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South Asian-specific *MYBPC3*$^{\Delta 25bp}$ Intronic Deletion and its role in Cardiomyopathies and Heart Failure

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Distribution of the 25bp Intronic Deletion in *MYBPC3* in South Asian populations

In 2001, a 25bp deletion in intron 32 (*MYBPC3*$^{\Delta 25bp}$) was described in two unrelated South Asian (SA) families associated with hypertrophic cardiomyopathy (HCM) (Fig. A).[1, 2] In these reports, *MYBPC3*$^{\Delta 25bp}$ was shown to segregate in an autosomal dominant manner with highly variable penetrance. Interestingly, *MYBPC3*$^{\Delta 25bp}$ was detected in 16 out of 229 unrelated healthy SA individuals, suggesting its prevalence in southern India and a possible role as a regional genetic polymorphism and potential "genetic modifier." A new paper by Harper and colleagues describes the *MYBPC3* splicing variant, c.1224–52G>A, in HCM.[3]

Prior population-based studies examined *MYBPC3*$^{\Delta 25bp}$ frequency and clinical correlates. A large case-control cohort reported findings from 6,273 individuals belonging to 107 ethnic populations across 35 Indian states and 2,085 indigenous individuals of 63 ethnic/racial groups from 26 countries, including all five continents.[4] This study concluded that *MYBPC3*$^{\Delta 25bp}$ is a South Asian-specific common variant (3.8%) associated with a range of outcomes, including HF in the setting of multiple forms of cardiomyopathy, not simply HCM. Secondary cardiovascular risk factors, including metabolic syndrome, age, ventricular arrhythmia and other gene mutations were enriched in *MYBPC3*$^{\Delta 25bp}$ carriers.[4] An

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ancestry-based study using 23andMe data confirmed *MYBPC3*$^{\Delta 25bp}$ as enriched in those with self-described SA ancestry (4.5%) and its infrequency in those of European ancestry (<0.05%).[5] Consistent with these findings, Simonson et al. (2010)[6] and Bashyam et al. (2012)[7] reported similar prevalence of *MYBPC3*$^{\Delta 25bp}$ with those of SA ancestry of 8% and 6%, respectively. A more recent study of those of SA ancestry living in the U.S. determined a 6% frequency of *MYBPC3*$^{\Delta 25bp}$.[8] In aggregate, *MYBPC3*$^{\Delta 25bp}$ is estimated to be present in approximately 100 million SA descendants worldwide, including ~350,000 living in the U.S. As a risk allele, then, it is important to clarify the clinical correlates and coassociating factors.

Association of *MYBPC3*$^{\Delta 25bp}$ variant with cardiovascular disease

As a risk allele, *MYBPC3*$^{\Delta 25bp}$ is found in other cardiomyopathies, left ventricular hypertrophy (LVH) and chronic HF,[4] as well as hypertension, ventricular tachyarrhythmias and aging.[4] Three independent studies showed that carriers of *MYBPC3*$^{\Delta 25bp}$ had worse coronary heart disease outcomes compared to noncarriers.[9–11] The 2018 study of South Asians living in the USA (US-SAs) took a genotype-first view to assess clinical findings associated with *MYBPC3*$^{\Delta 25bp}$ by examining carriers with electrocardiograms and echocardiograms.[8] Mild shifts in LV function were noted in *MYBPC3*$^{\Delta 25bp}$ compared to controls. Approximately 10% of *MYBPC3*$^{\Delta 25bp}$ carriers had an additional variant, *MYBPC3*^{D389V}. *MYBPC3*^{D389V} was only observed in the presence of *MYBPC3*$^{\Delta 25bp}$, and familial evaluation demonstrated the coinheritance of the two variants, consistent with their presence on the same allele. Those individuals with both D389V and Δ25bp had the most abnormal findings by echocardiography, compared to noncarriers and Δ25bp carriers.[8]

