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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

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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 39 healthy donor hearts. Expression profiling of 37,846 genes was performed using the Illumina Human HT-12v3 Expression BeadChip. All patients with HCM were genotyped for pathogenic variants causing HCM. Technical validation was performed using quantitative real-time polymerase chain reaction (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 of 37,846 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% (1502 of 37,846) to 6% (2336 of 37,846) of the transcriptome exhibiting differential expression between genotypic subgroups. The qRT-PCR confirmed differential expression in 92% (11 of 12 genes) of tested transcripts. Notably, in the context of coronavirus disease 2019 (COVID-19), the transcript for angiotensin I converting enzyme 2 (ACE2), a negative regulator of the angiotensin system, was the single most up-regulated gene in HCM (fold-change, 3.53; q-value = 1.30/10^23), which was confirmed by qRT-PCR in triplicate (fold change, 3.78; P=5.22×10^-4), and Western blot confirmed greater than 5-fold overexpression of ACE2 protein (fold change, 5.34; P=1.66×10^-6).

Conclusion: More than 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 antihypertrophic, antifibrotic response. However, given that the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) uses ACE2 for viral entry,
ACE2 UP-REGULATION IN HCM

Hypertrophic cardiomyopathy (HCM) affects approximately 1 in 500 individuals and is among the leading causes of identifiable sudden cardiac death (SCD) in the young. 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. However, genetic tests are negative in approximately 50% of all unrelated patients with HCM that are diagnosed by clinical studies. Additionally, the transcriptional changes that cause and result from HCM, with and without pathogenic variants, remain largely unknown because prior studies analyzed data from small numbers of patients. 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.

PATIENTS AND METHODS
We designed a case-control study to identify the messenger RNAs (mRNAs) differentially expressed in HCM-affected myocardium (n=106) vs control myocardium (n=39). All patients signed informed consent, and protocols were approved by Mayo Clinic 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.

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 HCM Clinic based on physical examination, electrocardiogram (ECG), and echocardiogram/cardiac magnetic resonance imaging 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 for 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 preoperative echocardiography study. Degree of endocardial and interstitial fibrosis was assessed semi-quantitatively at the time of resection by a cardiovascular pathologist.

A cohort of control tissue (n=39) 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 means of cardiac examination, ECG, and echocardiogram obtained within 24 hours before 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, MYBP3, MYH6, MYH7, MYL2, MYL3, TNNC1, TNNJ3, TNNT2, and TPM1; for expansion of gene

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symbols, use search tool at www.genenames.org) and 3 genes known to mimic HCM (GLA, LAMP2, and PRKAG2) were studied using filter-based hybridization capture, as described previously.\(^7\) 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- 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 (Broad Institute).\(^8\)

### 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 uses a phenol/chloroform extraction protocol and RNA purification columns. RNA quality was assessed by the Mayo Clinic Advanced Genomic Technology Center Microarray Shared Resource using the 2100 Bioanalyzer (Agilent) to obtain electropherograms from which an RNA integrity number\(^9\) was calculated. Only RNA samples with high enough quality (RNA integrity number ≥6.0) were used for transcriptome analyses.

### Microarray Hybridization

Complementary DNA (cDNA) corresponding to case and control mRNA was produced by reverse transcription. The cDNA was converted to biotin-labeled complementary RNA 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. The HCM and donor samples were randomly assigned to ensure that the groups were balanced over batches (Bead-Chips) 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 Polymerase Chain Reaction and Western Blot Validation

Quantitative real-time polymerase chain reaction (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. The 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

Tissue sections were deparaffinized in xylene, dipped in decreasing concentrations of ethyl alcohol, and 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. Tissue sections were treated with Peroxidase Blocking Reagent (Dako) for 15 minutes, washed with 1× 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, 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. Counterstaining with hematoxylin followed by dehydration in increasing concentrations of ethyl alcohol and xylene were performed before permanent coverslipping.

All slides were graded by a cardiac pathologist (J.J.M.) and graded for intensity (0 = no staining, 1+ = dot-like sarcoplasmic staining, and 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 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 were exported and analyzed in R (The R Foundation; http://www.r-project.org/). Briefly, the un-normalized raw data were first log2 transformed and evaluated for potential outlier samples and bead chip effects by graphic and dimension reduction approaches (density plot, MA plot, and principal components analysis).

