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RESEARCH LETTER

Promoter Deletion Confirms That MYBPC3 Haploinsufficiency Is Sufficient to Cause Hypertrophic Cardiomyopathy in Humans

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Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disorder of the heart muscle characterized clinically by cardiac hypertrophy and microscopically by myocyte disarray with interstitial fibrosis. It is most frequently caused by pathogenic variants in the *MYBPC3* gene, the majority of which are truncating (ie, nonsense, frameshift, or splice variants). This has led to the general hypothesis that haploinsufficiency of this gene is the pathogenic mechanism, and findings from studies of heterozygous human myocardium, such as decreased mutant to wild-type ratio of *MYBPC3* mRNA, reduction in cMyBP-C (cardiac myosin binding protein C), and nondetection of truncated protein products, support this.[1] However, these findings do not exclude the alternate hypothesis that truncated cMyBP-C products are required for HCM pathogenesis, for example, by leading to proteotoxicity or disruption of sarcomere structure/function, as nondetection of such proteins in human myocardium might be due to low quantity, transient expression, or inability of detection methods to recognize their misfolded structure.[2]

Perhaps, the most convincing evidence favoring the haploinsufficiency hypothesis is the knockout murine model described by Carrier et al.[3] Knockout mice heterozygous for an *Mybpc3* promoter deletion that specifically and completely ablates transcription of the gene developed a late-onset phenotype of asymmetrical septal hypertrophy associated with fibrosis. However, large-scale deletions of *MYBPC3* are a rare finding in human HCM probands, with only 11 (1–50 kb) described in the National Center for Biotechnology Information ClinVar archive to date. None of these deletions has significant cosegregation evidence to support pathogenicity. More notably, a deletion comparable to that of the murine model that results in specific and complete inactivation of 1 *MYBPC3* allele, with no possibility of a truncated product or proteotoxicity mechanism, has never previously been linked to HCM in humans. Here, we report on our copy number variant (CNV) analysis of the *MYBPC3* gene in probands with HCM and describe a deletion that fits this description.

We undertook genetic testing in 3870 HCM probands by targeted massively parallel sequencing of 16 to 22 genes. Testing was undertaken as part of routine clinical service; hence, proband consent was obtained by referring clinicians, and a research ethics review was not required. Standard sequencing methods, based on Haloplex target enrichment (Agilent) or Twist exome capture (Twist Bioscience) followed by Illumina sequencing, were used. CNVs were called by a custom-designed program that utilizes read-depth data to calculate a dosage quotient for each individual region of interest (or exon). Putative CNVs were confirmed or excluded by multiplex ligation–dependent probe amplification and by PCR and Sanger sequencing. Haplotype studies were undertaken by genotyping 12 markers spanning ≈4.6 Mb across the gene locus. All supporting data are provided in this article.

Large-scale *MYBPC3* deletions were confirmed in 18 probands (full details in Figure [A]). Among them

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Nonstandard Abbreviations and Acronyms

cMyBP-C	cardiac myosin binding protein C
CNV	copy number variant
HCM	hypertrophic cardiomyopathy

was a large deletion encompassing the entire *MYBPC3* gene and flanking genes (n=1) and deletions of exon 28 (n=1) and exons 23 to 26 (n=4). Our most pertinent finding was a founder variant: a deletion of the promoter and first 2 exons to codon 83 (p.Ala83; herein referred to as *MYBPC3*-Pr_02del; Figure [B]) detected in 12 probands of White British ancestry. This variant was found to be on a single haplotype that extends at least 1.95 Mb 5' to at least 2.63 Mb 3' of the gene. In cascade testing, this variant was cosegregated with HCM in a total of 8 affected relatives of 4 of the probands.

The *MYBPC3*-Pr_02del variant is remarkably similar to the aforementioned murine *Mybpc3* deletion, which extends from ≈0.8 kb 5' of the translation start site to the 88th codon (p.Ala88, second exon; equivalent to p.Ala80 in human cMyBP-C).[3] It is, therefore, expected to be functionally equivalent. Indeed, studies indicate that the sequence elements necessary for *MYBPC3* promoter activity are located <1.5 kb 5' of the translation start site, and deletion or mutation of these has been demonstrated to completely ablate *MYBPC3* expression in mouse and human cell lines.[4,5] Importantly, the *MYBPC3*-Pr_02del variant does not extend into neighboring genes or affect sequences known to influence their expression. Hence, although we were unable to obtain RNA to confirm, we think that it can be confidently assumed that this variant completely and specifically ablates in-cis expression of all *MYBPC3* gene products.