Molecular consequences of *MYBPC3*$^{\Delta 25bp}$

Experimental evidence supports a mechanistic role for *MYBPC3*$^{\Delta 25bp}$. Its expression in neonatal cardiomyocytes demonstrated skipping of exon 33.[2] Strikingly, evaluation of expression in a *MYBPC3*$^{\Delta 25bp}$–positive human heart confirmed exon 33 skipping *in vivo*.[4] Exon 33 skipping results in the loss of 62 amino acids to produce modified C10 domain of cardiac myosin binding protein-C (cMyBP-C$^{\Delta C10mut}$). Furthermore, this variant shifts the stop codon to the 3' UTR and adds a novel 55 amino acids in the C10 domain at the carboxyl terminus. cMyBP-C interacts *via* its C10 domain with the myosin LMM region, which is essential for cMyBP-C adherence, localization and stabilization in the sarcomere. If the C10 domain is modified, or truncated, cMyBP-C will not localize in the sarcomere.[12, 13]

The presence of exon 33 skipping in human cardiac biopsy samples[4], even if only partial, may alter cardiac properties. The underlying molecular mechanism could be attributed to haploinsufficiency or poison polypeptide.[13, 14] In theory, cMyBP-C$^{\Delta C10mut}$ would not bind to myosin LMM, resulting in removal through the ubiquitin proteasome system. Alternatively, through nonsense-mediated mRNA decay, the mutant RNA could be removed, and no translation would occur to synthesize cMyBP-C$^{\Delta C10mut}$, causing haploinsufficiency. A poison polypeptide effect can occur if cMyBP-C$^{\Delta C10mut}$ binds actin and the myosin S2 region through its N'-terminal interactions in the sarcomere. To test these two hypotheses,

we recently overexpressed cMyBP-C$^{\Delta C10mut}$ in adult cardiomyocytes *in vitro* and confirmed that it mislocalized in the Z-line of the sarcomere, rendering it nonfunctional.[13]

To determine the *in vivo* consequence of cMyBP-C$^{\Delta C10mut}$, a cardiac-specific transgenic mouse model was generated in which cMyBP-C$^{\Delta C10mut}$ was expressed under the control of cardiac-specific α-myosin heavy chain promoter.[13] In the transgenic line, mRNA levels of both endogenous and mutant *MYBPC3* were robustly detected (20-fold). Mutant protein comprised 31.6% of the total cMyBP-C protein, implying instability of the mutant compared to the wild type. It was revealed that (i) the expression of cMyBP-C$^{\Delta C10mut}$ was pathogenic in the heart, causing an HCM phenotype by 12 weeks of age, (ii) cMyBP-C$^{\Delta C10mut}$ was mislocalized in the sarcomere and caused a poison polypeptide effect, and (iii) the C10 domain was critical for cMyBP-C stability and localization. These experimental modeling studies concluded that cMyBP-C$^{\Delta C10mut}$ protein is toxic and leads to a cascade of events beginning with improper incorporation in the sarcomere, followed by contractile dysfunction, and, finally, the induction of pro-hypertrophic signaling and cardiac remodeling.[13]

A novel MYBPC3 mutant, *MYBPC3* c.1224–52G>A, disrupts splicing

The Harper study evaluated the coinheritance of c.1224–52G>A (*MYBPC3*$^{-52}$), another intronic variant in *MYBPC3*, in the etiology of HCM.[3] Two large HCM cohorts were evaluated with a combined total of 5,394 HCM cases.[15] Overall, very few SAs were included in these HCM cohorts and only 17 SA *MYBPC3*$^{\Delta 25bp}$ carriers. Panel gene analysis was used to identify pathogenic, likely pathogenic or variants of uncertain significance in the HCM cohorts. The authors focused on a previously identified, potentially pathogenic variant, *MYBPC3*$^{-52}$, that arose from the introduction of a cryptic splice acceptor site in intron 13, which includes a 50 bp intronic sequence in the mRNA, causing a frameshift and premature termination at codon 438.[15] *MYBPC3*$^{-52}$ was identified in 11 of 38 *MYBPC3*$^{\Delta 25bp}$ carriers. Haplotype analysis identified strong linkage disequilibrium between *MYBPC3*$^{-52}$ and *MYBPC3*$^{\Delta 25bp}$, suggesting that *MYBPC3*$^{-52}$ could be driving the HCM risk associated with *MYBPC3*$^{\Delta 25bp}$. However, the *MYBPC3*$^{-52}$ variant was also found in the absence of *MYBPC3*$^{\Delta 25bp}$, indicating that these two variants are separable and that *MYBPC3*$^{-52}$ may impart risk on its own. Expression analysis using RNA from lymphocytes from two *MYBPC3*$^{-52}$ carriers documented aberrant splicing. Future studies using cardiac-derived RNA and protein expression will be helpful to discern pathogenic mechanisms. Since *MYBPC3*^{D389V} was not included in the haplotype analysis, the role of D389V in the context of the *MYBPC3*$^{-52}$ remains to be explored. Dissection of specific *MYBPC* haplotypes, including the D389V allele, may help define ancestral population structures and/or functional consequences of the *MYBPC3*$^{\Delta 25bp}$ allele and its risk.