Outlier samples were excluded for further analyses and the remaining good samples were normalized together using fastlo, a model-based intensity-dependent normalization method that produces results essentially the same as those from cyclic loess but in a fraction of the time. Gene-level expression was compared between HCM and normal tissue or between genotype subgroups overall followed by pairwise contrasts through analysis of variance linear models together with false discovery rates (FDRs). Genes with a false discovery rate q-value less than 0.05 were considered statistically significant for HCM and normal tissue comparison.

For the pairwise comparisons between different genotype subgroups of HCM, P<.05 was considered statistically significant. A less stringent cutoff was used here in an effort to elucidate differences between 2 similar disease conditions, acknowledging that the chance of a false-positive result is higher. A cutoff for biological significance was set as an absolute fold change greater than 1.5 between genotype subsets of HCM.

The qRT-PCR data were analyzed after calculating $2^{-\Delta C_{T}}$ for the average $\Delta C_{T}$ value (transcript of interest minus GAPDH control) for the triplicate replicates of each sample. A 1-tailed $t$ test was used. A fold change was calculated by the $2^{-\Delta C_{T}}$ method, taking $2^{-\Delta C_{T}}$ for the overall average of all cases divided by $2^{-\Delta C_{T}}$ for the overall average of all controls.

**RESULTS**

**Cohorts**

All participants with HCM (n=121) provided written informed consent to participate in this Mayo Clinic Institutional Review Board–approved study. Among 121 tissue samples, 106 samples passed RNA and microarray quality controls. The HCM samples were obtained from mostly white individuals (54 men and 52 women) aged 9 to 78 years. Clinical manifestations of HCM and control participants are summarized in Table 1. For the 106 HCM cases, median age at diagnosis was 43 years (interquartile range [IQR], 27 to 55 years), median age at myectomy was 51 years (IQR, 32 to 60 years), 77% (n=82) had NYHA class III to IV symptoms, 27% (n=29) had a family history of HCM, and 13% (n=14) had a family history of SCD. Median left ventricular mass index was 171 g/m² (IQR, 139 to 225 g/m²), median left ventricular wall thickness was 22 mm (IQR, 18 to 26 mm), and median left ventricular outflow tract maximum instantaneous gradient was 68 mm Hg (IQR, 29 to 100 mm Hg). Histopathologic examination of myectomy samples showed moderate or severe interstitial fibrosis in 27% (n=29) and moderate or severe endocardial fibrosis in 63% (n=67) 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% (n=58 of 106) of the HCM cohort. In 45% (48/106) of participants, 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 patients with HCM, based on the 2 largest genotype groups (MYBPC3-positive and MYH7-positive) are shown in Table 2. These 2 groups were similar but differed from genotype-negative patients with HCM by younger age at diagnosis and myectomy, and higher prevalence of familial HCM, similarly to prior larger studies.4,13-15

Microarray Results
Cases vs Controls. Overall, 37,846 genes targeted by 48,804 probes were analyzed for all samples (Gene Expression Omnibus 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 less than 0.05. These differentially expressed genes represent 1075 molecular functions and 4272 biological processes as defined by the Gene Ontology Consortium. The top 10 up-regulated (2.5- to 3.5-fold increased expression compared with controls) and top 10 down-regulated genes (4.5- to 11.8-fold decreased expression compared with controls) along with fold changes and Gene Ontology classification are listed in Figure 1 and Tables 3 and 4.

Quantitative RT-PCR. Using qRT-PCR, mRNA results were validated for 12 select 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, and SMOC2. With the exception of CREB1, all other genes (n=11 of 12; 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 vascular constriction, was the most up-regulated gene in HCM tissues (3.53-fold increase vs controls, confirmed by qRT-PCR; Figures 1 and 2). Western blot analyses indicated 5.34-fold increased ACE2 protein expression.
compared with control (P<.001; Figure 3A). Additionally, immunohistochemistry cardiomyocyte staining of cardiac myectomy tissue from 14 patients with HCM showed significantly increased ACE2-antibody staining intensity (P¼.002) and distribution (P<.001) of ACE2 protein compared with 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 3.60-fold higher expression of ACE2 in HCM cases vs 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; P=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=.95), ACE2 transcript levels were 1.33-fold higher in female cases (P<.01).

