**SUPPLEMENTAL MATERIAL**

**METHODS**

**Oxford clinical laboratory cohort**

Analysis of core sarcomeric genes (*MYBPC3*, *MYH7*, *TNNI3*, *TNNT2*, *MYL2*, *MYL3*, *ACTC1*, *TPM1)* was undertaken in 2,757 probands referred by inherited cardiac condition specialists to the Oxford Medical Genetics Laboratory (OMGL) for HCM genetic testing between May 2013 and December 2018. Self-identified ancestry data is not available for this cohort; however, prior analysis of this cohort has suggested >80% of probands are of European ancestry.[1,2] Target DNA sequences were enriched using a custom-designed HaloPlex kit (Agilent) before being sequenced on an Illumina MiSeq instrument. Sequence data were adapter-trimmed using Cutadapt[3]; in addition, 5 bp from each end of the reads were cropped to remove any restriction enzyme footprints, and short as well as low-quality reads were discarded using Trimmomatic[4]. Filtered reads were mapped on the human reference genome (hs37d5 assembly) using BWA-mem.[5] Samples were haplotype called and jointly genotyped with an in-house pipeline adapted from the GATK Best Practices[6], using GATK version 4.0.11.0 and Picard version 2.9.2.[7] All variants were confirmed by Sanger sequencing. Variants were assigned a pathogenicity classification in accordance with published guidelines.[8]

**Hypertrophic Cardiomyopathy Registry cohort (HCMR)**

Analysis of core sarcomeric genes (*MYBPC3*, *MYH7*, *TNNI3*, *TNNT2*, *MYL2*, *MYL3*, *ACTC1*, *TPM1)* was undertaken in 2,636 probands with a clinical diagnosis of HCM recruited into the HCMR project from 2013 to 2018. Clinical details and study design of the HCMR project have been previously reported.[9] Target DNA sequences were enriched using a custom-designed TruSeq kit (Illumina) before being sequenced on an Illumina MiSeq instrument. Target-specific

primers and Illumina adapters were removed from the sequence data using Cutadapt.[3] Short as well as low-quality reads were discarded using Trimmomatic.[4] Filtered reads were mapped on the human reference genome (hs37d5 assembly) using BWA-mem.[5] Samples were haplotype called and jointly genotyped with an in-house pipeline adapted from the GATK Best Practices[6], using GATK version 4.0.11.0 and Picard version 2.9.2[7]. Variants were visually confirmed through inspection of BAM files. Variants were assigned a pathogenicity classification in accordance with published guidelines.[8] The HCMR cohort underwent genome-wide genotyping using the Axiom Precision Research Array (Affymetrix). Genotypes first underwent linkage disequilibrium pruning using PLINK (--indep-pairwise 1000 50 0.05), before FlashPCA2[10] was used to project ancestry-informative principal components present within the 1000 Genomes phase 3 cohort onto the HCMR cohort[11]. A multinomial logistic regression model classified ancestral groups as per the International Genome Sample Resource (http://www.internationalgenome.org/category/population/).

**Reference control data**

Reference control genotype data for case-control analyses was obtained from the Genome Aggregation Database (gnomAD, v2.1.1)[12,13], the NHLBI Trans-Omics for Precision Medicine (TOPMed) Whole Genome Sequencing Program BRAVO variant browser[14,15], and the Bioresource for Rare Disease (BRRD) cohort.[16] Details relating to the BRRD cohort have been previously published.[16]

**Statistical analysis**

Statistical analyses were undertaken using R version 3.5.3. Continuous normally distributed variables are presented as mean ± standard deviation (SD). Fishers exact test (FET) and odds

ratio (OR) analyses were undertaken to compare the proportion of individuals heterozygous for the *MYBPC3*$^{\Delta 25}$ variant in South Asian individuals from the HCMR cohort and the Genome Aggregation Database (gnomAD, v2.1.1). 95% confidence intervals (CI) were included for effect size estimates. Binomial confidence intervals for variant proportions were calculated using the exact (Clopper-Pearson) method. Data from the HCMR South Asian cohort and the BRRD South Asian cohort was analysed with the R *elrm* function (https://cran.r-project.org/src/contrib/Archive/elrm/), a Monte Carlo Markov Chain algorithm that approximates exact conditional inference for logistic regression models.[17] This method was used to model the independent effects of the *MYBPC3*$^{\Delta 25}$ and *MYBPC3* c.1224-52G>A variants whilst indirectly allowing for linkage disequilibrium between the two variants through their associations with HCM risk. An exact Mantel-Haenszel test was employed, using the R mantelhaen.test function, to test the association between *MYBPC3* c.1224-52G>A and HCM, adjusted for *MYBPC3*$^{\Delta 25}$.

