Supplemental Data

Results

The details of the pairwise gene expression change comparison of the major genotypic HCM subgroups are summarized in **Supplemental Figure 1 and Supplemental Table 1A-C**. In brief, 1502 genes (4% of transcriptome) were expressed differentially comparing the 17 MYH7+ cases with the 23 MYBPC3+ cases; 2163 genes (6% of transcriptome) when comparing the 17 MYH7+ cases and the 48 genotype negative-HCM cases, and 2336 (6% of transcriptome) genes when comparing the 23 MYBPC3+ cases and the 48 genotype negative-HCM cases (**Supplemental Figure 1)**. The up- and down-regulated genes for each of these subset analyses meeting an absolute and potentially biologically relevant fold-change > 1.5 are summarized in **Supplemental Tables 1A-C**.

Compared to the comparison between HCM and normal hearts, much smaller fold changes of expression were observed in these intra-disease subset analyses. In the MYH7+ versus MYBPC3+ comparison, the maximum absolute fold change was 1.61 and only 2 transcripts (*APOA1* and *HS.131412*) exhibited an absolute fold change > 1.5. For the MYH7+ and genotype negative-HCM comparison, the maximum absolute fold change was 2.10 and only 13 transcripts exhibited an absolute fold change > 1.5. For the MYBPC3+ and genotype negative-HCM comparison, the maximum absolute fold change was 1.76 and only 6 transcripts (*CENPA, FGF12, HS.390250, HBA2, F3,* and *HBB*) exhibited a fold change > 1.5 (**Supplemental Tables 1A-C**).

Discussion

Genotype Subgroup Analysis

Among our cases, 16% were caused by pathogenic/likely pathogenic variants in *MYH7* mutations, 22% of our HCM cases were caused by pathogenic/likely pathogenic variants in *MYBPC3*, and 45% of our HCM cases were unexplained genetically (genotype negative). Subset analyses showed those genes demonstrating an absolute difference in fold change > 1.5. The MYH7+ to MYBPC3+ comparison revealed only two genes. One was the major protein component of high density lipoprotein,[29] *APOA1* (1.51-fold in MYH7+), and one was an uncharacterized gene, *Hs.131412* (down 1.61-fold in MYH7+). While not directly associated with any known pro-hypertrophic pathway, *APOA1* protein could potentially have a heretofore undefined role in HCM; however, literature on a potential link is lacking. Given that 99.93% of genes in the MYH7+ versus MYBPC3+ comparison had a false discovery rate q-value >0.9, it is more likely to conclude that these two genetic subtypes of HCM are nearly indistinguishable, at the time of surgical septal myectomy, at least at the transcriptome level.

Similar observations were made comparing MYH7+ to the genotype negative-HCM subset as well as the MYBPC3+ vs genotype negative-HCM subset suggesting that pathophysiological differences between subtypes are likely subtle. For example, for the MHY7 versus genotype negative subset, there were two genes with potential theoretical relationships to a myocardial disease process. The first of these genes was *CORIN*, encoding a protein that produces biologically active atrial natriuretic peptide[35, 36], which was down-regulated 1.74-fold in MYH7+. The second was *COL3A1*, encoding type III collagen[37], up-regulated 1.53-fold in MYH7+, with possible relationship to the interstitial fibrosis observed in HCM. Other differentially expressed genes were related to glomerular injury[30] (*Thy1*), neurite outgrowth[31] (*SLITRK4*), lipoprotein catabolism[29] (*APOA1* and *APOE*), ketone body regulation[32] (*HMGCS2*), and hemoglobin[33] (*HBA2* and *HBB*).

Potential Role of Down-Regulated Genes

The most the down-regulated gene in our analysis was *SERPINA3* (down 11.8-fold in HCM compared to controls; validated by qRT-PCR). *SERPINA3* encodes a serine protease inhibitor that has been shown to have anti-inflammatory and anti-hypertrophic effects by blocking WNT signaling.[31] It normally functions to promote phosphorylation and degradation of beta-catenin, thus preventing the transcription of pro-inflammatory and pro-hypertrophic factors. The down-regulation of *SERPINA3A* in HCM suggests a subsequent upregulation of beta-catenin (up 1.1-fold in HCM compared to controls) and its associated pro-hypertrophic transcription factors, which could theoretically lead to pathological hypertrophy. Akin to *SERPINA3A*, we observed a down-regulation of *SERPINE1 (*4.1-fold in HCM compared to controls; validated by qRT-PCR). The gene encodes the protein plasminogen activator inhibitor 1 (PAI-1), an inhibitor of fibrinolysis thought to protect against vascular permeability and fibrosis**.**[32] Down-regulation of this gene could promote vascular permeability, thus facilitating the infiltration of macrophages and other inflammatory mediators into the myocardial interstitial cells. This, in turn, could result in fibrosis, a hallmark microscopic feature of HCM, and a compensatory hypertrophic response. Other genes potentially worth further study were *RASL11B*, up-regulated 3.3-fold in HCM and thought to be related to RAS proteins which have established roles in hypertrophy,[33] and *SMOC2*, up-regulated 2.8-fold in HCM and thought to potentiate the effect of growth factors and to activate matrix metalloproteinases.[34]

