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PII: S0025-6196(20)30398-0

DOI: https://doi.org/10.1016/j.mayocp.2020.04.028

Reference: JMCP 2878

To appear in: JMCP: Mayo Clinic Proceedings

Received Date: 13 April 2020
Revised Date: 21 April 2020
Accepted Date: 23 April 2020


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Marked Up-Regulation of ACE2 in Hearts of Patients with Obstructive Hypertrophic Cardiomyopathy: Implications for SARS-CoV-2-Mediated COVID-19

J. Martijn Bos, MD, PhD¹*, Virginia B. Hebl, MD, MS²*, Ann L. Oberg, PhD³, Zhifu Sun, MD, MS³, Daniel S. Herman, PhD⁴, Polakit Teekakirikul, MD⁵*, J. G. Seidman, PhD⁶, Christine E. Seidman, MD⁴, J. G. Maleszewski, MD, Hartzell V. Schaff, MD⁷, Joseph A. Dearani, MD⁸, Peter A. Noseworthy, MD², Paul A. Friedman, MD², Steve R. Ommen, MD², Frank V. Brozovich, MD, PhD², and Michael J. Ackerman, MD, PhD¹,²,¹⁰

¹) Department of Molecular Pharmacology & Experimental Therapeutics, Windland Smith Rice Sudden Death Genomics Laboratory, Mayo Clinic, Rochester, MN, USA.
²) Department of Cardiovascular Medicine, Mayo Clinic, Rochester, MN, USA.
³) Department of Health Sciences Research, Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN, USA.
4) Department of Genetics, Harvard Medical School, Boston, MA, USA.
5) Cardiovascular Division, Brigham and Women’s Hospital, Boston, MA, USA.
6) Howard Hughes Medical Institute, Chevy Chase, MD, USA.
7) Mechanobiology Laboratory, Victor Chang Cardiac Research Institute, Darlinghurst, Australia.
8) Department of Laboratory Medicine & Pathology, Mayo Clinic, Rochester, MN, USA.
9) Department of Cardiovascular Surgery, Mayo Clinic, Rochester, MN, USA.
10) Department of Pediatric and Adolescent Medicine/Division of Pediatric Cardiology, Windland Smith Rice Genetic Heart Rhythm Clinic, Mayo Clinic, Rochester, MN, USA.

* JMB and VBH contributed equally to this manuscript and should be considered co-equal first authors.

Corresponding author: Michael J. Ackerman, MD, PhD, Director, Mayo Clinic’s Windland Smith Rice Genetic Heart Rhythm Clinic and Windland Smith Rice Sudden Death Genomics Laboratory, Mayo Clinic, Guggenheim 501, 200 First St SW, Rochester, MN 55905, Fax: 507.284.3757; Phone: 507.284.0101 (ackerman.michael@mayo.edu).
@MJAckermanMDPhD
Key words: ACE2, Cardiomyopathy, COVID-19, Gene expression, Hypertrophic cardiomyopathy, Hypertrophy, Myocardial cardiomyopathy disease, SARS-CoV-2

ABBREVIATIONS

ACE2 Angiotensin converting enzyme 2
CHF Congestive heart failure
COVID Corona virus infectious disease
HCM Hypertrophic cardiomyopathy
HTN Hypertension
MYBPC3 Myosin binding protein C
MYH7 Beta myosin heavy chain
qRT-PCR quantitative real time polymerase chain reaction
SARS-CoV-2 Severe acute respiratory syndrome corona virus 2
SCD Sudden cardiac death
ABSTRACT

Objective: To explore the transcriptomic differences between patients with hypertrophic cardiomyopathy (HCM) and controls.

Patients and Methods: RNA was extracted from cardiac tissue flash frozen at therapeutic surgical septal myectomy for 106 patients with HCM and from 39 healthy donor hearts. Expression profiling of 37,846 genes was performed using the Illumina Human HT-12v3 Expression BeadChip. All HCM patients were genotyped for pathogenic variants causing HCM. Technical validation was performed using quantitative real-time PCR (qRT-PCR) and Western blot. This study was started on January 1, 1999 and final analysis was completed on April 20, 2020.

Results: Overall, 22% of the transcriptome (8443 genes) was expressed differentially between HCM and control tissues. Analysis by genotype revealed that gene expression changes were similar among genotypic subgroups of HCM, with only 4-6% of the transcriptome exhibiting differential expression between genotypic subgroups. qRT-PCR confirmed differential expression in 92% of tested transcripts. Notably, in the context of COVID-19, the transcript for ACE2, a negative regulator of the angiotensin system, was the single most up-regulated gene in HCM (fold-change 3.53, q-value=1.30x10^{-23}), which was confirmed with qRT-PCR in triplicate (fold-change 3.78; p=5.22x10^{-4}), and Western blot confirmed a >5-fold over-expression of ACE2 protein (fold-change 5.34, p=1.66x10^{-6}).

Conclusions: Over 20% of the transcriptome is expressed differentially between HCM and control tissues. Importantly, ACE2 was the most up-regulated gene in HCM indicating perhaps the heart’s compensatory effort to mount an anti-hypertrophic, anti-fibrotic response. However, given that the SARS-CoV-2 uses ACE2 for viral entry, this 5-fold increase in ACE2 protein may
confer increased risk for COVID-19 manifestations and outcomes in patients with increased ACE2 transcript expression and protein levels in the heart.

BACKGROUND

Hypertrophic cardiomyopathy (HCM) affects approximately 1 in 500 individuals\textsuperscript{1} and is among the leading causes of identifiable sudden cardiac death (SCD) in the young.\textsuperscript{2} HCM is often a genetic disease, typically with autosomal dominant inheritance, that is defined clinically as cardiac hypertrophy without physiologic explanation. Hundreds of pathogenic variants in many HCM-susceptibility genes have been identified, most of which encode components of the sarcomere.\textsuperscript{3-11} However, genetic tests are negative in approximately 50% of all unrelated patients with HCM that is diagnosed by clinical studies.\textsuperscript{4} Additionally, the transcriptional changes that cause and result from HCM, with and without pathogenic variants, remains largely unknown as prior studies analyzed data from small numbers of patients.\textsuperscript{5, 6} To better identify common transcriptional changes that represent fundamental, and heretofore unrecognized, pathogenic responses of human HCM, we performed transcriptome analysis of human HCM tissues.

METHODS

We designed a case-control study to identify the mRNAs differentially expressed in HCM-affected myocardium versus control myocardium. All patients signed informed consent, and protocols were approved by Mayo Clinic’s Institutional Review Board or the Human Research Ethics Committee of the University of Sydney. This study was started on January 1, 1999 and final analysis was completed on April 20, 2020.