Finally, because there are several RAAS-pathway antagonists, such as ACE inhibitors (ACEIs) 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 affected ACE2 expression. Overall, there were 13 patients treated with either an ACEi or ARB before their surgical myectomy. Although there was slightly lower ACE2 expression among treated patients (/C0 1.32-fold change), this did not reach statistical significance (P¼.082; q-value =1).

### Genotype Subgroup Analyses

Subgroup analyses were performed comparing the 3 largest genotypic subsets; MYBPC3-positive, MYH7-positive, and genotype-negative HCM. Pairwise comparisons of MYH7-positive and MYBPC3-positive, MYH7-positive and genotype-negative HCM, and MYBC3-positive 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

| TABLE 3. Top 10 Differentially Expressed Messenger RNA Transcripts Up-Regulated in HCM Compared With Controls |
|-------------------------------------------------|---------------------------------|-------------------------------------------------|-------------------------------------------------|
| Gene                             | Official Full Name | Fold Change | q-Value   | Gene Ontology Biological Process Term(s)                  | Gene Ontology Molecular Function Term(s) |
| ACE2                             | Angiotensin I converting enzyme (peptidyl-dipeptidase A) 2 | +3.53       | 1.30×10⁻²³ | Angiotensin catabolic process in blood; regulation of inflammatory response; regulation of vasoconstriction | Peptidase activity; glycoprotein binding; zinc ion binding |
| SFRP1                            | Secreted frizzled-related protein 1 | +3.32       | 5.93×10⁻²¹ | Regulation of cell growth; canonical Wnt receptor signaling pathway | Cysteine-type endopeptidase activity; Wnt-protein binding |
| RASL1/B                          | RAS-like, family 11, member B | +3.30       | 3.44×10⁻²² | Small GTPase-mediated signal transduction | GTPase activity |
| CENPA                            | Centromere protein A | +3.25       | 3.37×10⁻¹⁶ | Nucleosome assembly | Chromatin binding; DNA binding; protein binding |
| APOA1                            | Apolipoprotein A-I | +3.00       | 1.17×10⁻¹² | Lipid metabolic process | Cholesterol transport activity |
| HS.576694                        | Not available | +2.95       | 2.06×10⁻¹⁶ | Not available | Not available |
| SMO2                             | SPARC-related modular calcium binding 2 | +2.80       | 7.56×10⁻²⁷ | Extracellular matrix organization | Calcium ion binding |
| PROS1                            | Protein S (alpha) | +2.77       | 3.06×10⁻²⁹ | Blood coagulation | Calcium ion binding |
| FRZB                             | Frizzled-related protein | +2.64       | 2.75×10⁻²⁴ | Negative regulation of cell growth; negative regulation of Wnt receptor signaling pathway | Wnt-activated receptor activity; Wnt-protein binding |
| HSPA2                            | Heat shock 70 kDa protein 2 | +2.62       | 1.07×10⁻¹⁶ | Positive regulation of cyclin-dependent protein kinase activity involved in G2/M | Adenosine triphosphate binding |

HCM = hypertrophic cardiomyopathy; GTPase = hydrolase enzyme that binds to the nucleotide guanosine triphosphate; RAS = rat sarcoma.
subgroup might be subtle. Therefore, we accepted a higher false-positive rate to reveal potentially important differences in gene expression. After adjusting to meet \( P < .05 \), there were approximately 1000 to 2000 gene expression changes in each subgroup comparison, totaling approximately 4\% (1502 of 37,846) to 6\% (2336 of 37,846) of genes tested. Most differentially expressed genes had low absolute-fold changes and high false discovery rate q-values. The overall 3-way comparison of gene expression among these 3 genotypic subgroups of HCM revealed that 94\% (35,510 of 37,846) of gene expression changes were shared. Because additional differences between genotypic subgroups were subtle, we have summarized them in the Supplemental Data (available online at http://www.mayoclinicproceedings.org).