This new work suggests a role for *MYBPC3*$^{\Delta 25bp}$ in HCM in the setting of primary mutations. It further highlights *MYBPC3*$^{\Delta 25bp}$ as a risk allele for HF and other adverse cardiovascular outcomes, as observed in the population studies.[4] As a risk allele, the co-occurrence with pathogenic alleles, or even conventional risk factors like metabolic syndrome, is expected to worsen outcomes and disease trajectory. For example, the presence of *MYBPC3*$^{\Delta 25bp}$ would likely intensify other HCM mutations or further promote LVH in

the setting of hypertension. Whether the presence of these additional disease factors actually promotes more abnormal splicing around *MYBPC3*$^{\Delta 25bp}$ requires experimental study.

Questions for future studies

South Asians have significantly greater risk for cardiovascular diseases,[16–18] often developing heart disease up to a decade earlier than other ethnic groups and often with greater mortality.[16–19] This increased risk is not solely explained by hypertension, diabetes, obesity, sedentary activity, smoking or diet. Instead, some unusually prevalent non-modifiable risk factors, such as elevated lipoprotein(a) and clotting factors, appear to be present.[18] Added to this array of inherited predispositions is *MYBPC3*$^{\Delta 25bp}$. Overall, *MYBPC3*$^{\Delta 25bp}$ is a discrete marker shown to define a population at substantially increased risk for pathological LVH and other cardiovascular diseases and, thus representing a unique opportunity to improve outcomes and define novel mechanisms of cardiovascular disease (Fig. B–C). However, future studies will be needed to tease out the complex genotype-phenotype correlations specific to South Asian descendants with the aim of dissecting *MYBPC3*$^{\Delta 25bp}$ risk factors using various tools, such as humanized mouse models, human induced pluripotent stem cells-derived cardiomyocytes and organoids.[20]

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Nonstandard Abbreviations and Acronyms

bp	base pair
cMyBP-C	Cardiac myosin binding protein-C protein
cMyBP-C$^{\Delta C10mut}$	Modified C10 domain of cardiac myosin binding protein-C
LVH	Left ventricular hypertrophy
MYBPC3	Cardiac myosin binding protein-C gene
MYBPC3$^{\Delta 25bp}$	25bp deletion mutation in intron 32 of *MYBPC3* gene
MYBPC3^{-52}	c.1224–52G>A in *MYBPC3* gene
HCM	Hypertrophic cardiomyopathy
HF	Heart failure