Subjects

All patients undergoing therapeutic surgical septal myectomy for symptomatic relief of obstructive HCM between January 1, 1999 and December 31, 2010 were eligible for inclusion in
this study. The diagnosis of HCM was made by an experienced cardiologist from Mayo Clinic’s HCM Clinic based on physical exam, ECG, and echocardiographic/cardiac MRI findings. Diagnosis was corroborated by histologic examination of the patient’s surgical septal myectomy specimen. A representative portion of myectomy specimen was flash frozen at the time of excision and subsequently stored at -80°C. Data on patient age, sex, age at diagnosis, New York Heart Association (NYHA) classification, blood pressure, heart rate, family history of HCM, and family history of SCD were extracted from each patient’s electronic medical record. Echocardiographic parameters were extracted from each patient’s pre-operative echocardiography study. Degree of endocardial and interstitial fibrosis was assessed semi-quantitatively, at the time of resection, by a cardiovascular pathologist (JJM).

A cohort of control tissue was procured from the University of Sydney consisting of donor hearts for which there was not a suitable transplant recipient. A normal phenotype had been confirmed by cardiac examination, ECG, and echocardiogram obtained within 24 hours prior to explantation.

**DNA Extraction**

DNA was extracted from the HCM myectomy and control donor heart tissues using the Qiagen PureGene DNA Purification Kit (Qiagen, Inc.) according to the manufacturer’s protocol. Briefly, cells were lysed with detergent, RNA was removed using an RNase enzyme, proteins were removed by salt precipitation, and DNA was recovered with alcohol precipitation.

**Genotyping**

Damaging variants in 10 genes implicated in sarcomeric HCM (*ACTC1, MYBPC3, MYH6, MYH7, MYL2, MYL3, TNNC1, TNNI3, TNNT2, TPM1*) and 3 genes known to mimic HCM (*GLA, LAMP2, PRKAG2*) were studied using filter-based hybridization capture, as described
previously. In brief, genomic DNA extracted from the cardiac myectomy tissue was used to construct barcoded (3 base pairs) genomic DNA libraries. Ten or 20 barcoded genomic DNA libraries were then combined into each sample pool. DNA concatemers consisting of target gene segments were amplified and bound to 25 mm nitrocellulose membrane filters (Millipore). Sample genomic library pools were enriched by hybridization to filter-bound DNA concatemers and subjected to either single-end or paired-end sequencing using either Genome Analyzer II (Illumina) or HiSeq (Illumina). Sequences were aligned with Novoalign (Novocraft Technologies) and analyzed using The Genome Analysis Toolkit (GATK, Broad Institute).

**Tissue RNA Extraction and Quality Assessment**

Total RNA was extracted from each tissue sample using the Qiagen miRNeasy Kit (Qiagen, Inc.) according to the manufacturer’s protocol. The technique utilizes a phenol/chloroform extraction protocol and RNA purification columns. RNA quality was assessed by the Mayo Clinic Advanced Genomic Technology Center (AGTC) Microarray Shared Resource (MSR) using the 2100 Bioanalyzer (Agilent) to obtain electropherograms from which an RNA integrity number (RIN) was calculated. Only RNA samples with high enough quality (RIN ≥ 6.0) were used for transcriptome analyses.

**Microarray Hybridization**

Complementary DNA (cDNA) corresponding to case and control mRNA was produced by reverse transcription. cDNA was converted to biotin-labeled complementary RNA (cRNA) and hybridized to the Human HT-12 v3 Expression BeadChip (Illumina) to quantify the expression level of 48,804 mRNA transcripts representing 37,846 genes by streptavidin-Cy3 staining and laser excitation fluoroscopy. HCM and donor samples were randomized to ensure the groups were balanced over batches (BeadChips) to avoid potential confounding from batches.
Laboratory quality control measures included simultaneously hybridizing housekeeping controls, hybridization controls, negative controls, and technical replicates.

**Quantitative Real-Time PCR and Western Blot Validation**

Quantitative real-time PCR (qRT-PCR) was performed on randomly selected samples from each comparison group (18 HCM cases and 13 controls) to validate some of the microarray results. cDNA was generated from each RNA sample using the iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer’s protocol. TaqMan Gene Expression Assays (Applied Biosystems) for the genes of interest and the endogenous control GAPDH were used per the manufacturer’s protocol, and were run on the ABI Prism 7900HT Real Time System (Applied Biosystems). Each reaction was run in triplicate. Protein expression of ACE2 was confirmed by Western blot analysis with loading normalized for actin.

**Immunohistochemistry**

The tissue sections were de-paraffinized in xylene, dipped in decreasing concentrations of ethyl alcohol, and then rehydrated in distilled water. Antigen retrieval for ACE2 was performed by placing slides in preheated Citrate as the retrieval solution in a steamer at 98°C for 40 minutes. The staining procedure was carried out in the Dako Autostainer Plus as follows. The tissue sections were treated with Peroxidase Blocking Reagent (Dako) for 15 minutes, washed with 1x Wash Buffer (Dako), and treated with Protein Block SNIPER (Biocare Medical LLC) for 10 minutes. The primary antibody for ACE2 (R&D Systems) was diluted 1:400 in Background Reducing Diluent (Dako) and incubated for 60 minutes at room temperature. After rinsing with wash buffer, the sections were incubated in secondary antibody and tertiary reagent from the Goat HRP Kit (Biocare Medical LLC) for 15 minutes. Betazoid diaminobenzidine chromogenic substrate system (Biocare Medical LLC) was used for colorimetric visualization. Counter
staining with hematoxylin followed by dehydration in increasing concentrations of ethyl alcohol
and xylene were performed prior to permanent coverslipping. All slides were graded by a cardiac
pathologist (JJM), and graded for intensity (0 = no staining, 1+ = dot-like sarcoplasmic staining,
2+=diffuse sarcoplasmic staining) and distribution (scoring 0 = 0% of cells, 1 = < 25%, 2 = 26-
75% and 3 ≥ 75% of cells) of ACE2 staining.

**Analytical and Statistical Methods**

Specimens were allocated randomly to arrays using randomized block methods in order to avoid
confounding of biological and experimental effects. Illumina BeadStudio Version 3.1.3 with
gene expression module 3.4 was used to process raw data without background correction and
normalization. The gene level expression data was exported and analyzed in R (http://www.r-
project.org/). Briefly, the un-normalized raw data was first log2 transformed and evaluated for
potential outlier samples and bead chip effects by graphic and dimension reduction approaches
(density plot, M-A plot, and principal components analysis).

Outlier samples were excluded for further analyses and the remaining good samples were
normalized together using fastlo10, a model-based, intensity-dependent, normalization method
that produces results essentially the same as those from cyclic loess10, but in a fraction of the
time. Gene level expression was compared between HCM and normal tissue or between
genotype subgroups overall followed by pair-wise contrasts via ANOVA linear models together
with false discovery rates (FDR)11. Genes with a FDR q-value < 0.05 were considered
statistically significant for HCM and normal tissue comparison.