**Haplotype analysis**

Using gene panel sequence data specific to individuals of South Asian genetic ancestry from the HCMR cohort, haplotype analysis was conducted using Haploview (version 4.2). Genetic markers located within *MYBPC3*, specifically between positions 47353825 and 47364865 (GRCh37), minimum genotyping rate of 90%, Hardy-Weinberg p-value cut-off of 0.001 and a maximum of one Mendel error, were considered alongside *MYBPC3* c.1224-52G>A and *MYBPC3*$^{\Delta 25}$. Additionally, haplotype analysis using short tandem repeats that span ~4.6 Mb around *MYBPC3* was performed using PHASE (v2.1.1) in all individuals of South Asian ancestry from the HCMR cohort, and in all individuals in the HCMR and OMGL cohorts that carried the *MYBPC3*$^{\Delta 25}$ variant and / or the *MYBPC3* c.1224-52G>A variant.

**Investigating the pathogenicity of *MYBPC3* c.1224-52G>A**

**RNA analysis**

A combination of *in silico* tools (SpliceSiteFinder-like, MaxEntScan, NNSplice and Human Splicing Finder), designed to predict the impact of variants on 3' and 5' splice sites were consulted through the splicing module of Alamut® Visual 2.7.1. Total RNA was extracted from lymphocytes derived from peripheral blood of two affected individuals with the *MYBPC3* c.1224-52G>A variant and reverse-transcribed to cDNA using standard protocols. PCR was performed using primers targeted at exon 12 and exon 16 of the *MYBPC3* gene and electrophoresed on an agarose gel. PCR amplicons were gel-purified and Sanger sequenced.

| | | | Counts | |
|---|---|---|---|---|
| **Gene** | **HGVS.c** | **HGVS.p** | **OMGL (n=2,757)** | **HCMR (n=2,636)** |
| *MYBPC3* | c.1504C>T | p.Arg502Trp | 58 | 39 |
| *MYBPC3* | c.2373dup | p.Trp792ValfsTer41 | 11 | 45 |
| *MYBPC3* | c.772G>A | p.Glu258Lys | 23 | 40 |
| *MYBPC3* | c.1224-52G>A | - | 32 | 23 |
| *MYBPC3* | c.1624+4A>T | - | 19 | 16 |
| *MYBPC3* | c.1484G>A | p.Arg495Gln | 8 | 17 |
| *MYH7* | c.2389G>A | p.Ala797Thr | 12 | 16 |
| *MYBPC3* | c.1624G>C | p.Glu542Gln | 15 | 13 |
| *MYBPC3* | c.1928-2A>G | - | 7 | 14 |
| *MYBPC3* | c.3330+2T>G | - | NA | 13 |

**Supplementary Table 1. Most frequent pathogenic or likely pathogenic variants seen across OMGL and HCMR cohorts**

**A**

| | | MYBPC3 c.1224-52G>A | | |
|---|---|---|---|---|
| | | -52/-52 | -52/+ | +/+ |
| MYBPC3$^{\Delta 25}$ | Del/del | 0 | 0 | 0 |
| | Del/+ | 0 | 5 | 12 |
| | +/+ | 0 | 1 | 116 |

**B**

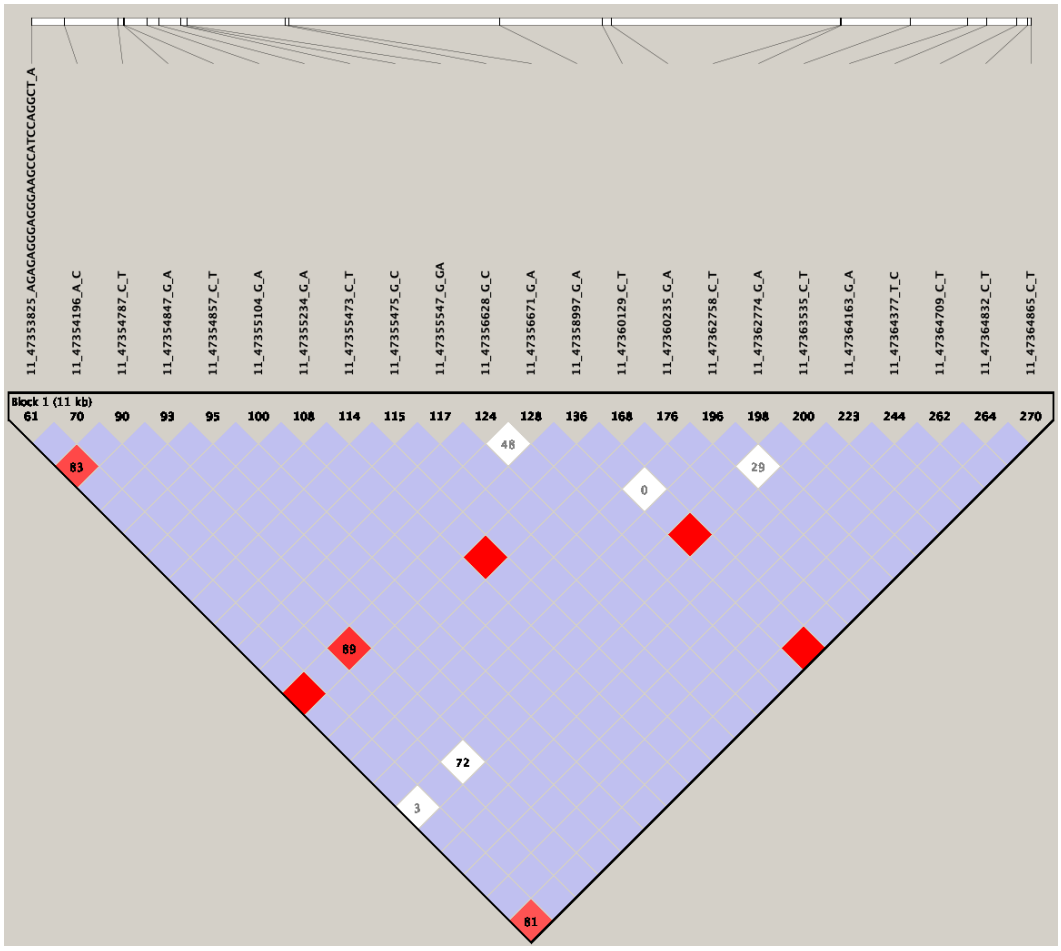
| Haplotypes | Haplotype frequencies | |
|---|---|---|
| Haplotypes MYBPC3$^{\Delta 25}$ - MYBPC3 c.1224-52G>A | LD model | Equilibrium model |
| Del/-52 | 0.018 | 0.001 |
| +/-52 | 0.004 | 0.021 |
| Del/+ | 0.045 | 0.062 |
| +/+ | 0.933 | 0.916 |