Supplemental Table 1: Differentially expressed genes in HCM, genotype subset comparisons

A. MYH7+ versus MYBPC3+

Gene	Official Full Name	Fold Change	Q-Value	P-Value	GO Biological Process Term(s)	GO Molecular Function Term(s)
APOA1	Apolipoprotein A-I	+1.51	1.00	4.98×10^{-2}	Lipid metabolic process	Cholesterol transport activity
HS.131412	Not available	-1.61	1.00	3.63×10^{-2}	Not available	Not available

Supplemental Table 1: Differentially expressed genes in HCM, genotype subset comparisons

B. MYH7+ versus Genotype-Negative HCM

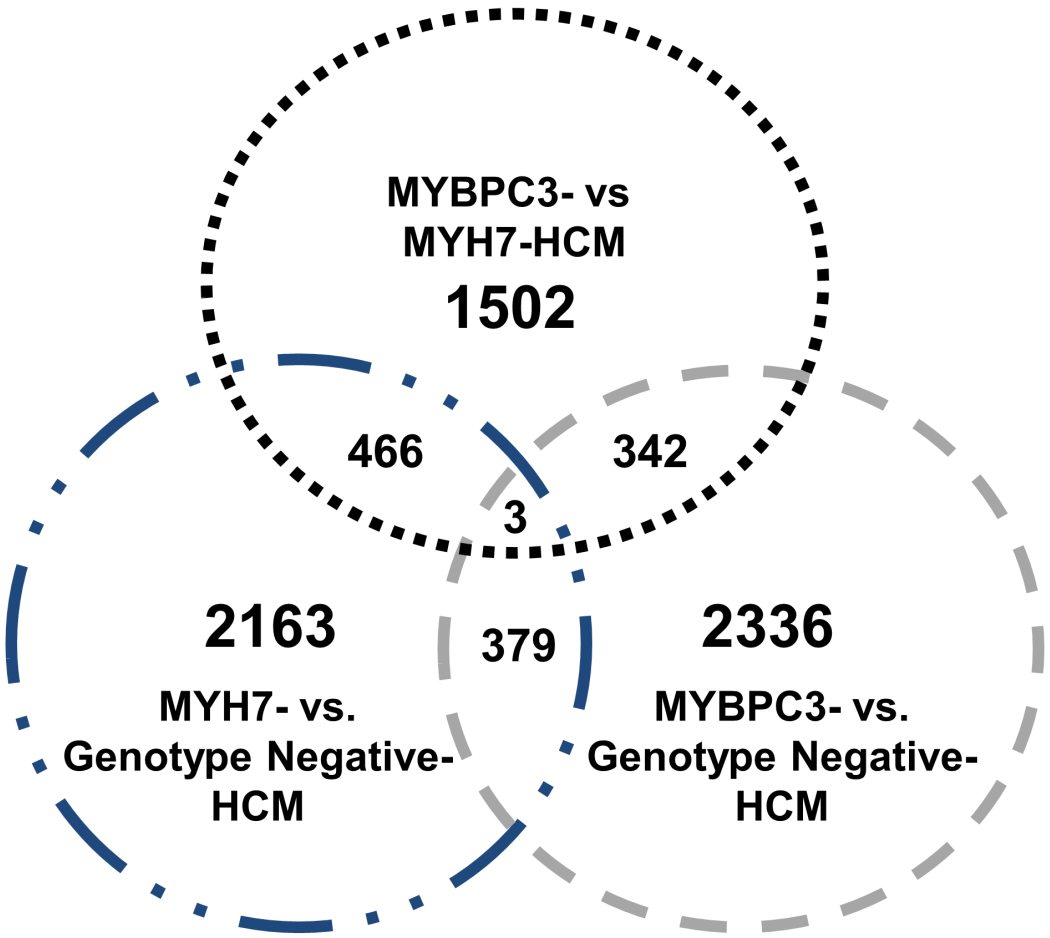
Gene	Official Full Name	Fold Change	Q-Value	P-Value	GO Biological Process Term(s)	GO Molecular Function Term(s)
THY1	Thy-1 cell surface antigen	+1.68	0.39	$1.47x10^{-4}$	Positive regulation of release of sequestered calcium ion into cytosol; angiogenesis	Enzyme binding; GPI anchor binding; Rho GTPase activator activity
SLITRK4	SLIT and NTRK-like family, member 4	+1.61	0.71	$8.43x10^{-3}$	Axonogenesis	Not available
HS.131412	Not available	+1.59	0.81	$2.20\text{-}x10^{-2}$	Not available	Not available
APOE	Apolipoprotein E	+1.53	0.58	$2.13x10^{-3}$	Cholesterol homeostasis; cellular calcium ion homeostasis; response to growth factor stimulus	Cholesterol transporter
COL3A1	Collagen, type III, alpha 1	+1.53	0.51	$8.02x10^{-4}$	Extracellular matrix structural constituent; transforming growth factor beta receptor signaling pathway	Extracellular matrix structural constituent; protein binding
HMGCS2	3-Hydroxy-3-methylglutaryl-CoA synthase 2	-2.10	0.63	$4.10x10^{-3}$	Isoprenoid biosynthetic process	Hydroxymethylglutaryl-CoA synthase activity
HBA2	Hemoglobin, alpha 2	-1.92	0.46	$3.75x10^{-4}$	Transport	Oxygen transporter activity
HS.390250	Fibroblast growth factor 12	-1.90	0.75	$1.19x10^{-2}$	Signal transduction; heart development	Growth factor activity