For the pair-wise comparisons between different genotype subgroups of HCM, a p-value
< 0.05 was considered statistically significant. A less stringent cut-off was used here in an effort
to elucidate differences between two similar disease conditions, acknowledging the fact that the
chance of a false positive result is higher. A cut-off for biological significance was set as an absolute fold change > 1.5 between genotype subsets of HCM.

qRT-PCR data was analyzed after calculating $2^{-\Delta Ct}$ for the average $\Delta Ct$ value (transcript of interest minus GAPDH control) for the triplicate replicates of each sample. A one-tailed t-test was used. A fold-change was calculated by the $2^{-\Delta\Delta Ct}$ method, taking $2^{-\Delta Ct}$ for the overall average of all cases divided by $2^{-\Delta Ct}$ for the overall average of all controls.

RESULTS

Cohorts

All HCM participants ($n=121$) provided written informed consent to participate in this Mayo Clinic IRB-approved study. Among 121 tissue samples, 106 samples passed RNA and microarray quality controls. HCM samples were obtained from mostly Caucasian subjects (54 men and 52 women), ages 9-78 years. Clinical manifestations of HCM and control subjects are summarized in Table 1. For HCM cases, median age at diagnosis was 43 years (interquartile range (IQR) 27-55 years), median age at myectomy was 51 years (IQR 32-60 years), 77% had NYHA Class III to IV symptoms, 27% had a family history of HCM, and 13% had a family history of SCD. Their median left ventricular mass index was 171 g/m^2 (IQR 139-225), median left ventricular wall thickness was 22 mm (IQR 18-26 mm), and median left ventricular outflow tract maximum instantaneous gradient was 68 mmHg (IQR 29-100 mm Hg). Histopathology of the myectomy samples showed moderate or severe interstitial fibrosis in 27%, and moderate or severe endocardial fibrosis in 63% of patients.

From the donor hearts, 39 of 44 RNA samples passed RNA and microarray quality control and were used for microarray analyses. These 39 donors included 19 men and 20 women,
with an average age of 37 ± 15 years, approximately 10 years younger than HCM cases (Table 1).

Pathogenic variants were identified in 55% of HCM cohort. In approximately 45% of subjects (48/106), the 13-gene test panel did not identify the cause for HCM. As anticipated, mutations in *MYBPC3* (encoding cardiac myosin binding protein C) and *MYH7* (encoding beta myosin heavy chain) genes were the most commonly identified (23/106; 22% and 17/106; 16%, respectively). Clinical parameters of the patients with HCM, based on the two largest genotype groups (MYBPC3+ and MYH7+) are shown in Table 2. These two groups were similar but differed from genotype-negative HCM patients by younger age at diagnosis and myectomy, and higher prevalence of familial HCM, similarly to prior larger studies. There was no difference in NYHA classification, systolic blood pressure, heart rate, ejection fraction, left ventricular mass index, left ventricular wall thickness, left ventricular outflow tract maximum instantaneous gradient, or interstitial or endocardial fibrosis among the three primary genotypic subgroups (MYH7+, MYBPC3+, and genotype negative-HCM).

**Microarray Results**

**Cases versus controls**

Overall, 37,846 genes targeted by 48,804 probes were analyzed for all samples (GEO accession number GSE36961), and 8443 genes (22% of the transcriptome) were expressed differentially between HCM and controls based on a false discovery rate q-value < 0.05. These differentially expressed genes represent 1075 molecular functions and 4272 biological processes as defined by the Gene Ontology (GO) Consortium. The top 10 up-regulated (2.5- to 3.5-fold increased expression compared to controls) and top 10 down-regulated genes (4.5- to 11.8-fold decreased
expression compared to controls) along with fold changes and GO classification are listed in Figure 1 and Tables 3A/B.

Quantitative Real-Time PCR

Using qRT-PCR, the mRNA results were validated for 12 selected genes with differential expression (fold change >1.5) in HCM and control samples. Genes were selected based on potential functional roles in cardiac hypertrophy and included ACE2, CAMK2A, CAMK2G, CCND1, CREB1, LDHA, MAP2K1, RASD1, RASL11B, SERPINE1, SERPINA3, SMOC2. With the exception of CREB1, all other genes (92%) showed similar magnitude and directionality of differential expression by both techniques (Figure 2).

Overexpression of ACE2 in HCM

Notably, the ACE2-encoded angiotensin I/angiotensin II converting enzyme subtype 2, an important counter-regulator of the renin-angiotensin-aldosterone system (RAAS) involved in hypertrophy, fibrosis, and vasoconstriction, was the most up-regulated gene in HCM tissues (3.5-fold increase vs. controls, confirmed by qRT-PCR; Figures 1 and 2). Western blot analyses indicated 5.3-fold increased ACE2 protein expression compared to control (p<0.001; Figure 3A). Additionally, immunohistochemistry cardiomyocyte staining of cardiac myectomy tissue from 14 HCM patients showed significantly increased ACE2-antibody staining intensity (p=0.002) and distribution (p<0.001) of ACE2 protein compared to 8 control samples (Figure 3B).

Interestingly, ACE2 is located on the X-chromosome and therefore sex-differences could be expected. Thus, we performed a sex-corrected analysis of our expression data for ACE2 and observed that there was still a 3.60-fold higher expression of ACE2 in HCM cases versus controls (p = 2.66E-27). Further, after adding age as an adjustment variable to account for a
younger control cohort, $ACE2$ overexpression in cases still persisted (3.66-fold, 1.01E-26).

Overall, although there was no significant difference in $ACE2$ expression between males and females in the control cohort (fold-change -1.01; $p = 0.95$), $ACE2$ transcript levels were 1.33-fold higher in female cases ($p<0.01$).

Finally, as there are several RAAS-pathway antagonists, such as ACE-inhibitors (ACEi) and angiotensin receptor blockers (ARBs), that are commonly used in the treatment program for various cardiovascular diseases, we examined whether the presence of such a drug before surgical myectomy impacted $ACE2$ expression. Overall, there were 13 patients treated with either an ACEi or ARBs before their surgical myectomy. While there was a slightly lower $ACE2$-expression among treated patients (-1.32 fold-change), this did not reach statistical significance ($p$-value = 0.082; q-value = 1).

**Genotype Subgroup Analyses**

Subgroup analyses were performed comparing the three largest genotypic subsets; MYBPC3+, MYH7+, and genotype negative-HCM. Pair-wise comparisons of MYH7+ and MYBPC3+, MYH7+ and genotype negative-HCM, and MYBC3+ and genotype negative-HCM were performed. There were no gene expression changes that met a false discovery rate q-value $< 0.1$, suggesting a high probability of false positive findings.

However, given that the disease states under comparison are, as previously documented, phenotypically indistinguishable (at clinical, gross anatomic, and microscopic levels), we hypothesized that gene expression changes due to genotype subgroup might be subtle. Therefore, we accepted a higher false positive rate in order to reveal potentially important differences in gene expression. After adjusting to meet a p-value $< 0.05$, there were ~1000 –
2000 gene expression changes in each subgroup comparison totaling approximately 4-6% of
genes tested. Most differentially expressed genes had low, absolute fold changes and high false
discovery rate q-value. The overall 3-way comparison of gene expression among these 3
genotypic subgroups of HCM revealed that 94% of gene expression changes were shared. Since
additional differences between genotypic subgroups were subtle, we have summarized them in
the Supplemental data.

