17); however, as comparators, all variants were also tested using CardioClassifier (v0.2.0) [28] and InterVar (v2.2.2) [29]. CardioClassifier has a limited selection of genes available for analysis, and some complications in extracting B variants from the CardioClassifier web interface. As such, the results of the comparative analysis are split into two. The first section includes only variants assessed by all three tools and without CardioClassifier, with all variants available from cases and controls. The ACMG classifications from genebe were included in the main analysis, with data curated using R scripts (v4.3.1).

The Consequence field was used to guide the annotation of truncating variants, non-truncating variants, and probable B variants. Truncating variants were defined as:

feature_truncation, frameshift_truncation, inframe_deletion, conservative_inframe_deletion, disruptive_inframe_deletion, stop_gained, stop_gained_NMD_escaping, stop_gained_NMD_triggering, splice_donor_variant, splice_acceptor_variant, frameshift_variant, inframe_insertion, inframe_deletion

Non-truncating but damaging mutations were defined as:

inframe_deletion, inframe_insertion, missense_variant, stop_lost, start_lost, protein_altering_variant.

All others were removed from further analysis since they were expected to have no deleterious effect on the gene. This is the approach followed in the Egyptian HCM study, mentioned previously [6]. All mutations found fitting this criterion were retained for analysis. Furthermore, co-occurring mutations in any one sample were also collected as potentially having an oligogenic effect.

Variants were also assessed at a variety of MAFs to better understand the possible architecture of the disease. For this, three brackets of rare variants were created, and the upper limit was 0.01, generally seen to be the threshold for what is considered a 'rare' allele. The second and third brackets were defined based

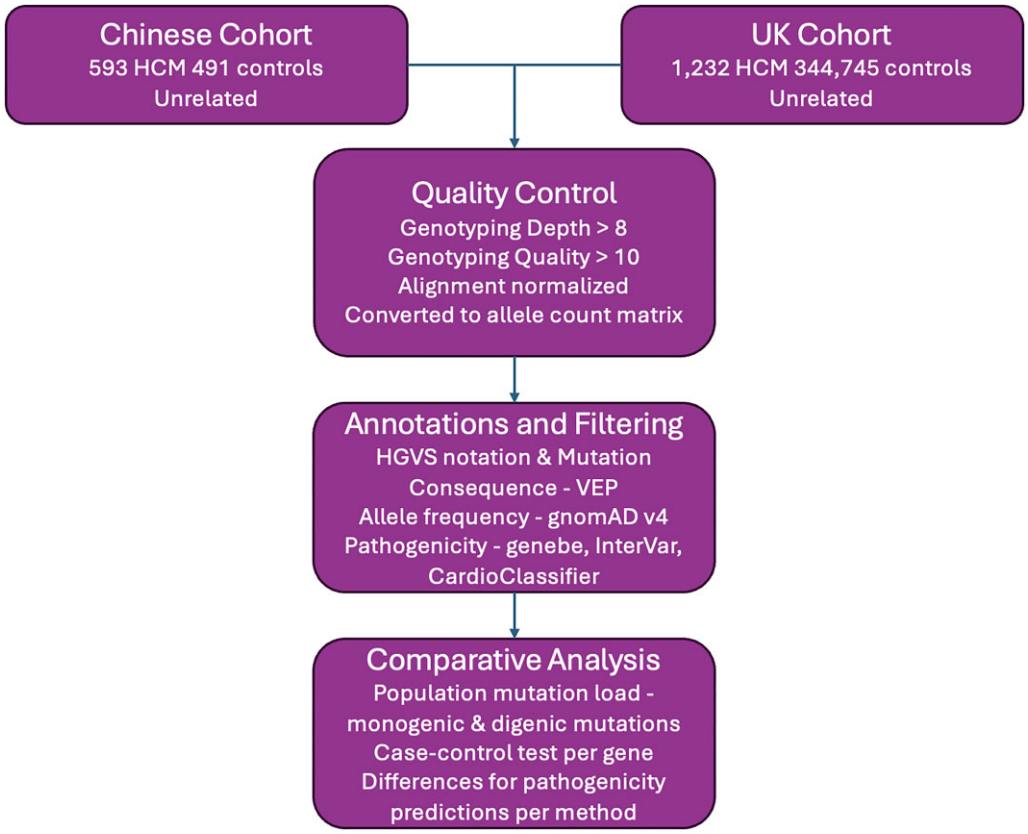


Figure 1. Workflow for rare variant mutation analysis.

on figures derived from the alleleFrequencyApp [30], with an allele frequency between 0.01 and 1.26E−3 simply being rare, between 1.26E−3 and 4E−5 seen as possibly part of a digenic pair of mutations, and an allele frequency <4E−5 seen as potentially a monogenic causal mutation. The MAFs were sourced from the maximum allele frequency of all populations from gnomAD v4 in exome and whole genome samples.

Statistical analysis

The number of mutations with a filtering allele frequency < 4E−5 was counted for cases and controls per gene. These were used to generate contingency tables to perform a one-sided Fisher's exact test to assess the significance of the mutation burden. Since this involved 11 tests, a stringent Bonferroni correction was applied for multiple testing to give a significance threshold of 0.00454. This process was repeated for only the variants that were predicted to be pathogenic/likely pathogenic (P/LP) by genebe.