**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.

**TABLE 4. Top 10 Differentially Expressed mRNA Transcripts, Down-Regulated in HCM Compared With Controls**

| Gene       | Official Full Name                                           | Fold Change | q-Value       | Gene Ontology Biological Process Term(s)                                                                 | Gene Ontology Molecular Function Term(s)                                      |
|------------|---------------------------------------------------------------|-------------|---------------|----------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| SERPINA3   | Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3 | -11.81 | 1.73 \times 10^{-37} | Inflammatory response; regulation of proteolysis; regulation of lipid metabolic process                       | Peptidase inhibitor activity; DNA binding; protein binding                     |
| RASD1      | RAS, dexamethasone-induced                                  | -11.43       | 2.26 \times 10^{-40} | Small GTPase-mediated signal transduction                                                                     | GTP binding; GTPase activity                                                  |
| S100A9     | S100 calcium-binding protein A9                             | -7.54        | 7.18 \times 10^{-36} | Cell-cell signaling; leukocyte chemotaxis; actin cytoskeleton reorganization                                        | Calcium ion binding; protein binding; signal transducer activity                |
| S100A8     | S100 calcium-binding protein A8                             | -6.27        | 1.23 \times 10^{-30} | Inflammatory response; response to zinc ion; response to ethanol                                               | Calcium ion binding; protein binding                                           |
| MT1X       | Metallothionein 1X                                          | -5.18        | 1.02 \times 10^{-30} | Response to metal ion                                                                                         | Zinc ion binding                                                               |
| CEBPD      | CCAAT/enhancer binding protein (C/EBP), delta                | -4.95        | 1.13 \times 10^{-40} | Transcription from RNA polymerase II promoter                                                                 | Protein dimerization activity; sequence-specific DNA binding                    |
| ZFP36      | Zinc finger protein 36, C3H type, homolog (mouse)           | -4.72        | 2.07 \times 10^{-40} | 3'-UTR-mediated mRNA stabilization                                                                         | DNA binding; mRNA binding; protein binding; zinc ion binding                    |
| MT1M       | Metallothionein 1M                                          | -4.67        | 2.49 \times 10^{-35} | Negative regulation of growth                                                                                | Zinc ion binding                                                               |
| TUBA3D     | Tubulin, alpha 3d                                           | -4.61        | 1.75 \times 10^{-39} | Microtubule-based movement                                                                                    | GTP binding; GTPase activity; structural molecule activity                     |
| TUBA3E     | Tubulin, alpha 3e                                           | -4.51        | 0.00            | Microtubule-based movement                                                                                    | GTP binding; GTPase activity; structural molecule activity                     |

HCM = hypertrophic cardiomyopathy; GTP = guanosine triphosphate; GTPase = hydrolase enzyme that binds to the nucleotide guanosine triphosphate; mRNA = messenger RNA; RAS = rat sarcoma; UTR = untranslated region.
ACE2 and HCM

Given the current and devastating coronavirus disease 2019 (COVID-19) pandemic (>2.5 million confirmed cases) that has claimed more than 172,000 lives worldwide in less than 4 months (April 21, 2020), it was noteworthy that the most up-regulated gene in HCM samples was ACE2 (3.53-fold; q-value = 1.30 × 10⁻²³). ACE2 protein was also increased more than 5-fold by Western blot analyses (P < .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 prohypertrophic polypeptides angiotensin I and angiotensin II to angiotensin-(1-9) and angiotensin-(1-7), respectively, thereby producing counter-regulating, vasodilating, and potentially antihypertrophic/antifibrotic polypeptides (Figure 3C).¹⁹

Accordingly, we speculate that up-regulation of both ACE2 transcript and ACE2 protein levels might be a compensatory counter-regulatory signaling response (pathoresponsive) in patients with obstructive HCM. This was echoed in a recent article by Liu et al²⁰ in which the investigators studied the transcriptome of HCM mouse models and found that profibrotic pathways initiated by an increase of endothelin-1 were the main drivers of HCM pathogenesis in mice through microRNA-29 and transforming growth factor β signaling. However, using our preliminary microarray data that were derived from the patients in our study and made publicly available (GSE36961), this differential expression of transformed growth factor β—signaling genes was not observed. Instead, the increased transcript levels of ACE2 were noted, prompting the speculation that ACE2 overexpression might be a compensatory response.²⁰

Last, ACE2, located in the X-chromosome, was upregulated significantly in female patients with HCM (1.33-fold compared with males; P < .01) and although only 1 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.²¹-²³