DISCUSSION

The Human HCM mRNA Transcriptome

Our analysis of 106 HCM myectomy tissues and 39 control tissues identified 8443 differentially
expressed genes, 22% of all genes analyzed. These genes participate in 1075 Molecular
Functions and 4272 Processes as defined by the Gene Ontology Consortium. Remarkably, the
most differentially expressed genes were not previously identified in hypertrophic pathways.
Whether this reflects the relatively small sizes of study groups, variability in age and treatments,
background genotypes, or other factors is unknown. Previous genotype-phenotype studies have
not identified a gene-specific profile.16-18 Nevertheless, we suggest that the newly identified
differentially expressed genes warrant further investigation.

ACE2 and HCM

Given the current and devastating COVID-19 pandemic (> 2.5 million confirmed cases) that has
claimed over 172,000 lives worldwide in less than 4 months (04/21/2020), it was noteworthy that
the most up-regulated gene in HCM samples was ACE2 (3.5-fold; q-value = 1.30x10−23). ACE2
protein was also increased > 5-fold by Western blot analyses (p< 0.001). ACE2 encodes
angiotensin converting enzyme subtype 2, which has important compensatory roles in
modulating excessive activation of the RAAS as occurs in hypertension (HTN), congestive heart
failure (CHF), and atherosclerosis. In its soluble form, ACE2 acts as a carboxypeptidase cleaving the pro-hypertrophic polypeptides angiotensin I and angiotensin II to angiotensin 1-9 and angiotensin 1-7 respectively, thereby producing counter-regulating, vasodilating, and potentially anti-hypertrophic/anti-fibrotic polypeptides (Figure 3C).\textsuperscript{19}

Accordingly, we speculate that up-regulation of both \textit{ACE2} transcript and ACE2 protein levels might be a compensatory, counter-regulatory signaling response (‘patho-responsive’) in patients with obstructive HCM. This was echoed in a recent paper by Liu \textit{et al.} in which the investigators studied the transcriptome of HCM mouse models and found that pro-fibrotic pathways initiated by increase of endothelin-1(ET1) were the main drivers of HCM pathogenesis in mice through miRNA-29 and TGFβ signaling. However, using our preliminary microarray data that was derived from the patients in our study and made publicly available (GSE36961), this differential expression of the TGFβ-signaling genes was not observed. Instead, the increased transcript levels of \textit{ACE2} were noted prompting the speculation that \textit{ACE2} overexpression might be a compensatory response.\textsuperscript{20}

Lastly, \textit{ACE2}, located in the X-chromosome, was upregulated significantly in female patients with HCM (1.33 fold compared to males: p<0.01), and while only one gene, findings like these could start to shed light and form the basis to understanding some of the underlying (epigenetic) contributions to the significantly different outcomes that are observed for women with HCM.\textsuperscript{21-23}

\textbf{\textit{ACE2} and its Possible Role in COVID-19-Related Morbidity and Mortality}

Beyond the potential relevance of \textit{ACE2} expression in HCM hearts and its disease pathogenesis, in its membrane-bound state, ACE2 plays an important role as a functional receptor required for viral entry and subsequent viral replication for the SARS-CoV family of viruses\textsuperscript{24, 25}, and thereby
may in fact contribute to the increased morbidity and mortality from SARS-CoV-2 in adult patients with a variety of heart diseases. The currently endemic SARS-CoV-2 is a member of the SARS-family of coronaviruses that bind to membrane-bound ACE2 via its viral spike protein. Recently, Zhou et al. assessed virus infectivity in HeLa cell transfected with ACE2 and in fact demonstrated preferential binding to ACE2 over other coronavirus receptors, such as aminopeptidase N (APN) or dipeptidyl peptidase 4 (DPP4). After binding to ACE2, cleavage of the spike protein (possibly by the transmembrane protease-2 serine (TMPRSS2) enzyme) primes viral internalization by endocytosis. This viral internalization may result in loss of membrane ACE2 and a subsequent increase in the Angiotensin II:Ang (1-7) ratio, which in turn allows excessive angiotensin II and unopposed angiotensin type 1 receptor (AT1R)-mediated lung injury in patients with COVID-19 and the development of severe acute respiratory distress syndrome (ARDS).

ACE2 is expressed in many other tissues including the intestinal and vascular epithelium, kidneys, and the heart. However, expression in cardiomyocytes is quite low. In fact, in a study of cardiac cell samples from donor hearts, both ACE2 and TMPRSS2 showed highest expression in the heart tissue’s pericyte sub-population rather than in the cardiomyocytes. Nevertheless, widespread expression of ACE2 may contribute to multi-organ dysfunction seen in patients with COVID-19.

The marked 5-fold increase in ACE2 protein in HCM may provide a mechanism to explain higher rates of severe outcomes in COVID19 patients who also have cardiovascular co-morbidities, as well as the direct cardiac damage caused by SARS-CoV-2 infections. While the incidence of COVID-19 seems highest in the elderly or immunocompromised, a large number of affected patients and those requiring hospitalization suffer from significant co-morbidities,
including highly prevalent cardiovascular diseases, such as HTN or CHF. A large meta-analysis of over 46,000 patients in China showed the most common co-morbidities were HTN (17±7%, 95% CI 14 – 22%), diabetes mellitus (8±6% 95% CI 6-11%), and cardiovascular disease (5±4%, 95% CI 4-7%)\(^3\)\(^1\). These percentages were much higher in patients requiring hospitalization or even ICU admission. In two separate inpatient studies, pre-existing HTN was present in 30% (and up to 60% for ICU or non-surviving patients) as were concomitant cardiovascular disease (15%, increased to 13-25% in ICU or non-surviving patients)\(^3\)\(^2\), \(^3\)\(^3\).

There is mounting evidence for cardiotropism with SARS-CoV-2 infection and direct cardiac toxicity.\(^3\)\(^2\) In early studies, 7.2% of all COVID-19 patients and 22% of patients admitted to ICUs showed evidence of myocardial injury with elevated high sensitivity Troponin I (hs-cTnI) or new ECG abnormalities with clinical manifestations of myocardial ischemia or myocarditis. Based on the significantly increased expression of membrane-bound ACE2 in HCM hearts, we speculate that obstructive HCM, and perhaps other ACE2-generating heart diseases, sensitizes the myocardium to increased SARS-CoV-2 viral entry and subsequent viral replication, while the subsequent decrease of surface ACE2 (after viral internalization) leads to increase of damaging angiotensin II and AT1R activity and loss of the protective effects of Ang(1-7) (Figure 3). Just recently, Liu et al. proposed a similar hypothesis, describing the resulting unopposed increase of angiotensin II and subsequent downstream increase of detrimental inflammation, reactive oxygen species, vasoconstriction, and thrombosis as the basis for cardiac damage stemming from SARS-CoV-2 infection.\(^3\)\(^4\)