The computations described in this paper were performed using the University of Birmingham's Bluebeard HPC service, which provides a High-Performance Computing service to the University's research community. See http://www.birmingham.ac.uk/bear for more details.

Results

Cohort characteristics

The Chinese cohort was made up of 593 cases and 491 controls. The cases had a mean age of 49 ± 15.2 years and were 65.2% male. The UK Biobank cohort included 1 232 cases and 344 745 controls. The cases had a mean age of onset of 64 ± 11.2 years and were 62.5% male. Of this group, 946 (76.8%) had a record of hypertension, with an ICD10 code of I10, I11, I13, I15, or I16. There was a record of 6 individuals with a myectomy and 89 who had undergone cardioverter defibrillator implantation.

Table 1. Rare variants in the 16 genes related to HCM.

Gene[a]	Chinese	UK
ACTC1	2 (0.34%)	0 (0%)
ACTN2	9 (1.52%)	8 (0.649%)
ALPK3	29 (4.89%)	17 (1.38%)
CSRP3	2 (0.34%)	4 (0.32%)
FHOD3	14 (2.36%)	19 (1.52%)
JPH2	8 (1.35%)	11 (0.89%)
MYBPC3	122 (20.6%)	52 (4.22%)
MYH7	104 (17.5%)	48 (3.90%)
MYL2	4 (0.67%)	2 (0.16%)
MYL3	8 (1.35%)	4 (0.32%)
PLN	1 (0.17%)	1 (0.08%)
TNNC1	4 (0.67%)	3 (0.24%)
TNNI3	19 (3.20%)	1 (0.08%)
TNNT2	23 (3.88%)	8 (0.65%)
TPM1	11 (1.85%)	0 (0%)
TRIM63	0 (0%)	0 (0%)

[a]Rare mutations are defined as having a filtering allele frequency score < 4E−5. All mutations have a 'Consequence' likely to be deleterious to the resultant protein.

HCM variance prevalence

In the Chinese HCM patients, a total of 256 potentially P, rare variants were identified from 52.8% of cases (Table 1 and supplementary Table S1 and S2, see online supplementary material). In comparison, the UK patients had a total of 168 of these

Table 2. Overview of the presence of rare variants in candidate genes in Chinese and UK cases.

Gene	Chinese HCM	UK HCM	Chinese Ctrl	UK Ctrl
ACTC1	0.34% (2)[a]	0	0.20% (1)	0
ACTN2	1.52% (9)	0.65% (8)	1.63% (8)	0.53% (1825)
ALPK3	4.89% (29)	1.38% (17)	2.24% (11)	0.91% (3151)
CSRP3	0.34% (2)	0.32% (4)	0.61% (3)	0.19% (667)
FHOD3	2.36% (14)	1.54% (19)	2.65% (13)	1.03% (3555)
JPH2	1.35% (8)	0.89% (11)	1.43% (7)	0.38% (1314)
MYBPC3	20.6% (122)	4.22% (52)	3.26% (16)	0.74% (2557)
MYH7	17.5% (104)	3.9% (48)	1.02% (5)	0.99% (3406)
MYL2	0.67% (4)	0.16% (2)	0.20% (1)	0
MYL3	1.35% (8)	0.24% (3)	0	0.15% (513)
PLN	0.17% (1)	0.08% (1)	0	0.03% (112)
TNNC1	0.67% (4)	0.24% (3)	0.20% (1)	0.06% (210)
TNNI3	3.20% (19)	0.08% (1)	0.20% (1)	0.15% (517)
TNNT2	3.89% (25)	0.65% (8)	0.20% (1)	0.17% (599)
TPM1	1.85% (11)	0	0.20% (1)	0
TRIM63	0	0	0	0

[a]Frequency in cohort, with count in brackets.

variants, found in 13.6% of cases (Table 1 and supplementary Table S3 and S4, see online supplementary material). The most commonly mutated genes in both cohorts were *MYBPC3* and *MYH7*. From the Chinese group, 122 individuals (20.6%) had rare *MYBPC3* variants (28 missense mutations, 24 nonsense mutations, 57 frameshift mutations, and 20 other truncating mutations). Meanwhile, 52 UK individuals (4.22%) had rare *MYBPC3* variants (27 missense mutations, 1 nonsense mutation, 16 frameshift mutations, and 8 other truncating mutations). Additionally, there were 104 Chinese individuals (17.5%) with *MYH7* variants, compared to 48 UK cases (3.9%). These were all missense mutations, with three in the UK cohort also predicted to be splice region variants. Mutations for all genes are summarised in Table 2, including those for controls.

In addition, the number of carriers of rare variants in other HCM pathogenic genes included *FHOD3* (19 cases, 1.54%), *ALPK3* (17 cases, 1.38%), *JPH2* (11 cases, 0.89%), *ACTN2* and *TNNT2* (8 cases, 0.65%), *MYL3* and *CSRP3* (4 cases, 0.32%), *TNNC1* (3 cases, 0.24%), *MYL2* (2 cases, 0.16%), and *TNNI3* and *PLN* (1 case, 0.08%). In addition, there were no rare variants in the genes of *ACTC1*, *TPM1*, and *TRIM63*.

