Population Sampling and *in vitro* Modeling of a 25bp Deletion in MYBPC3 Associated With Hypertrophic Cardiomyopathy

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Introduction

Hypertrophic cardiomyopathy (HCM) is a heart condition that results in left ventricular hypertrophy (muscle thickening) (Figure 1), difficulties with pumping blood, arrhythmias, and is the leading cause of sudden cardiac death (SCD) in young adults and athletes. The prevalence of HCM has been estimated to be as high as 1 in 500 within the general population [1]. However, due to cost and sensitivity problems, it is not routinely screened for in the United States.

The majority of mutations leading to HCM have been identified in sarcomeric proteins, mainly the cardiac isoform of myosin-binding protein C (*MYBPC3*). Of particular interest is a 25bp deletion (rs36212066) in intron 32 of *MYBPC3* (*MYBPC3*$^{\Delta 25}$), which has been associated with left ventricle dysfunction and the development of HCM. This 25bp intronic deletion results in a mis-splicing event and exclusion of exon 33 during translation (Figure 6). *MYBPC3*$^{\Delta 25}$ is known to have a high carrier frequency in South Asians (up to 6%) [2], but was previously unobserved in Europeans.

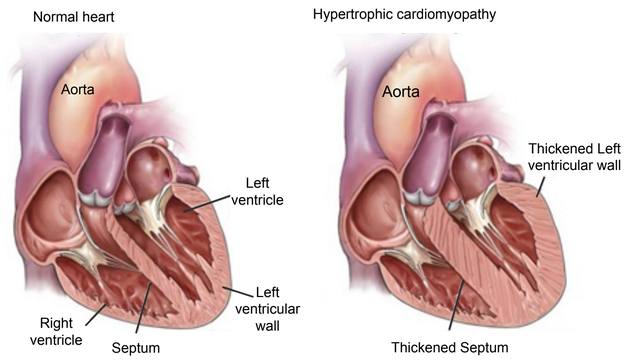

Figure 1. Characteristic features of hypertrophic cardiomyopathy consist of a thickened left ventricular wall and septum, and a reduced volume of left ventricular chamber. [3]

While *in vitro* models of HCM have been created in human induced pluripotent stem (iPS) cells, none have been made so far for *MYBPC3*. Such models can be used in conjunction with population phenotyping to help explain the pathology and incomplete penetrance of rs36212066 and may provide targets for pharmaceutical therapy.

Methods

23andMe developed a custom probe to assay rs36212066 on the latest iteration of its genotyping chip (V3).

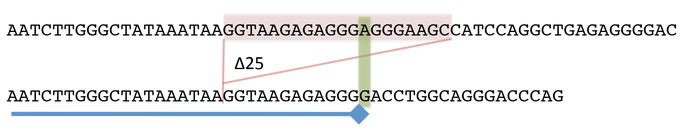

Figure 3. The deleted form leads to a G at the assay location (green), while the inserted (reference) form has an A. Probe orientation is indicated by the blue line.

A total of 110,751 customers were genotyped on the V3 chip containing these probes. The accuracy of our genotyping was validated through bi-directional Sanger sequencing; all homozygotes, 60 heterozygote carriers, and 60 controls were validated. All samples were concordant.

Results

Out of the 23andMe customer database, we detected 165 heterozygote carriers and 5 homozygotes. As expected, the carriers were predominantly individuals of South Asian ancestry and our observed allele frequency was consistent with previous studies conducted in India. Worldwide genotyping had previously observed the deletion in South and East Asia but not in Europe or Africa. Our data suggests that the deletion is present in Europeans as well, though at a low frequency that would have made it undetectable in earlier work.

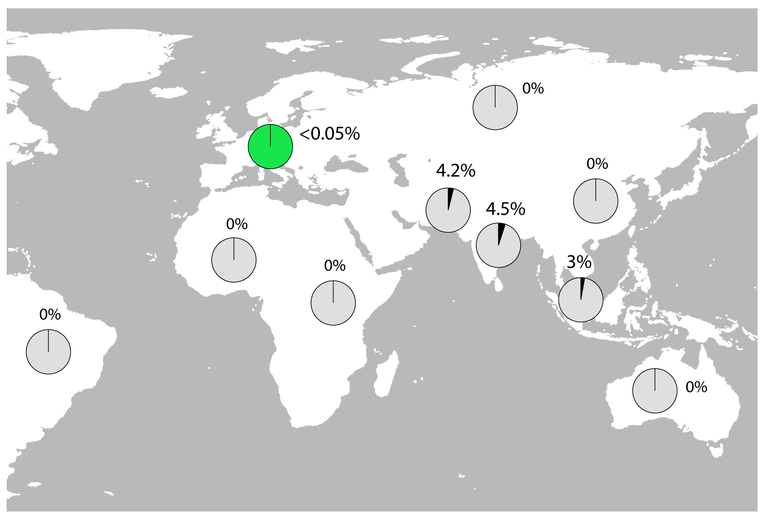

Figure 4. World map showing the observed distribution of rs36212066. Frequency data in Europe (green) is based on 23andMe customer data.