The effect of ACE (ACEi) inhibitors or angiotensin receptor blockers (ARBs) is under active investigation. Although initial reports suggested potentially worse outcomes in patients with COVID-19 who were on ACE inhibitors,\(^3\)\(^5\), \(^3\)\(^6\) a subsequent review article concluded that
there was insufficient evidence for this claim prompting all major cardiac societies to advise that heart disease patients treated with these medications should continue them. On the other hand, ARBs could attenuate the impact of SARS-CoV-2 by blocking the damaging effects resulting from a viral-mediated decrease of ACE2 and subsequent increase of damaging angiotensin II. In fact, experimental studies of the related virus, SARS-CoV, showed that down-regulation of ACE2 exacerbated lung injury, and treatment with the ARB losartan mitigated these effects. Furthermore, a large study among 1128 patients with HTN and diagnosed with COVID-19 showed that unadjusted mortality rate was significantly lower in those whose HTN was treated with ACEi/ARBs (3.7% vs. 9.8%; p = 0.001). This risk remained consistently lower when performed as a propensity score-matched analysis with adjustment of imbalanced variables, such as age gender, co-morbidities, and in-hospital medications (adjusted HR, 0.37; 95% CI, 0.15-0.89; p = 0.03).

In light of these findings, a clinical trial has been launched testing the effect of losartan in study eligible patients with COVID-19 (NCT04312009, Figure 4). In addition, clinical grade human recombinant soluble ACE2 (hrsACE2) can block early stages of SARS-CoV-2 infection significantly by preventing the virus from entering the cell highlighting the crucial and dual role of ACE2 in health and disease (Figure 4). A clinical trial to test hrsACE2 in patients was commenced recently in Europe (EudraCT2020-001172-15).

**Study Limitations**

We studied only HCM patients undergoing therapeutic surgical septal myectomy, representing one phenotypic subset of HCM. However, this study design had the inherent bias as the procedure provided the only means to ethically obtain heart tissue. It therefore remains to be determined whether ACE2 elevation was a marker of this state of the disease alone (obstructive
HCM) and whether it persisted following the relief of LVOTO. Whether ACE2 elevation is present in the hearts of patients with non-obstructive HCM, other cardiomyopathies, HTN, or other forms of acquired heart disease is unknown.

To overcome the logistical and ethical issue of obtaining healthy heart tissue, we used a common source for healthy heart tissue as controls, both due to the scarcity of this reagent and the need for these control tissues to be procured and flash-frozen to preserve RNA (immediate flash-freezing close to the time of death). While confounding of specimen source and case/control status must be considered as a possible explanation for the results herein, as these are patients from a different ethnical background (although likely both Euro-Caucasian), this collaboration provided us with the best source of flash frozen cardiac tissue guaranteeing preserved, high-quality RNA necessary for these types of analyses.

CONCLUSION

Using a high-throughput gene expression profiling technology, we discovered that > 20% of the transcriptome is expressed differentially between HCM and control heart tissue, while 5% of transcriptomic changes differ within the three most common HCM genotypes. Importantly, the single most up-regulated gene in the cardiac transcriptome for patients with obstructive HCM was ACE2. Further, the proven 5-fold increase in ACE2 protein levels in the heart may shed light on the increased morbidity and mortality in COVID-19 patients with underlying cardiovascular diseases. However, it remains to be demonstrated whether non-obstructive HCM, other cardiomyopathies, other acquired cardiovascular diseases, or hypertension precipitate an overexpression of ACE2 protein in the heart as shown here for obstructive HCM. Whether an angiotensin receptor blocker like losartan or delivery of an ACE2 decoy with human recombinant soluble ACE2 might be effective therapeutic strategies for COVID-19 patients with
ACE2-accentuating diseases like obstructive HCM warrants further investigation.

**FUNDING SOURCES**

This work is supported by the Mayo Clinic Windland Smith Rice Comprehensive Sudden Cardiac Death Program, the Paul and Ruby Tsai and Family Hypertrophic Cardiomyopathy Research Fund, the National Institutes of Health (HL084553 and HL133165 to CES and JGS) and the Howard Hughes Medical Institute (CES).

**DISCLOSURES**

MJA is a consultant for Abbott, Audentes Therapeutics, Boston Scientific, Daiichi Sankyo, Invitae, LQT Therapeutics, Medtronic, MyoKardia Inc., and UpToDate. PAN, PAF, MJA and Mayo Clinic have financial interest in AliveCor. CES and JGS are founders and own shares in Myokardia Inc., a startup company that is developing therapeutics that target the sarcomere. None of these entities were involved in this study in any manner.
REFERENCES


FIGURES

Figure 1. Top up-regulated and down-regulated genes in HCM. Bar diagram showing the top 10 up-regulated (left) and down-regulated (right) genes in tissues of patients with obstructive HCM compared to controls.

Figure 2. Technical validation of the microarray technique. Microarray fold-change and quantitative real-time PCR (qRT-PCR) fold change are plotted side-by-side for the 12 genes tested. The qRT-PCR data validated the microarray data for 11/12 (92%).

Figure 3. Marked accentuation of ACE2 in HCM. Bar diagram showing a 5.3-fold increase of ACE2 protein in HCM patients compared to controls by Western blot analysis (A) and significant staining of ACE2-antibody in the myectomy specimen from a patient with obstructive HCM (B). Flow chart showing the role of ACE2 in converting angiotensin I to ang (1-9) and angiotensin II to ang (1-7) to counter the effects of angiotensin II (C).

Figure 4. ACE2 overexpression and SARS-CoV-2 infection. Central illustration showing possible mechanism behind ACE2 overexpression and SARS-CoV-2 infection picturing normal ACE2 expression on the left and ACE2 protein overexpression in obstructive HCM on right. The SARS-CoV-2 virus hijacks membrane-bound ACE2 for cellular entry. Aside from allowing cellular invasion and viral replication, internalization of the SARS-CoV-2-ACE2 complex causes a decrease of surface ACE2. Loss of surface ACE2 i) increases the angiotensin II:angiotensin (1-7) ratio and ii) increases angiotensin type 1 receptor (AT1R) activity with a resultant increase in damaging angiotensin II activity. Shown are potential therapeutic targets (and clinical trials).
using either angiotensin receptor blockers (ARBs, losartan specifically) or human recombinant soluble ACE2 (hrsACE2). For patients with ACE2-accentuating heart diseases like obstructive HCM, the speculated increase in viral infectivity of the heart muscle remains to be proven.