Analysis of 491 controls (supplementary Table S2, see online supplementary material) found 71 individuals with LP mutations, with 16 having mutations in *MYBPC3*, 13 in *FHOD3*, 11 in *ALPK3*, 8 in *ACTN2*, 7 in *JPH2*, 6 in *TPM1*, 5 in *MYH7*, 3 in *CSRP3*, and 1 in *ACTC1*, *MYL2*, *TNNC1*, *TNNI3*, and *TNNT2*.

Comparisons in the genetic architecture of HCM between Chinese and UK HCM patients

The initial analysis focused on variants that had a filtering allele frequency in gnomAD of < 4E−5, identifying rare variants in 52.8% of Chinese cases and 13.6% of UK cases, compared to 14.5% in Chinese controls and 5.32% in UK controls (supplementary Tables S1, S2, and S3). Within the context of affected genes (Fig. 2), the most common mutations occurred in the *MYBPC3* and *MYH7* genes, as well as in genes that constitute the thin filament. Supplementary Tables S1 and S3 present the numbers of cases with mutations in each gene, while supplementary Tables S4–S7 (see online supplementary material) present *P-values* from the incidence comparison of rare variants between cases and controls, asserting significant deviation from the null hypothesis for mutations in several genes. Additionally, Supplementary Tables S5 and S7 include only mutations that are P/LP.

Within the Chinese cohort (Fig. 2 and Table 3), the presence of *MYBPC3* truncating mutations (P = 1.01E−26), *MYH7* non-truncating mutations (P = 2.41E−23), non-truncating mutations in the thin filament genes (P = 1.29E−9), *ALPK3* truncating mutations (P = 0.00149), and non-truncating mutations in the *MLC* genes (P = 0.00443) was observed. However, if only those predicted to be pathogenic are taken into account, the presence of the *MLC* genes is no longer a significant finding.

In the UK cohort (Fig. 2 and Table 3), significance was found for the presence of *MYH7* non-truncating mutations (P = 2.06E−16), *MYBPC3* truncating mutations (P = 7.72E−29) and non-truncating mutations (P = 2.99E−7), and *ALPK3* truncating mutations (P = 2.65E−5). When making the comparison with only P/LP mutations, there is a weak but significant association with the thin filament genes (P = 0.0022).

Also notable was the difference in the number of rare variants with an allele frequency from gnomAD. Of the 251 unique rare mutations found in the Chinese cases, 142 lacked a record in gnomAD. The UK Biobank is included in gnomAD, so the majority of variants were found, with 19 of 151 unique mutations lacking an annotation.

In summary, the two disease cohorts shared significant statistical association with *ALPK3* and *MYBPC3* truncating mutations, as well as non-truncating mutations in *MYH7* (Fig. 2, and supplementary Tables S5 and S7). However, an association with the Chinese cohort was also found with non-truncating mutations in the genes that constitute the *MLC* and thin filament, and in the UK cohort with non-truncating mutations in the *MYBPC3* gene (supplementary Tables S6 and S7).

Co-inherited mutations identified in the cohorts

Additional analysis, aimed at identifying co-inherited mutations (supplementary Tables S8 and S9, see online supplementary material), found 46 possible individuals with two or more rare mutations in the Chinese cohort and 425 in the UK cohort. Of these, 11 pairs contained two variants classified as P/LP, and all included a mutation in *MYBPC3* in the Chinese cohort. These were co-occurring with *MYH7* (4), *ALPK3* (3), *TNNI3* (2), *ACTN2*, *TNNC1*, and *TNNI3*. There were only 8 co-inherited P/LP mutations in the UK cohort, with all except 2 including a mutation in *MYH7*. This could