As far as we are aware, this is the first description of a relatively frequent, disease-causing, founder CNV in any cardiomyopathy gene. We think that it can be confidently classified as pathogenic according to the American College of Medical Genetics criteria whether by sequence variant guidelines or CNV guidelines (see Figure [A]). More importantly, we propose that this

A

Deletion:	*MYBPC3*-Pr_02del	*MYBPC3*-23_26del	*MYBPC3*-28del	*MYBPC3*-Alldel
HGVS (NM_000256.3):	c.-1497_248delinsTTGGCTTCTCCGCTCAGCT	c.2148+158_2737+258delinsT	c.(2905+1_2906-1)_(2994+1_2995-1)del	Not determined
Genomic Coordinates*:	g.47351283_47354144	g.47335619_47339166	g.47333922_47334010†	g.47308591 to g.47426279‡
No. Probands:	12	4	1	1
Shared Haplotype§:	>1.95 Mb 5' to >2.63 Mb 3'	>167 kb 5' to >677 kb 3'	Not determined	Not determined
Segregation‖:	8 (4)	1 (1)	0	0
ACMG Criteria (Seq)#:	PVS1, PS4(MOD), PM2, PP1(STR)	PVS1, PS4(SUP), PM2	PVS1, PM2	PVS1, PM2
ACMG Criteria (CNV)**:	1A: 0, 2C-1: 1.00, 3A: 0, 5D: 0.45	1A: 0, 2E: 0.90, 3A: 0	1A: 0, 2E: 0.90, 3A: 0	1A: 0, 2A: 1.00, 3A: 0
Classification:	Pathogenic	Pathogenic	Pathogenic	Pathogenic

B

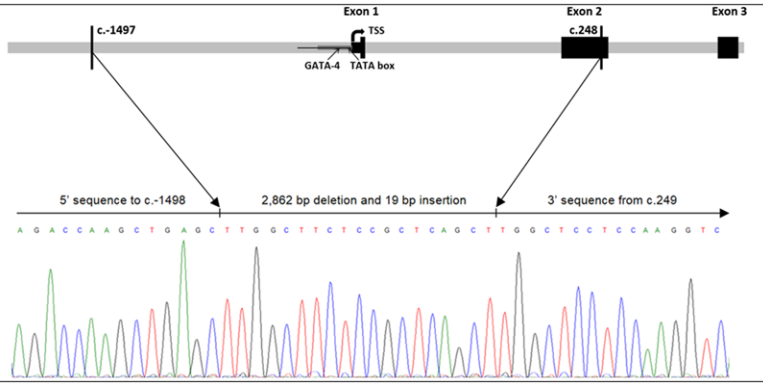

Figure. XXX.

A, Large-scale deletions detected in 3870 hypertrophic cardiomyopathy probands referred for genetic testing. *Genomic coordinates are stated according to Chr11 (GRCh38). †,‡These deletions were confirmed by multiplex ligation–dependent probe amplification; hence, the precise breakpoints have not been determined. These genomic coordinates indicate the first and last nucleotides of *MYBPC3* exon 28 or the multigene deletion called ExomeDepth. §Minimum extent of haplotype common to probands with the deletion, relative to first and last exons of *MYBPC3*. ‖Number of affected relatives in whom deletion has been detected (number of pedigrees). #Pathogenicity evidence criteria according to the American College of Medical Genetics (ACMG) 2015 sequence variant guidelines (PMID: 25741868). MOD, criteria applied at a moderate level. STR, criteria applied at a strong level. **Pathogenicity evidence scores according to ACMG 2020 copy number variant (CNV) guidelines (PMID: 31690835). **B**, **Top**, Diagram of the promoter region and first 3 exons of the *MYBPC3* gene, with the extent of the *MYBPC3*-Pr_02del variant delimited. The minimal promoter region (including the transcription start site, a TATA box motif, a GATA-4 site, and other transcription factor binding sites, deletion of which results in ablation of *MYBPC3* transcription) as previously defined is indicated by a thin black horizontal line[4] and a thicker dark gray line.[5] **Bottom**, Sanger sequence trace showing break-insertion point of the c.-1497_248delinsTTGGCTTCTCCGCTCAGCT (*MYBPC3*-Pr_02del) variant.

variant provides robust evidence that isolated *MYBPC3* haploinsufficiency is sufficient to cause HCM and, thereby, provides a solid basis for developing therapies that bolster compensation of normal cMyBP-C protein levels from the nonvariant allele in heterozygous individuals.

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