## TABLES

### Table 1: Demographics of HCM and control cohorts

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
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<td>Number</td>
<td>106</td>
<td>39</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>54/52</td>
<td>19/20</td>
</tr>
<tr>
<td>Age, diagnosis (years)</td>
<td>43 (27-55)</td>
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</tr>
<tr>
<td>Age, myectomy (years)</td>
<td>51 (32-60)</td>
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</tr>
<tr>
<td>Age, death (years)</td>
<td>N/A</td>
<td>38 (23-48)</td>
</tr>
<tr>
<td>NYHA Class III-IV</td>
<td>82 (77)</td>
<td>N/A</td>
</tr>
<tr>
<td>Family History of HCM</td>
<td>29 (27)</td>
<td>N/A</td>
</tr>
<tr>
<td>Family History of SCD</td>
<td>14 (13)</td>
<td>N/A</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>120 (109-130)</td>
<td>N/A</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>70 (62-80)</td>
<td>N/A</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>68 (60-76)</td>
<td>N/A</td>
</tr>
<tr>
<td>Ejection Fraction</td>
<td>0.74 (0.68-0.77)</td>
<td>N/A</td>
</tr>
<tr>
<td>LV Mass Index (g/m²)</td>
<td>171 (139-225)</td>
<td>N/A</td>
</tr>
<tr>
<td>LV Wall Thickness (mm)</td>
<td>22 (18-26)</td>
<td>N/A</td>
</tr>
<tr>
<td>LVOT MIG (mmHg)</td>
<td>68 (29-100)</td>
<td>N/A</td>
</tr>
<tr>
<td>Interstitial Fibrosis Score of Moderate or Severe</td>
<td>29 (27)</td>
<td>N/A</td>
</tr>
<tr>
<td>Endocardial Fibrosis Score of Moderate or Severe</td>
<td>67 (63)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Values are n, n (%), or median (IQR). M, Male; F, Female; N/A, not available; NYHA, New York Heart Association; HCM, hypertrophic cardiomyopathy; SCD, sudden cardiac death; BP, blood pressure; bpm, beats per minute; LV, left ventricular; OT, outflow tract; MIG, maximum instantaneous gradient.
Table 2: Baseline characteristics by major genotype subgroups

<table>
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<tr>
<th></th>
<th>MYBPC3-HCM</th>
<th>MYH7-HCM</th>
<th>Genotype Negative</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Number</td>
<td>23</td>
<td>17</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>13/10</td>
<td>7/10</td>
<td>27/21</td>
<td>NS</td>
</tr>
<tr>
<td>Age, diagnosis (years)</td>
<td>37 (23-45)</td>
<td>37 (13-45)</td>
<td>52 (33-64)</td>
<td>0.003</td>
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<tr>
<td>Age, myectomy (years)</td>
<td>38 (30-53)</td>
<td>43 (16-52)</td>
<td>57 (44-67)</td>
<td>0.003</td>
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<tr>
<td>NYHA Class III-IV</td>
<td>19 (83)</td>
<td>12 (75)</td>
<td>35 (78)</td>
<td>NS</td>
</tr>
<tr>
<td>Family History of HCM</td>
<td>11 (48)</td>
<td>7 (41)</td>
<td>8 (17)</td>
<td>0.01</td>
</tr>
<tr>
<td>Family History of SCD</td>
<td>6 (27)</td>
<td>1 (6)</td>
<td>5 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>120 (110-122)</td>
<td>118 (102-127)</td>
<td>125 (113-137)</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>70 (62-75)</td>
<td>66 (57-70)</td>
<td>74 (64-82)</td>
<td>0.01</td>
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<tr>
<td>Heart Rate (bpm)</td>
<td>68 (60-73)</td>
<td>70 (60-78)</td>
<td>68 (59-76)</td>
<td>NS</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>0.70 (0.65-0.76)</td>
<td>0.75 (0.75-0.80)</td>
<td>0.73 (0.70-0.76)</td>
<td>NS</td>
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<tr>
<td>LV Mass Index (g/m²)</td>
<td>192 (144-238)</td>
<td>183 (130-258)</td>
<td>171 (134-217)</td>
<td>NS</td>
</tr>
<tr>
<td>LV Wall Thickness (mm)</td>
<td>24 (18-27)</td>
<td>25 (18-29)</td>
<td>21 (16-24)</td>
<td>NS</td>
</tr>
<tr>
<td>LVOT MIG (mmHg)</td>
<td>40 (16-106)</td>
<td>81 (70-121)</td>
<td>64 (30-94)</td>
<td>NS</td>
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<tr>
<td>Interstitial Fibrosis Score moderate or severe</td>
<td>5 (22)</td>
<td>4 (24)</td>
<td>13 (27)</td>
<td>NS</td>
</tr>
<tr>
<td>Endocardial Fibrosis Score moderate or severe</td>
<td>15 (65)</td>
<td>13 (76)</td>
<td>30 (63)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are n, n(%), or median (IQR). M, Male; F, Female; N/A, not available; NYHA, New York Heart Association; HCM, hypertrophic cardiomyopathy; SCD, sudden cardiac death; BP, blood pressure; bpm, beats per minute; LV, left ventricular; OT, outflow tract; MIG, maximum instantaneous gradient.
Table 3A: Top 10 differentially expressed mRNA transcripts, up-regulated in HCM compared to controls

<table>
<thead>
<tr>
<th>Gene</th>
<th>Official Full Name</th>
<th>Fold Change</th>
<th>q-Value</th>
<th>Gene Ontology Biological Process Term(s)</th>
<th>Gene Ontology Molecular Function Term(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE2</td>
<td>Angiotensin I converting enzyme (peptidyl-dipeptidase A) 2</td>
<td>+3.53</td>
<td>1.30x10^{-23}</td>
<td>Angiotensin catabolic process in blood; regulation of inflammatory response; regulation of vasoconstriction</td>
<td>Peptidase activity; glycoprotein binding; zinc ion binding</td>
</tr>
<tr>
<td>SFRP1</td>
<td>Secreted frizzled-related protein 1</td>
<td>+3.32</td>
<td>5.93x10^{-21}</td>
<td>Regulation of cell growth; canonical Wnt receptor signaling pathway</td>
<td>Cysteine-type endopeptidase activity; Wnt-protein binding</td>
</tr>
<tr>
<td>RASL11B</td>
<td>RAS-like, family 11, member B</td>
<td>+3.30</td>
<td>3.44x10^{-22}</td>
<td>Small GTPase mediated signal transduction</td>
<td>GTPase activity</td>
</tr>
<tr>
<td>CENPA</td>
<td>Centromere protein A</td>
<td>+3.25</td>
<td>3.37x10^{-16}</td>
<td>Nucleosome assembly</td>
<td>Chromatin binding; DNA binding; protein binding</td>
</tr>
<tr>
<td>APOA1</td>
<td>Apolipoprotein A-I</td>
<td>+3.00</td>
<td>1.17x10^{-12}</td>
<td>Lipid metabolic process</td>
<td>Cholesterol transport activity</td>
</tr>
<tr>
<td>HS.576694</td>
<td>Not available.</td>
<td>+2.95</td>
<td>2.06x10^{-16}</td>
<td>Not available</td>
<td>Not available</td>
</tr>
<tr>
<td>SMO2C</td>
<td>SPARC related modular calcium binding 2</td>
<td>+2.80</td>
<td>7.56x10^{-27}</td>
<td>Extracellular matrix organization</td>
<td>Calcium ion binding</td>
</tr>
<tr>
<td>PROS1</td>
<td>Protein S (alpha)</td>
<td>+2.77</td>
<td>3.06x10^{-29}</td>
<td>Blood coagulation</td>
<td>Calcium ion binding</td>
</tr>
<tr>
<td>FRZB</td>
<td>Frizzled-related protein</td>
<td>+2.64</td>
<td>2.75x10^{-24}</td>
<td>Negative regulation of cell growth; negative regulation of Wnt receptor signaling pathway</td>
<td>Wnt-activated receptor activity; Wnt-protein binding</td>
</tr>
<tr>
<td>HSPA2</td>
<td>Heat shock 70kDa protein 2</td>
<td>+2.62</td>
<td>1.07x10^{-16}</td>
<td>Positive regulation of cyclin-dependent protein kinase activity involved in G2/M</td>
<td>ATP binding</td>
</tr>
</tbody>
</table>
Table 3B: Top 10 differentially expressed mRNA transcripts, down-regulated in HCM compared to controls