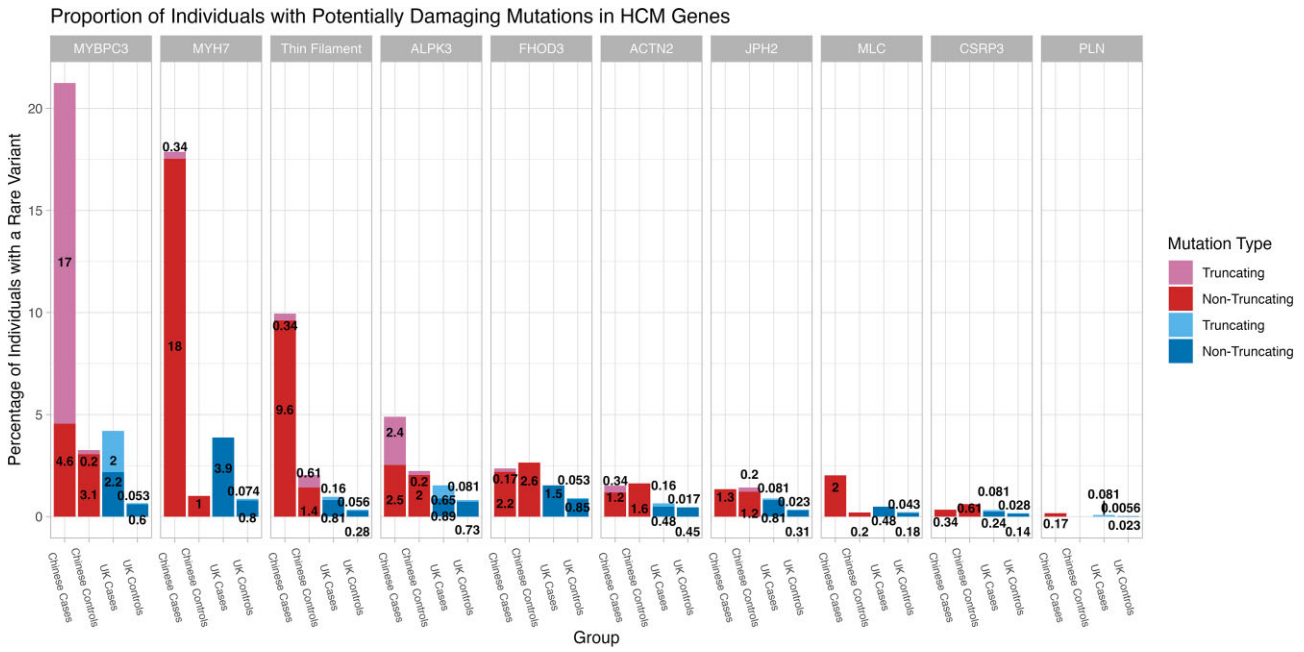


Figure 2. Percentage of individuals in each cohort with rare mutations in each gene. Rare mutations are defined as having a filtering allele frequency score < 4E−5. All mutations carry a 'Consequence' likely to be deleterious to the resultant protein.

Table 3. Comparison of the frequency of rare variants in HCM-associated genes between HCM cases and controls.

Gene	Mutation type	Cohort	*P* value[a] (P/LP only)
ALPK3	Truncating	Chinese	0.00149 (6.83E−4)
ALPK3	Truncating	UK	2.65e−5 (4.9E−6)
MYBPC3	Truncating	Chinese	1.01e−26 (1.01E−26)
MYBPC3	Truncating	UK	7.72e−29 (1.72E−32)
MYBPC3	Non-truncating	UK	2.99e−7 (4.48E−15)
MYH7	Non-truncating	Chinese	2.41e−23 (6.01E−23)
MYH7	Non-truncating	UK	2.06e−16 (5.36E−24)
MLC[b]	Non-truncating	Chinese	0.00443 (0.159)
Thin filament[c]	Non-truncating	Chinese	1.29e−09 (3.4E−10)
Thin filament	Non-truncating	UK	0.0078 (0.0022)

[a]Fisher's exact 1-sided test level of significance = 0.00454, with Bonferroni correction for 11 tests = 0.00454. [b]MLC: *MYL2* and *MYL3*. [c]Thin filament: *ACTC1*, *TNNC1*, *TNNI3*, *TNNT2*, *TPM1*.

indicate some genetic interaction that increases susceptibility in the etiology of the disease.

Classification of rare variants

Variants were filtered by a set of sequence ontology [24] terms, indicating likely damaging consequences. Additionally, ACMG classifications [26] were used to indicate the likelihood of pathogenicity based on prior knowledge and set criteria (Fig. 3), using the classifications B, likely B (LB), LP, or P. As expected, both cohorts included a much greater number and proportion of P/LP mutations compared to controls. For the Chinese cohort, total rare mutations were 51.7% P and 22.6% LP from a total of 403, whereas only 3 P mutations (4.2%) and 7 LP mutations (9.9%) were found from a total of 71 in the control participants. For the UK cohort, rare mutations were 41.7% P and 16.7% LP from a total of 180, compared to 5.9% P and 10.7% LP mutations from a total of 18 962 mutations in the control cases. The major contributors to P and LP mutations were *MYBPC3* and *MYH7*. In the Chinese cases, *MYBPC3* was 40.5% of these, and *MYH7* was 36.8%. The remaining 22.6% consisted of nine genes, with *TNNT2*, *TNNI3*, and *ALPK3* the main contributors. For the UK cohort, *MYBPC3* contributed 40.0% of these mutations, and *MYH7* another 35.2%. The other 23.8% consisted of 7 genes, most notably *ALPK3*.

Different analysis approaches affect the interpretation of clinically actionable variants

A comparison was made of ACMG classifications using genebe, InterVar, and CardioClassifier (supplementary Tables S10 and S11, see online supplementary material) [26–28, 29]. The latter offers a web interface that renders the extraction of some data less straightforward, such as variants classified as B, and to compensate for this, these data, as well as genes that were not included in CardioClassifier's database, were ignored in our comparison. Comparing variants assessed by all classifiers, a notable difference is the number of variants that are classified as being of uncertain significance (VUS), with genebe assigning the fewest (1 459), then InterVar (1 835), and finally CardioClassifier (2 778). All figures for classifications by each tool can be found in Table 4. In this, an analysis is also included without CardioClassifier, in which genebe found 116 more to be P/LP and 18 336 to be B/LB than InterVar.