ACE2 and Its Possible Role in COVID-19–Related Morbidity and Mortality

Beyond the potential relevance of 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 severe acute respiratory syndrome coronavirus (SARS-CoV) family of viruses²⁴,²⁵ and thereby may contribute to the increased morbidity and mortality from SARS-CoV-2 in adult patients with a variety of heart diseases.²⁶ The currently endemic SARS-CoV-2 is a member of the SARS family of coronaviruses that bind to membrane-bound ACE2 through its...
viral spike protein.\textsuperscript{27} Recently, Zhou et al.\textsuperscript{27} assessed virus infectivity in HeLa cells transfected with ACE2 and demonstrated preferential binding to ACE2 over other coronavirus receptors, such as aminopeptidase N or dipeptidyl peptidase 4. After binding to ACE2, cleavage of the spike protein (possibly by the transmembrane protease-2 serine enzyme) primes viral internalization by endocytosis. This viral internalization may result in loss of membrane ACE2 and a subsequent increase in the angiotensin II to angiotensin-(1-7) ratio, which in turn allows excessive angiotensin II and unopposed angiotensin type 1 receptor—mediated lung injury in patients with COVID-19 infection and the development of severe acute respiratory distress syndrome.\textsuperscript{24,25}

ACE2 is expressed in many other tissues, including the intestinal and vascular epithelium, kidneys, and heart. However, expression in cardiomyocytes is low. In a study of cardiac cell samples from donor hearts, both ACE2 and transmembrane protease-2 serine showed the highest expression in the heart tissue’s pericyte subpopulation rather than in cardiomyocytes.\textsuperscript{28} Nevertheless, widespread ACE2 expression may contribute to the multiorgan dysfunction seen in patients with COVID-19 infection.\textsuperscript{29,30}

The marked 5-fold increase in ACE2 protein in HCM may provide a mechanism to

FIGURE 3. Marked accentuation of angiotensin-converting enzyme type 2 (ACE2) in hypertrophic cardiomyopathy (HCM). Bar diagram shows: (A) 5.3-fold increase in ACE2 protein in HCM patients compared with controls by Western blot analysis and (B) significant staining of ACE2 antibody in the myectomy specimen from a patient with obstructive HCM. Flow chart shows (C) the role of ACE2 in converting angiotensin (ang) I to ang (1-9) and angiotensin II to ang (1-7) to counter the effects of angiotensin II. SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.
explain higher rates of severe outcomes in COVID-19–infected patients who also have cardiovascular comorbid conditions, as well as the direct cardiac damage caused by SARS-CoV-2 infections. Although the incidence of COVID-19 infection seems highest in the elderly or immunocompromised, a large number of affected patients and those requiring hospitalization have significant comorbid conditions, including highly prevalent cardiovascular diseases such as HTN or CHF. A large meta-analysis of more than 46,000 patients in China showed that the most common comorbid conditions were HTN (17%±7%; 95% CI, 14% to 22%), diabetes mellitus (8%±6%; 95% CI, 6% to 11%), and cardiovascular disease (5%±4%; 95% CI, 4% to 7%). These percentages were much higher in patients requiring hospitalization or even intensive care unit (ICU) admission. In 2 separate inpatient studies, preexisting HTN was present in 30% (and up to 60% for ICU or nonsurviving patients), as were concomitant cardiovascular disease (15%, increased to 13% to 25% in ICU or nonsurviving patients).32,33

There is mounting evidence for cardiotropism with SARS-CoV-2 infection and direct cardiac toxicity. In early studies, 7.2% of all COVID-19–infected patients and 22% of patients admitted to ICUs showed evidence of myocardial injury with elevated high-sensitivity troponin I levels 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 an increase of damaging angiotensin II and angiotensin type 1 receptor activity and loss of the protective effects of angiotensin-(1-7) (Figure 3).71 Just recently, Liu et al34 proposed a similar hypothesis, describing the resulting unopposed increase in angiotensin II and subsequent downstream increase in detrimental inflammation, reactive oxygen species, vasoconstriction, and thrombosis as the basis for cardiac damage stemming from SARS-CoV-2 infection. Similarly, Chen et al35 describe this as a potential mechanism, illustrated by ACE2 overexpression in tissue from heart failure patients.