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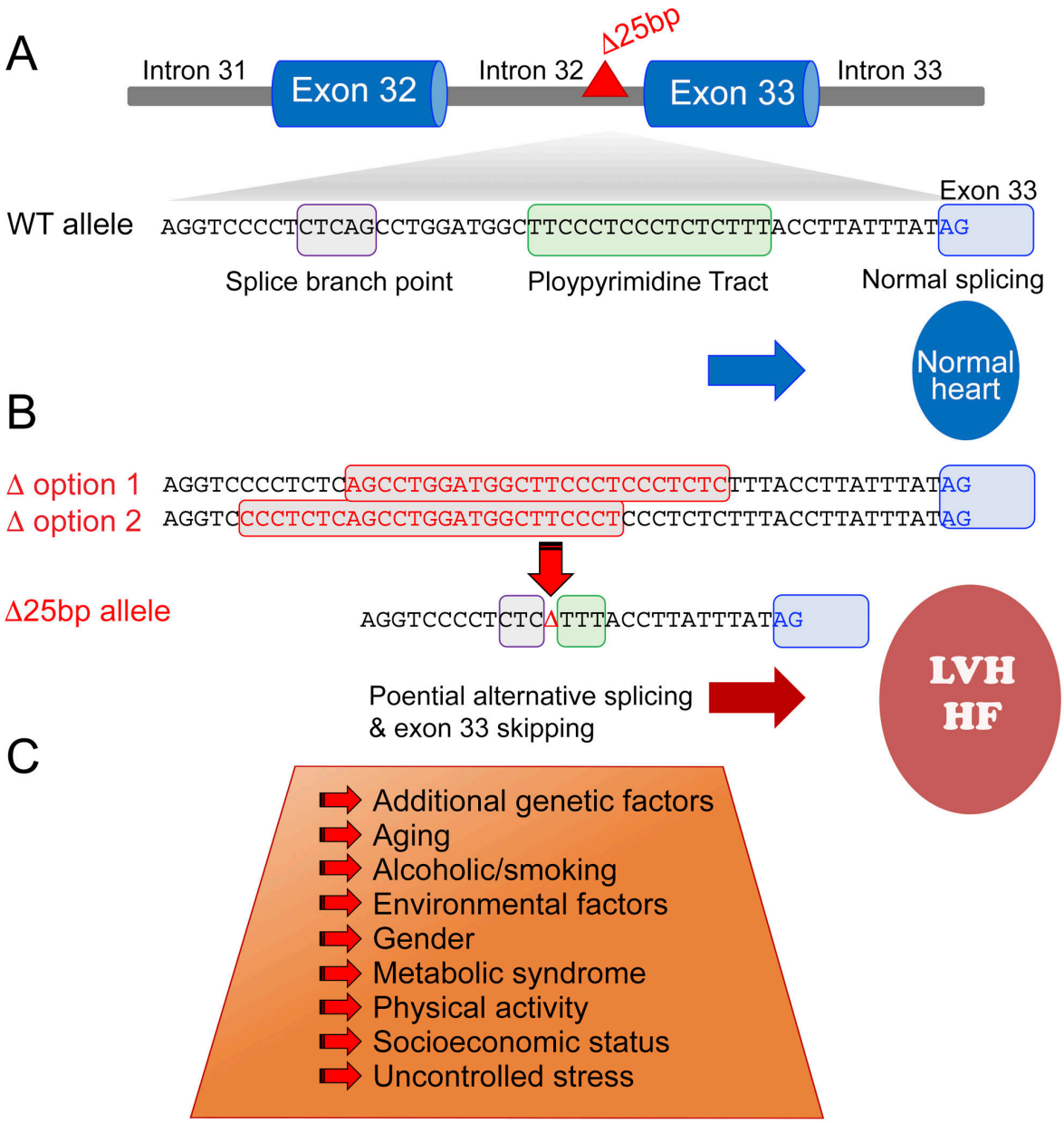

Figure. Genotype and potential contributing factors to *MYBPC3*$^{\Delta 25bp}$ pathogenicity.

(A) *MYBPC3*$^{\Delta 25bp}$ maps to intron 32 between the splice branch point and polypyrimidine track. In the absence of *MYBPC3*$^{\Delta 25bp}$, normal exon splicing occurs. (B) The presence of the *MYBPC3*$^{\Delta 25bp}$ variant causes one of two possible changes (red boxes) both resulting in the same partial deletion of the splice branch point and polypyrimidine track sequences. (C) The presence of additional risk factors may increase the potential for alternative splicing and exon 33 skipping leading to left ventricular hypertrophy (LVH) and heart failure (HF), a key focus of current and future work.