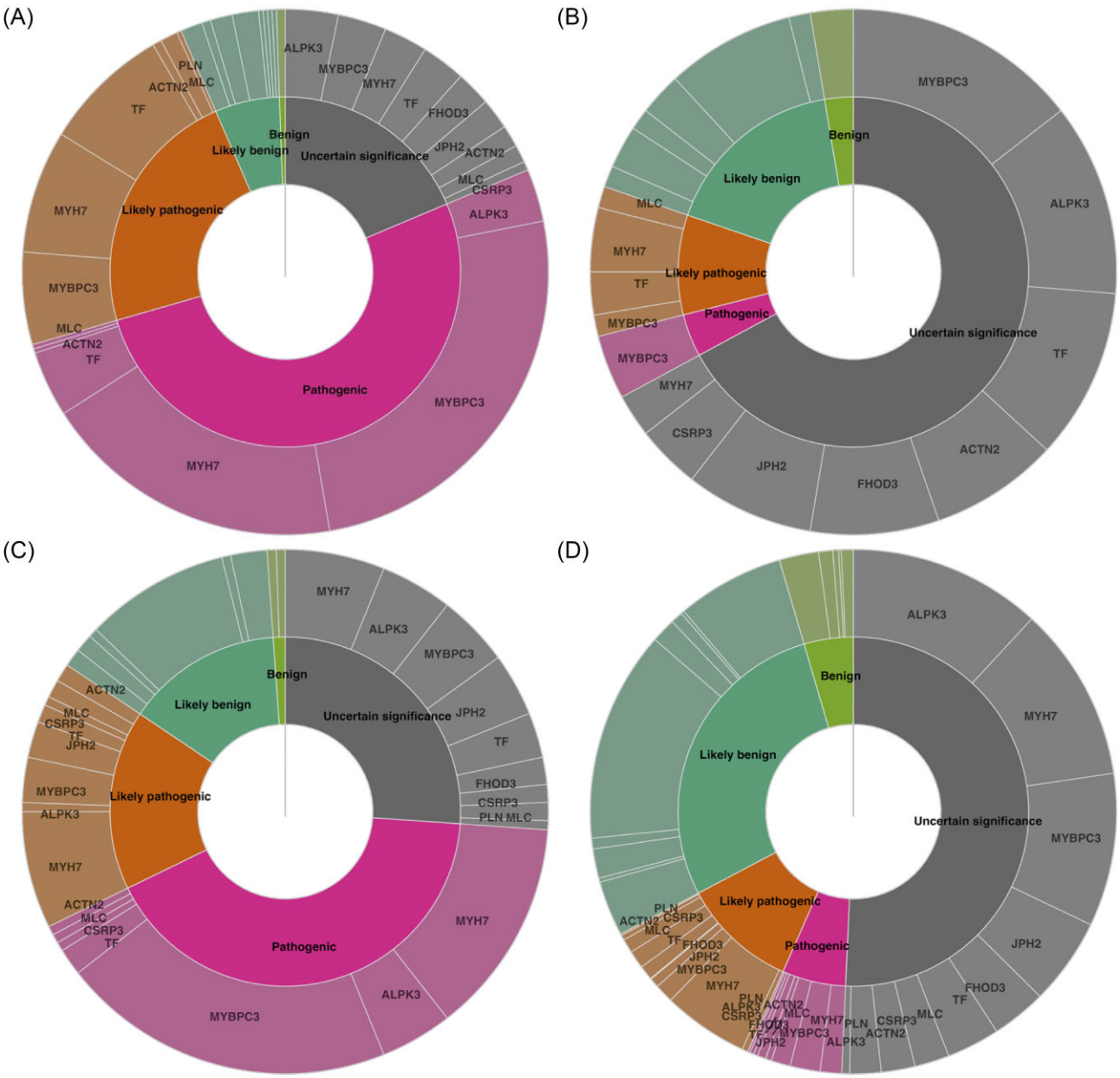


Figure 3. These plots show the ACMG predicted pathogenicity of the mutations. These are in the categories of P, LP, uncertain significance, LB, or B. Plots show the type of mutation found in (**A**) Chinese cases, (**B**) Chinese controls, (**C**) UK cases, and (**D**) Chinese controls.

Table 4. Classification given for variants by different tools.

Tool	P/LP	VUS	B/LB
CardioClassifier	243	2 778	21
genebe	1007 (1 347)[a]	1 459 (5 038)	577 (24 998)
InterVar	1 084 (1 231)	1 835 (24 371)	114 (6 662)

[a]Figures in brackets indicate when CardioClassifier was not included.

Rare variants in normal controls

There were 3 114 (0.903%) controls in the UK cohort with P or LP mutations. Of these, 1 996 had a record of another cardiovascular disease code (ICD10 code Chapter IX). The most common was primary hypertension (1 303), followed by chronic ischaemic heart disease (459), and angina pectoris (378). Each code present in this group of controls was tested for increased incidence of each condition given exposure to pathogenic risk alleles. However, after correction for multiple tests using the Benjamini–Hochberg false discovery rate, no single term achieved significance. The most significant was atrial fibrillation and atrial flutter ($P = 0.000317$, false discovery rate = 0.697), followed by rheumatoid arthritis, and unspecified and placental transfusion syndromes.

Different allele frequencies

The threshold for variant rarity was relaxed to take into account digenic and oligogenic models, in order to display a monogenic model as has been used previously in this study, with an MAF of < 4E−5, as well as a digenic model with MAFs up to 1.26E−3, and finally a rare bracket for a more additive model (Fig. 4). This provides further evidence for the involvement of *MYBPC3* with