The effect of ACEIs or ARBs is under active investigation. Although initial reports suggested potentially worse outcomes in patients with COVID-19 infection who were using ACEIs,36,37 a subsequent review article concluded that there was insufficient evidence for this claim, prompting all major cardiac societies to advise that patients with heart disease treated with these medications should continue to use them as prescribed.38 However, ARBs could attenuate the impact of SARS-CoV-2 by blocking the damaging effects resulting from a viral-mediated decrease in ACE2 and subsequent increase in damaging angiotensin II level. 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.24 Furthermore, a large study among 1128 patients with HTN and diagnosed with COVID-19 infection showed that the unadjusted mortality rate was significantly lower in those for whom HTN was treated with ACEi/ARBs (3.7% vs 9.8%; P=.001). This risk remained consistently lower when performed as a propensity score–matched analysis with adjustment of imbalanced variables such as age, sex, comorbid conditions, and in-hospital medications (adjusted hazard ratio, 0.37; 95% CI, 0.15-0.89; P=.03).39

In light of these findings, a clinical trial has been launched testing the effect of losartan in study-eligible patients with COVID-19 infection (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,40 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 patients with HCM undergoing therapeutic surgical septal myectomy, representing 1 phenotypic subset of HCM. However, this study design has therefore an inherent bias because this procedure provides 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 left ventricular outflow tract obstruction. Whether ACE2 elevation is present in the hearts of patients with

FIGURE 4. Angiotensin-converting enzyme type 2 (ACE2) overexpression and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Central illustration shows possible mechanism behind ACE2 overexpression and SARS-CoV-2 infection picturing normal ACE2 expression (left) and ACE2 protein overexpression in obstructive hypertrophic cardiomyopathy (HCM; 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 in surface ACE2. Loss of surface ACE2: (1) increases the angiotensin II to ang (1-7) ratio and (2) 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 such as obstructive HCM, the speculated increase in viral infectivity of the heart muscle remains to be proven. (Portion of figure adapted from: Simmons G, Zmora P, Gierer S, Heurich A, Pöhlman S. Proteolytic activation of the SARS-coronavirus spike protein: Cutting enzymes at the cutting edge of antiviral research. Antivir Res. 2013;100(3):605-614 with permission from Elsevier, license number 4814880904484).
nonobstructive 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). Although confounding of specimen source and case/control status must be considered as a possible explanation for the results herein, because these are patients from a different ethnic 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.

CONCLUSIONS
Using a high-throughput gene expression profiling technology, we discovered that greater than 20% of the transcriptome is expressed differentially between HCM and control heart tissue, whereas 5% of transcriptomic changes differ within the 3 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—infected patients with underlying cardiovascular diseases. However, it remains to be demonstrated whether nonobstructive HCM, other cardiomyopathies, other acquired cardiovascular diseases, or HTN precipitate overexpression of ACE2 protein in the heart as shown here for obstructive HCM. Whether anARB such as losartan or delivery of an ACE2 decoy with hrsACE2 might be effective therapeutic strategies for COVID-19—infected patients with ACE2-accentuating diseases such as obstructive HCM warrants further investigation.

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Drs Bos and Hebl contributed equally to this manuscript and should be considered co-equal first authors.

SUPPLEMENTAL ONLINE MATERIAL
Supplemental material can be found online at http://www.mayoclinicproceedings.org. Supplemental material attached to journal articles has not been edited, and the authors take responsibility for the accuracy of all data.

Abbreviations and Acronyms: ΔCt = transcript of interest minus GAPDH control; ACE2 = angiotensin I converting enzyme 2; ACEi = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; AT1R = angiotensin type 1 receptor; BP = blood pressure; cDNA = complementary DNA; CHF = congestive heart failure; COVID-19 = coronavirus disease 2019; ECG = electrocardiogram; GTP = guanosine triphosphate; HCM = hypertrophic cardiomyopathy; hrsACE2 = human recombinant soluble angiotensin I converting enzyme 2; HTN = hypertension; ICU = intensive care unit; IQR = interquartile range; LV = left ventricular; MIG = maximum instantaneous gradient; mRNA = messenger RNA; MYBPC3 = myosin binding protein C; MYH7 = beta myosin heavy chain; NA = not available; NS = not significant; NYHA = New York Heart Association; qRT-PCR = quantitative real-time polymerase chain reaction; RAAS = renin-angiotensin-aldosterone system; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SCD = sudden cardiac death; UTR = untranslated region

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