HBB	Hemoglobin, beta	-1.85	0.51	9.06×10^{-4}	Oxygen transport	Oxygen transport activity
CORIN	Corin, serine peptidase	-1.74	0.80	2.09×10^{-2}	Regulation of systemic arterial blood pressure by atrial natriuretic peptide; peptide hormone processing	Peptidase activity; scavenger receptor activity
FGF12	Fibroblast growth factor 12	-1.63	0.75	1.28×10^{-2}	Signal transduction; heart development	Growth factor activity
LOC644322	Similar to Ribosome biogenesis protein BMS1 homolog	-1.53	0.33	8.02×10^{-5}	Not available	Not available
APOA1	Apolipoprotein A-I	-1.51	0.83	2.62×10^{-2}	Lipid metabolic process	Cholesterol transport activity

Supplemental Table 1: Differentially expressed genes in HCM, genotype subset comparisons

C. MYBPC3+ versus Genotype-Negative HCM

Gene	Official Full Name	Fold Change	Q-Value	P-Value	Selected GO Biological Process Term(s)	GO Molecular Function Term(s)
CENPA	Centromere protein A	+1.76	0.65	2.93×10^{-3}	Nucleosome assembly	Chromatin binding; DNA binding; protein binding
FGF12	Fibroblast growth factor 12	-1.67	0.65	3.57×10^{-3}	Signal transduction; heart development	Growth factor activity
HS.390250	Fibroblast growth factor 12	-1.67	0.73	2.45×10^{-2}	Signal transduction; heart development	Growth factor activity
HBA2	Hemoglobin, alpha 2	-1.61	0.65	3.46×10^{-3}	Transport	Oxygen transporter activity
F3	Coagulation factor III (thromboplastin, tissue factor)	-1.53	0.34	8.53×10^{-5}	Blood coagulation; positive regulation of platelet-derived growth factor receptor signaling pathway	Cell surface binding; phospholipid binding; protease binding
HBB	Hemoglobin, beta	-1.50	0.70	1.33×10^{-2}	Oxygen transport	Oxygen transport activity



Supplemental Figure 1. Venn diagram depicting the number of genes that were expressed differentially among the major genetic subgroups of HCM: myosin binding protein C-HCM (MYBPC3-HCM), beta myosin heavy chain (MYH7-HCM), and Genotype Negative-HCM. The number of differentially expressed genes in the comparison between MYBPC3- and MYH7-HCM (1502 genes, or 4% of the transcriptome), MYBPC3- and Genotype Negative-HCM (2336 genes, or 6% of the transcriptome), and MYH7-HCM and Genotype Negative-HCM (2163 genes, or 6% of the transcriptome) are shown. Overlapping numbers of differentially expressed genes between each of the pairwise comparisons and among all three pairwise comparisons are also shown.