<table>
<thead>
<tr>
<th>Gene</th>
<th>Official Full Name</th>
<th>Fold Change</th>
<th>Q-Value</th>
<th>Gene Ontology Biological Process Term(s)</th>
<th>Gene Ontology Molecular Function Term(s)</th>
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</thead>
<tbody>
<tr>
<td>SERPINA3</td>
<td>Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3</td>
<td>-11.81</td>
<td>1.73x10⁻³⁷</td>
<td>Inflammatory response; regulation of proteolysis; regulation of lipid metabolic process</td>
<td>Peptidase inhibitor activity; DNA binding; protein binding</td>
</tr>
<tr>
<td>RASD1</td>
<td>RAS, dexamethasone-induced 1</td>
<td>-11.43</td>
<td>2.26x10⁻⁴⁰</td>
<td>Small GTPase mediated signal transduction</td>
<td>GTP binding; GTPase activity</td>
</tr>
<tr>
<td>S100A9</td>
<td>S100 calcium binding protein A9</td>
<td>-7.54</td>
<td>7.18x10⁻³⁶</td>
<td>Cell-cell signaling; leukocyte chemotaxis; actin cytoskeleton reorganization</td>
<td>Calcium ion binding; protein binding; signal transducer activity</td>
</tr>
<tr>
<td>S100A8</td>
<td>S100 calcium binding protein A8</td>
<td>-6.27</td>
<td>1.23x10⁻³⁰</td>
<td>Inflammatory response; response to zinc ion; response to ethanol</td>
<td>Calcium ion binding; protein binding</td>
</tr>
<tr>
<td>MT1X</td>
<td>Metallothionein 1X</td>
<td>-5.18</td>
<td>1.02x10⁻³⁰</td>
<td>Response to metal ion</td>
<td>Zinc ion binding</td>
</tr>
<tr>
<td>CEBPD</td>
<td>CCAAT/enhancer binding protein (C/EBP), delta</td>
<td>-4.95</td>
<td>1.13x10⁻⁴⁰</td>
<td>Transcription from RNA polymerase II promoter</td>
<td>Protein dimerization activity; sequence-specific DNA binding</td>
</tr>
<tr>
<td>ZFP36</td>
<td>Zinc finger protein 36, C3H type, homolog (mouse)</td>
<td>-4.72</td>
<td>2.07x10⁻⁴⁰</td>
<td>3'-UTR-mediated mRNA stabilization</td>
<td>DNA binding; mRNA binding; protein binding; zinc ion binding</td>
</tr>
<tr>
<td>MT1M</td>
<td>Metallothionein 1M</td>
<td>-4.67</td>
<td>2.49x10⁻³⁵</td>
<td>Negative regulation of growth</td>
<td>Zinc ion binding</td>
</tr>
<tr>
<td>TUBA3D</td>
<td>Tubulin, alpha 3d</td>
<td>-4.61</td>
<td>1.75x10⁻³⁹</td>
<td>Microtubule-based movement</td>
<td>GTP binding; GTPase activity; structural molecule activity</td>
</tr>
<tr>
<td>TUBA3E</td>
<td>Tubulin, alpha 3e</td>
<td>-4.51</td>
<td>0.00</td>
<td>Microtubule-based movement</td>
<td>GTP binding; GTPase activity; structural molecule activity</td>
</tr>
</tbody>
</table>
Figure 1

Top 10 Up-Regulated Genes in HCM

Top 10 Down-Regulated Genes in HCM
Figure 2

- ACE2
- RASL11B
- SMOC2
- CAMK2A
- CAMK2G
- CCND1
- CREB
- MAP2K1
- LDHA
- SERPINE1
- RAS1D
- SERPINA3

**Microarray**
* = p < 0.05 for cases vs. controls

**qRT-PCR**
† = p < 0.05 for cases vs. controls
Figure 3

A) Relative Normalized Expression

B) HCM Control

C) Angiotensinogen
   Renin
   Angiotensin I
   ACE
   Angiotensin II
   Vasoconstriction, hypertrophy, fibrosis
   ACE2
   SARS-CoV-2
   Ang (1-9)
   Ang (1-7)
   Vasodilation, anti-hypertrophic, anti-inflammatory
Figure 4

Normal Myocardium  Hypertrophic Cardiomyopathy

SARS-CoV-2 attachment to membrane-bound ACE2

hrsACE2 EudraCT2020-001172-15

Decreased ACE2 expression

Increased activity of AT1R

Viral entry via endocytosis

Viral replication and injury

Increased activity of angiotensin II

Average susceptibility and disease response

Increased susceptibility and disease response

ARBs NCT043612509
hrsACE2 EudraCT2020-001172-15
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,\textsuperscript{29} \textit{APOA1} (1.51-fold in MYH7+), and one was an uncharacterized gene, \textit{Hs.131412} (down 1.61-fold in MYH7+). While not directly associated with any known pro-hypertrophic pathway, \textit{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 MYH7 versus genotype negative subset, there were two genes with potential theoretical relationships to a myocardial disease process. The first of these genes was \textit{CORIN}, encoding a protein that produces biologically active atrial natriuretic peptide\textsuperscript{35, 36}, which was down-regulated 1.74-fold in MYH7+. The second was \textit{COL3A1}, encoding type III collagen\textsuperscript{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\textsuperscript{30} (\textit{Thy1}), neurite outgrowth\textsuperscript{31} (\textit{SLITRK4}), lipoprotein catabolism\textsuperscript{29} (\textit{APOA1} and \textit{APOE}), ketone body regulation\textsuperscript{32} (\textit{HMGCS2}), and hemoglobin\textsuperscript{33} (\textit{HBA2} and \textit{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. 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 SERPINA3 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 SERPINA3, 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. 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, and SMOC2, up-regulated 2.8-fold in HCM and thought to potentiate the effect of growth factors and to activate matrix metalloproteinases.
Supplemental Table 1: Differentially expressed genes in HCM, genotype subset comparisons