**C**

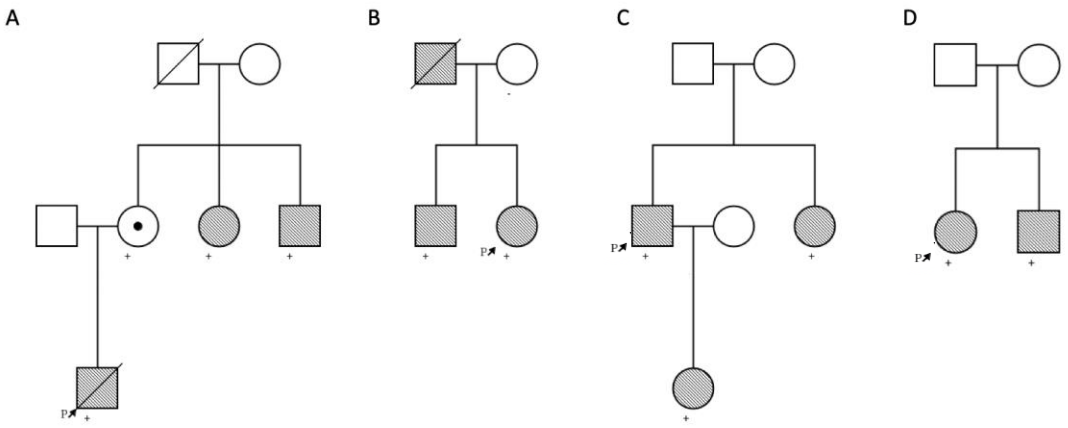
| | Predicted haplotype counts | |
|---|---|---|
| | MYBPC3 c.1224-52G>A | + |
| MYBPC3$^{\Delta 25}$ | 4.8 | 12.1 |
| + | 1.1 | 250.0 |

**Odds ratio measure of "allelic association" = 93.3**

**Supplementary Table 2. Haplotype analysis.** Panel A: Observed occurrences of *MYBPC3* c.1224-52G>A relative to *MYBPC3*$^{\Delta 25}$ within the HCMR South Asian cohort (n=134); Panel B: Reported haplotype frequencies under a linkage disequilibrium model; Panel C: Predicted haplotype counts given observed counts and linkage disequilibrium estimates between genetic markers. + indicates the presence of the wild-type (common, assumed ancestral) allele




**Supplementary Figure 1. Linkage disequilibrium plot for *MYBPC3*.** Generated using Haploview, red squares indicate high linkage disequilibrium (LD) between markers. Between marker 61 (MYBPC3$^{\Delta 25}$) and marker 270 (MYBPC3 c.1224-52G>A) there is evidence of high LD ($D' = 0.81$ and $r^2 = 0.22$).




**Supplementary Figure 2. Segregation analysis *MYBPC3* c.1224-52G>A variant.** Shaded squares represent males with a clinical diagnosis of HCM. Shaded circles represent females with a clinical diagnosis of HCM. White circle with a black dot represent a genotype +ve, phenotype negative female. Letter P and arrow identifies the Proband, defined as the first individual referred for genetic testing. + represents heterozygous for the *MYBPC3* c.1224-52G>A variant.

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