in vitro Modeling with iPS cells

Using Transcription Activator-Like Effector Nucleases (TALEN) technology, we have generated putative MYBPC3 knockout (K/O) iPS cell clones (Figure 5). These will be differentiated into beating cardiomyocytes and screened to establish a cellular *in vitro* HCM phenotype. The wild-type and mutant iPS cell lines will be differentiated into beating, functional cardiomyocytes. Phenotypic differences between the WT and mutant cardiomyocytes will be measured using various functional assays such as the IN Cell 2000 (cell size), Seahorse FX (mitochondrial function), and Orbi-trap Mass spectrometry (protein-protein interactions). Once a robust *in vitro* cellular phenotype has been established, a high-throughput drug screening platform will be established to attempt to revert the cellular "diseased" phenotype (Figure 6).

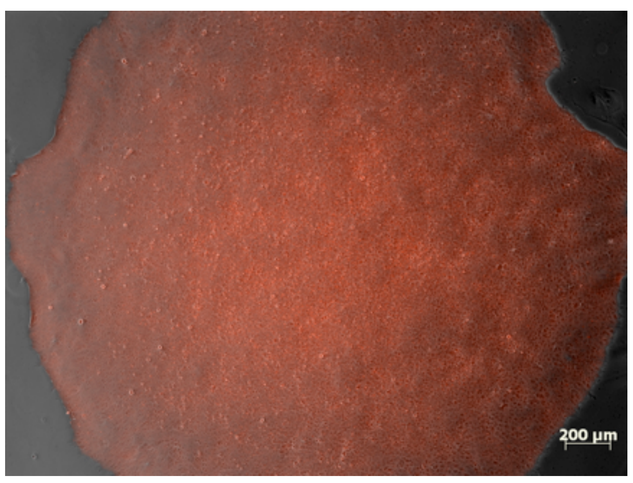

Figure 5. Putative MYBPC3 K/O iPS cell clone expressing mCherry as a marker to indicate appropriate on-site K/O targeting.

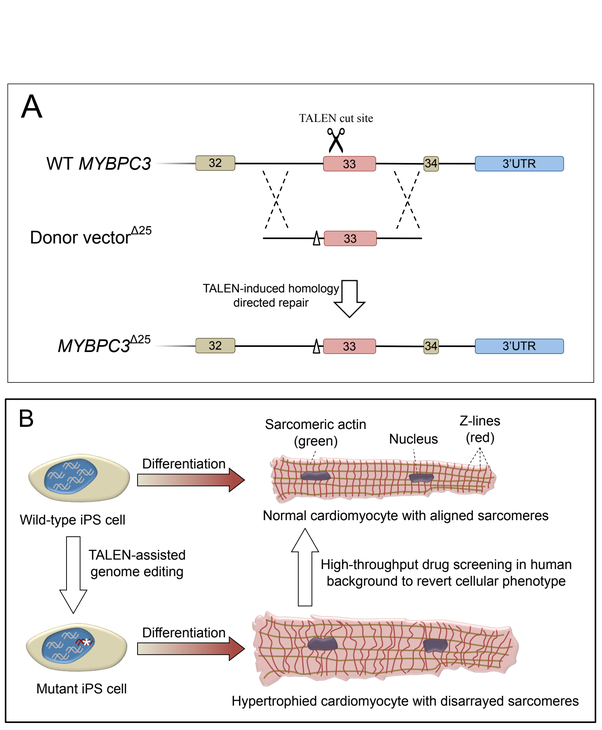

Figure 6. A) TALEN-induced homology directed repair will be used to create the *MYBPC3*$^{\Delta 25}$ mutation. B) Both WT and mutant cell lines will be differentiated into functional cardiomyocytes.

Discussion

Using the 23andMe database, we were able to detect rs36212066 in Europeans, a population in which it had not been previously observed. We also returned results to participants using an HCM report on the 23andMe genome service [4].

It is possible that the European carriers acquired it through recent South Asian admixture. To rule this mechanism out, we are in the process of phasing and haplotype analysis using 23andMe's ancestry painting algorithm.

Due to the lack of widespread screening for HCM, self-reported status for the condition is unlikely to be an effective proxy for phenotyping the carriers in the 23andMe customer database. Follow-up echocardiograms in consultation with physicians may be an effective way to replicate this association in a cohort of South Asians living in the United States.

The HCM status of the homozygotes is of particular interest, as they are hypothesized to have a more severe phenotype. To date, the cardiomyopathy status of living homozygotes for rs36212066 has not been studied.

Acknowledgments

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References and Resources

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