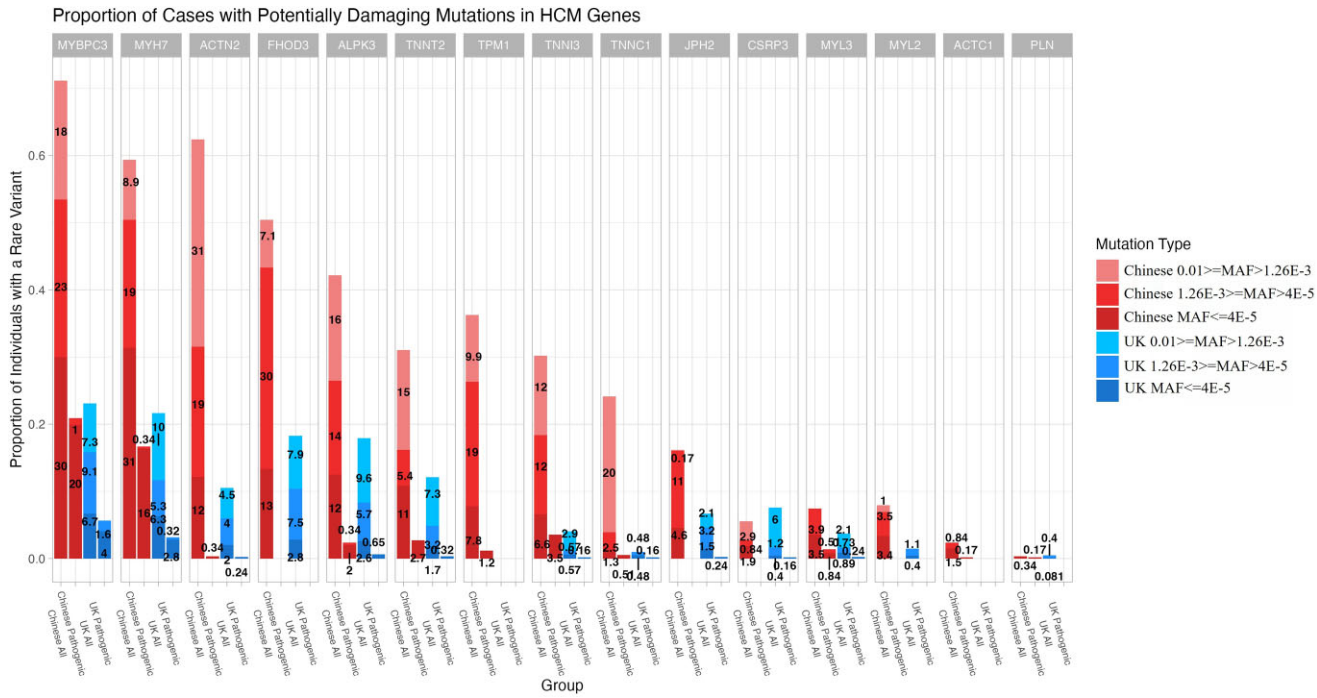


Figure 4. Percentage of individuals in each cohort of cases with mutations that have a minor allele frequency < 4E−5 and have a 'Consequence' indicating a deleterious mutation or a consistent record in ClinVar indicating pathogenicity based on the different allele frequencies.

a very high prevalence of mutations in *MYBPC3* and *MYH7* in both cohorts, as well as *ALPK3* and *FHOD3* (Fig. 4). As would be expected, many of the more common variants were not found to be P/LP, with the striking example seen in *ACTN2*, which had many mutations in both cohorts, but very few were predicted to be pathogenic.

Most frequent variants identified in Chinese cohorts

Nine individuals were found to have the same LP*TNNT2* gene (c.300C > G). Interestingly, 17 individuals were found to have the same LP truncating *MYBPC3* mutation in the Chinese cases, attributed to deletion of a single base, recorded twice in gnomAD v4.0.0 in the East Asian and non-Finnish European group, but not reported in either dbSNP or dbVar. In gnomAD v4.0.0, the allele frequency in the East Asian group is predicted to be larger due to fewer genomes being assessed; however, this is still very rare at 2.24E−5. In addition, the computational simulation of the protein structure and functional analysis of the variant (c.3624del in *MYBPC3*) are demonstrated in supplementary Fig. S2 (see online supplementary material). The variant results in disruption of the N-terminal Ig domain, resulting in compromised binding to myosin. Furthermore, the presence of this mutation was confirmed in these patients using Sanger sequencing (supplementary Fig. S3, see online supplementary material).

Discussion

We report for the first time the rare variants and diagnostic genetic testing yield for a large sample of HCM probands across Chinese HCM patients. Compared to the UK ancestry cohort, the Chinese HCM probands have a higher rare variant identified than European probands, but there was a similar proportion of P or LP in the two cohorts. In addition, classifying variants through genebe enabled us to re-evaluate the classification of identified VUSs in patients, which increased the diagnosis rate of the variant interpretation. Finally, we report that c.3624del in *MYBPC3* and c.300C > G in *TNNT2* account for 2.9% and 1.5% of all HCM cases (17/593; 9/593) in the Chinese cohort, respectively, which are predominantly present in patients of East Asian ancestry. These findings highlight the necessity of ethnicity-specific genetic databases and refined variant interpretation frameworks.