A. MYH7+ versus MYBPC3+

<table>
<thead>
<tr>
<th>Gene</th>
<th>Official Full Name</th>
<th>Fold Change</th>
<th>Q-Value</th>
<th>P-Value</th>
<th>GO Biological Process Term(s)</th>
<th>GO Molecular Function Term(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOA1</td>
<td>Apolipoprotein A-I</td>
<td>+1.51</td>
<td>1.00</td>
<td>4.98x10^{-2}</td>
<td>Lipid metabolic process</td>
<td>Cholesterol transport activity</td>
</tr>
<tr>
<td>HS.131412</td>
<td>Not available</td>
<td>-1.61</td>
<td>1.00</td>
<td>3.63x10^{-2}</td>
<td>Not available</td>
<td>Not available</td>
</tr>
</tbody>
</table>
## Supplemental Table 1: Differentially expressed genes in HCM, genotype subset comparisons

### B. MYH7+ versus Genotype-Negative HCM

<table>
<thead>
<tr>
<th>Gene</th>
<th>Official Full Name</th>
<th>Fold Change</th>
<th>Q-Value</th>
<th>P-Value</th>
<th>GO Biological Process Term(s)</th>
<th>GO Molecular Function Term(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>THY1</td>
<td>Thy-1 cell surface antigen</td>
<td>+1.68</td>
<td>0.39</td>
<td>1.47x10^{-4}</td>
<td>Positive regulation of release of sequestered calcium ion into cytosol; angiogenesis</td>
<td>Enzyme binding; GPI anchor binding; Rho GTPase activator activity</td>
</tr>
<tr>
<td>SLITRK4</td>
<td>SLIT and NTRK-like family, member 4</td>
<td>+1.61</td>
<td>0.71</td>
<td>8.43x10^{-5}</td>
<td>Axonogenesis</td>
<td>Not available</td>
</tr>
<tr>
<td>HS.131412</td>
<td>Not available</td>
<td>+1.59</td>
<td>0.81</td>
<td>2.20x10^{-2}</td>
<td>Not available</td>
<td>Not available</td>
</tr>
<tr>
<td>APOE</td>
<td>Apolipoprotein E</td>
<td>+1.53</td>
<td>0.58</td>
<td>2.13x10^{-3}</td>
<td>Cholesterol homeostasis; cellular calcium ion homeostasis; response to growth factor stimulus</td>
<td>Cholesterol transporter</td>
</tr>
<tr>
<td>COL3A1</td>
<td>Collagen, type III, alpha 1</td>
<td>+1.53</td>
<td>0.51</td>
<td>8.02x10^{-4}</td>
<td>Extracellular matrix structural constituent; transforming growth factor beta receptor signaling pathway</td>
<td>Extracellular matrix structural constituent; protein binding</td>
</tr>
<tr>
<td>HMGCS2</td>
<td>3-Hydroxy-3-methylglutaryl-CoA synthase 2</td>
<td>-2.10</td>
<td>0.63</td>
<td>4.10x10^{-5}</td>
<td>Isoprenoid biosynthetic process</td>
<td>Hydroxymethylglutaryl-CoA synthase activity</td>
</tr>
<tr>
<td>HBA2</td>
<td>Hemoglobin, alpha 2</td>
<td>-1.92</td>
<td>0.46</td>
<td>3.75x10^{-4}</td>
<td>Transport</td>
<td>Oxygen transporter activity</td>
</tr>
<tr>
<td>HS.390250</td>
<td>Fibroblast growth factor 12</td>
<td>-1.90</td>
<td>0.75</td>
<td>1.19x10^{-2}</td>
<td>Signal transduction; heart development</td>
<td>Growth factor activity</td>
</tr>
<tr>
<td>HBB</td>
<td>Hemoglobin, beta</td>
<td>-1.85</td>
<td>0.51</td>
<td>9.06x10^{-4}</td>
<td>Oxygen transport</td>
<td>Oxygen transport activity</td>
</tr>
<tr>
<td>CORIN</td>
<td>Corin, serine peptidase</td>
<td>-1.74</td>
<td>0.80</td>
<td>2.09x10^{-2}</td>
<td>Regulation of systemic arterial blood pressure by atrial natriuretic peptide; peptide hormone processing</td>
<td>Peptidase activity; scavenger receptor activity</td>
</tr>
<tr>
<td>FGF12</td>
<td>Fibroblast growth factor 12</td>
<td>-1.63</td>
<td>0.75</td>
<td>1.28x10^{-2}</td>
<td>Signal transduction; heart development</td>
<td>Growth factor activity</td>
</tr>
<tr>
<td>LOC644322</td>
<td>Similar to Ribosome biogenesis protein BMS1 homolog</td>
<td>-1.53</td>
<td>0.33</td>
<td>8.02x10^{-5}</td>
<td>Not available</td>
<td>Not available</td>
</tr>
<tr>
<td>APOA1</td>
<td>Apolipoprotein A-I</td>
<td>-1.51</td>
<td>0.83</td>
<td>2.62x10^{-2}</td>
<td>Lipid metabolic process</td>
<td>Cholesterol transport activity</td>
</tr>
</tbody>
</table>
### Supplemental Table 1: Differentially expressed genes in HCM, genotype subset comparisons

#### C. MYBPC3+ versus Genotype-Negative HCM

<table>
<thead>
<tr>
<th>Gene</th>
<th>Official Full Name</th>
<th>Fold Change</th>
<th>Q-Value</th>
<th>P-Value</th>
<th>Selected GO Biological Process Term(s)</th>
<th>GO Molecular Function Term(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CENPA</td>
<td>Centromere protein A</td>
<td>+1.76</td>
<td>0.65</td>
<td>2.93x10^{-3}</td>
<td>Nucleosome assembly</td>
<td>Chromatin binding; DNA binding; protein binding</td>
</tr>
<tr>
<td>FGF12</td>
<td>Fibroblast growth factor 12</td>
<td>-1.67</td>
<td>0.65</td>
<td>3.57x10^{-3}</td>
<td>Signal transduction; heart development</td>
<td>Growth factor activity</td>
</tr>
<tr>
<td>HS.390250</td>
<td>Fibroblast growth factor 12</td>
<td>-1.67</td>
<td>0.73</td>
<td>2.45x10^{-2}</td>
<td>Signal transduction; heart development</td>
<td>Growth factor activity</td>
</tr>
<tr>
<td>HBA2</td>
<td>Hemoglobin, alpha 2</td>
<td>-1.61</td>
<td>0.65</td>
<td>3.46x10^{-3}</td>
<td>Transport</td>
<td>Oxygen transporter activity</td>
</tr>
<tr>
<td>F3</td>
<td>Coagulation factor III (thromboplastin, tissue factor)</td>
<td>-1.53</td>
<td>0.34</td>
<td>8.53x10^{-5}</td>
<td>Blood coagulation; positive regulation of platelet-derived growth factor receptor signaling pathway</td>
<td>Cell surface binding; phospholipid binding; protease binding</td>
</tr>
<tr>
<td>HBB</td>
<td>Hemoglobin, beta</td>
<td>-1.50</td>
<td>0.70</td>
<td>1.33x10^{-2}</td>
<td>Oxygen transport</td>
<td>Oxygen transport activity</td>
</tr>
</tbody>
</table>
**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.