Genetic testing yield in different ethnic HCM cohorts

In a genetic study that included 2291 HCM patients in Norway, 273 patients (11.9%) were found to carry P variants [5]. Among them, 98% of variants were found in the sarcomeric genes, most often in the *MYBPC3* (66.7%) and *MYH7* (22.7%) genes. In addition, the most common HCM variant was the splice-site variant c.3190 + 2T > G in *MYBPC3*, accounting for 14% of all HCM variants [5]. In another study that included a heterogeneous cohort of 1376 HCM patients, 369 patients (26.8%) carried LP or P variants, most often in *MYBPC3* (39.7%, $n = 148$) and *MYH7* (29.0%, $n = 108$) [31]. In addition, within the Sarcomeric Human Cardiomyopathy Registry, comprising 2405 patients from 8 centers in Europe, and North and South America, a P/LP sarcomere variant was identified in 41.5% of all patients tested ($n = 1279$), of which most were identified in *MYBPC3* (57%) and *MYH7* (32%) [32]. The rate of genetic positivity varied among different studies, which could be accounted for by the variation of pathogenicity interpretations between researchers. On the other hand, race and gender may also contribute to the differences in genetic explainability. For example, in an American cohort, fewer Black patients carried sarcomeric mutations compared with White patients [29 (26.1%) vs 569 (40.5%); $P = 0.006$] [7]. In addition, male HCM patients are less likely to have sarcomeric mutations [33]. Furthermore, the proportion of

patients with rare variants in validated HCM genes was higher in the Egyptian HCM cohort compared with the UK HCM cohort (52.8% vs. 32.0%) [6]. The results from our study also suggest that Chinese HCM patients have a higher proportion of rare variants compared to the UK cohort (52.8% vs 13.6%); however, Chinese patients with HCM have a similar proportion of P (51.7% vs 41.7%) or LP (22.6% vs 16.7%) variants compared to UK probands. Since there is a much greater mutational yield in the Chinese cohort, this could be due to a lack of information in the allele frequency databases of these mutations. This could also have increased the likelihood of a pathogenic prediction. As such, further investigation into the genetics of the Chinese population would be beneficial for more accurate classification.

It is crucial to account for rare variants. Of the 28 studies in the GWAS Catalog [34] (Feb 2025), associations were found with only two of the genes from this study, *FHOD3* and *ALPK3*. This suggests that the most important genes for HCM, *MYBPC3* and *MYH7*, are being overlooked by GWAS studies that focus entirely on common variants.

MYH7 and MYBPC3 variants

In previous studies, ~70% of HCM patients with a pathogenic sarcomeric gene variant were found to carry pathogenic variants in the *MYH7* and *MYBPC3* genes [1]. Among them, the mutation type of the *MYH7* gene is mainly a missense mutation, while the mutation type of the *MYBPC3* gene is mainly a truncation mutation [35, 36]. As expected, *MYBPC3* and *MYH7* accounted for the majority of rare variations, with variants in *MYH7* more likely to present as non-truncating mutations while variants in *MYBPC3* were more likely to present as truncating mutations. In addition, some key differences were observed between the Chinese and UK HCM cohorts. For example, non-truncating mutations in the *MYBPC3* gene were significantly enriched in UK HCM cases as compared to controls.

Rare variants in the ALPK3 and FHOD3 genes

Previous studies reported that *ALPK3* was associated with the occurrence of HCM phenotypes [37], while another study found that heterozygous rare variants in *ALPK3* were associated with HCM in East Asians (truncating: 4/793 vs. 4/4523, $P = 0.02$; missense: 25/793 vs. 46/4523, $P = 2.56E{-}5$) [38]. In addition, a multi-center study found that 2.32% of 3189 HCM patients carried pathogenic variants of the *FHOD3* gene [39]. In another study, 3.3% of 1000 Chinese patients were found to carry pathogenic mutations in the *FHOD3* gene [40]. Our study reported a high prevalence of mutations in *ALPK3* (truncating: Chinese $P = 0.00149$ vs UK $P = 2.65E{-}5$), but our results did not support the involvement of *FHOD3* since neither cohort reached significance. In addition, when only the predicted P/LP variants were included, *P*-values decreased as a number of non-P variants were removed from controls.

Variant interpretation

The current understanding of HCM genetic architecture has primarily been shaped by data derived from European ancestry cohorts. Given the larger number of European samples in gnomAD, the allele frequency thresholds used are likely more accurate for that population than for Chinese cohorts. Consequently, some variants that are classified as rare in European populations may be more prevalent in the Chinese population. Additionally, since mutations in Chinese individuals are less reported, the predictions of pathogenicity may be fewer due to the limited available evidence. Expanding ethnic-specific genetic data sets in resources such as gnomAD and ClinVar could enhance the interpretation of VUSs in these populations.

Furthermore, newly discovered genes associated with HCM might be overlooked due to a lack of representation in certain databases. Notably, when a less stringent allele frequency filter was applied, the number of identified variants increased, suggesting that genes such as *TRIM63* may play a role in disease etiology, even if it is not fully penetrant as part of an additive inheritance model. Finally, a comparison of tools such as genebe, InterVar, and CardioClassifier showed that genebe classified fewer variants as VUS, with most being reclassified as LB/B, although some additional P/LP variants were identified. Combined evidence emphasizes that genetic architecture and variant pathogenicity in HCM differ significantly across ethnicities. Current reference databases, heavily skewed toward European populations, inadequately represent global diversity, leading to the misclassification of variants in underrepresented groups. Clinically, this study advocates for ethnicity-specific guidelines in genetic testing and interpretation to improve diagnostic accuracy. Future research must focus on elucidating the functional and clinical impacts of ethnic-specific variants and exploring oligogenic models in diverse cohorts to advance personalized medicine for HCM.

Our results show that genebe reduces VUS classifications significantly, improving clinical utility. In disorders like HCM, distinguishing LB from potentially P variants is critical for diagnosis and management. Evidence against pathogenicity can alleviate patient anxiety [41], while identifying P variants enables proactive monitoring and early intervention [42]. Though ACMG criteria provide rigor, further validation of tools like genebe is needed to optimize their use in genetic counseling and therapeutic decision-making.

Most frequent variants identified in different ethnic-specific HCM cohorts

A recent study reported that the most common HCM variant in Norway was the splice-site variant c.3190 + 2T.G in *MYBPC3*, accounting for 14% of all HCM variants [14]. The most common HCM variant in a Netherlands cohort was c.2373dup in *MYBPC3*, accounting for the variant in 25% of the probands [43], while a Finnish study reported p.Q1061X in *MYBPC3* as the most common HCM variant across the study cohort [44]. In our study, a c.3624del in *MYBPC3* and c.300C > G in *TNNT2* account for 2.9% and 1.5% of Chinese HCM cases, respectively.

Future directions

Given the limitations of rarity-based pathogenicity predictions, future studies should leverage familial data through co-segregation analyses. Estimating penetrance is crucial, as many variants may not be fully penetrant, impacting risk prediction and clinical management. For example, Yin *et al*. demonstrated incomplete penetrance of the R58Q mutation in Chinese families, highlighting the need for pedigree-based validation [45].

Additionally, experimentation can be carried out using iPSC-derived cardiomyocytes with CRISPR-Cas9 gene editing to introduce mutations of interest. Mosqueira *et al.* [46] demonstrated this approach by observing phenotypic effects via electrophysiology, calcium imaging, and transcriptomics. Potential genetic interactions could be further investigated using techniques like immunoprecipitation [47] or transcriptomic co-expression analysis [48]. While animal models may be limited due to mutation specificity, comparative biopsy studies with tissue staining [49] could also elucidate structural impacts.

Our study has several limitations. First, there was a lack of quantification of the penetrance of a mutation for pathogenicity predictions. As such, there is little indication as to whether the mutation could cause the disease in a monogenic etiology or if it is simply caused by a partial delay of disease onset. Second, within the ACMG classification framework, rarity serves as evidence suggesting the mutation may be pathogenic, which introduces a bias toward categorizing rare variants as pathogenic, as this is the subset of variants selected for analysis in this study. This could lead to more of these variants being predicted to be pathogenic and shows the importance of validation beyond this study. One approach could be to use a family co-segregation study to leverage pedigree information and more clearly demonstrate transmission of the mutation with disease onset.

According to our findings, the study is the first to identify genotypes associated with HCM in a Chinese cohort, providing insights into ethnicity-specific genetic mutations in HCM. Chinese patients with HCM have a higher proportion of rare variants, but have a similar proportion of P/LP variants in HCM genes than those of European ancestry due to a lack of representation of Chinese ancestry in current reference resources. The c.3624del variant in *MYBPC3* and the c.300C > G variant in *TNNT2* appear to be specific to Chinese individuals, offering important insights into ethnic differences and the complex genetic architecture of HCM.

Ethics statement

This prospective cohort study was approved by the Ethical Committee of West China Hospital, Sichuan University (No. 2019-166). Written informed consent of the Chinese cohort was obtained from each participant. This prospective Chinese cohort study was registered in the Chinese Clinical Trial Registry (URL: https://www.chictr.org.cn/searchprojEN.html; Registry number: ChiCTR1900022965).

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Author contributions

Jie Wang (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing—original draft, Writing—review & editing), Dominic Russ (Data curation, Formal analysis, Methodology, Resources, Software, Visualization, Writing—original draft), Yongsan Yang (Data curation, Formal analysis, Methodology, Validation, Visualization, Writing—original draft, Writing—review & editing), Lutong Pu (Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing—original draft), Mengdi Yu (Data curation, Formal analysis, Investigation, Methodology, Writing—original draft), Jinquan Zhang (Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing—original draft), Jiajun Guo (Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing—review & editing), Yuanwei Xu (Data curation, Investigation, Methodology, Validation, Visualization, Writing—review & editing), Ke Wan (Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing—review & editing), Heng Xu (Conceptualization, Formal analysis, Methodology, Supervision, Writing—review & editing), Yuchi Han (Conceptualization, Methodology, Project administration, Supervision, Writing—review & editing), Georgios V. Gkoutos (Conceptualization, Project administration, Resources, Supervision, Validation, Writing—review & editing), and Yucheng Chen (Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing—review & editing)

Supplementary data

Supplementary data are available at *PCMEDI* online.

Conflict of interest

None declared. In addition, as an Editorial Board Member of *Precision Clinical Medicine*, the corresponding author G.V.G. was blinded from reviewing and making decisions on this manuscript.

Data availability

Data from the UKB (https://www.ukbiobank.ac.uk/use-our-data/apply-for-access/) are available to all researchers upon making an application. This research has been conducted using the UK Biobank Resource under application number 31224. In addition, data from the Chinese cohort are available under accession numbers GVM000832 and GVM000833 via the Genome Variation Map (GVM) at the National Genomics Data Center, Beijing Institute of Genomics, Chinese Academy of Sciences, and China National Center for Bioinformation (China). While restrictions apply due to licensing agreements, external researchers may request access through the corresponding author (Y.C.), subject to approval by the West China Hospital Ethics Committee and compliance with data transfer agreements.

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