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CLINICAL RESEARCH

Dynamic Regulation of SARS-Cov-2 Binding and Cell Entry Mechanisms in Remodeled Human Ventricular Myocardium

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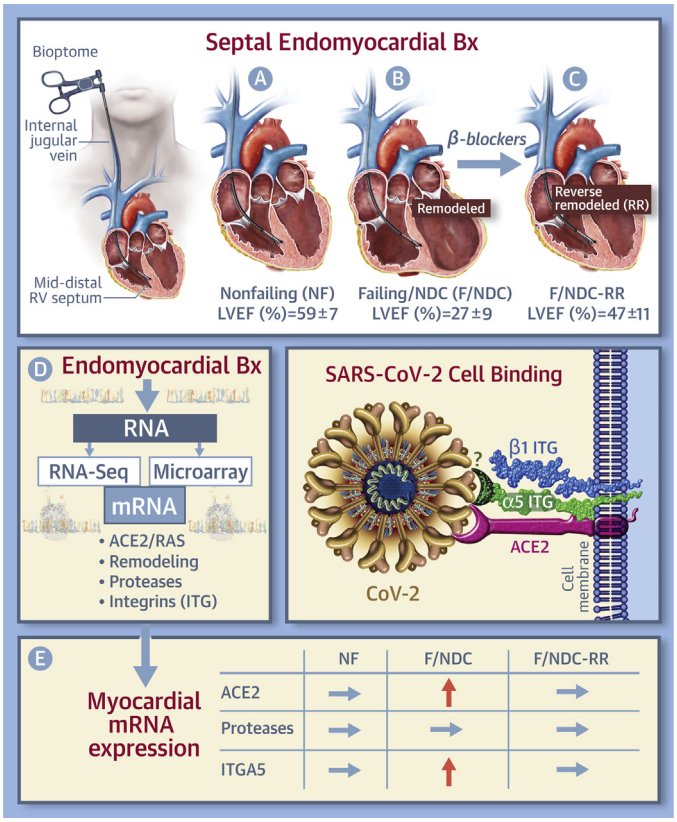
VISUAL ABSTRACT



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HIGHLIGHTS

- The CoV-2 cellular receptor *ACE2* and 5 proteases implicated in fusion of virus and cell membranes that are vital to cell entry were expressed at the mRNA level in RNA extracted from septal EmBx of patients with F/NDC and NF control patients.
- *ACE2* was up-regulated by 1.97 fold in 46 patients with F/NDC compared with NF control patients, but proteases showed similar degrees of expression.
- On LV reverse remodeling effected by beta-blocking agents, ACE2 expression, in the presence of unchanged doses of ACE inhibitors or ARBs, down-regulated into the normal range.
- *ITGA5*, which encodes an integrin that binds to ACE2 and to a motif in the CoV-2 spike protein binding domain, was expressed in both NF control subjects and subjects with F/NDC, was up-regulated in the latter at baseline, was decreased in expression on reverse remodeling similar to *ACE2*, and is a candidate for facilitating CoV-2 binding and cell entry in LV myocardium.

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ABBREVIATIONS AND ACRONYMS

ACE = angiotensin converting enzyme

ACE2 = angiotensin converting enzyme 2

ARB = angiotensin receptor blocker

BNP = B-type natriuretic peptide

COVID-19 = coronavirus disease-2019

EmBx = endomyocardial biopsies

F/NDC = nonischemic dilated cardiomyopathy with heart failure

HFrEF = heart failure with reduced (<0.50) left ventricular ejection fraction

IQR = interquartile range

LOCF = last observation carried forward

LV = left ventricle (ventricular)

LVEF = left ventricular ejection fraction

mRNA = messenger ribonucleic acid

NF = nonfailing

NR = nonresponder

PCR = polymerase chain reaction

R = responder

RAS = renin-angiotensin system

RGD = arginine-glycine-aspartic acid

RNA-Seq = ribonucleic acid sequencing

RV = right ventricle (ventricular)

SARS-CoV-2 = severe acute respiratory syndrome-coronavirus-2

SUMMARY

Using serial analysis of myocardial gene expression employing endomyocardial biopsy starting material in a dilated cardiomyopathy cohort, we show that mRNA expression of the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) cardiac myocyte receptor ACE2 is up-regulated with remodeling and with reverse remodeling down-regulates into the normal range. The proteases responsible for virus-cell membrane fusion were expressed but not regulated with remodeling. In addition, a new candidate for SARS-CoV-2 cell binding and entry was identified, the integrin encoded by *ITGA5*. Up-regulation in ACE2 in remodeled left ventricles may explain worse outcomes in patients with coronavirus disease 2019 who have underlying myocardial disorders, and counteracting ACE2 up-regulation is a possible therapeutic approach to minimizing cardiac damage. (J Am Coll Cardiol Basic Trans Science 2020;5:871–83)

The 2020 coronavirus disease-2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome-coronarvirus-2 (SARS-CoV-2) has many unique clinical features, including high infectivity, a protean clinical presentation and course, and life-threatening potential (1). Myocardial involvement is an important pathophysiologic component of some critically ill patients with CoV-2 infections (2–6), either due to myocarditis (5,6) or myocardial dysfunction without evidence of inflammation (2–4). The prevalence of cardiac complications in patients without underlying heart disease ranges from 20% to 30% (7–9), which when present worsens prognosis. In the initial report of patients receiving intensive care, evidence of cardiac injury was associated with a 50% mortality compared with a <10% mortality without such evidence (3). The prognosis worsens further when evidence of myocardial injury is superimposed on pre-existing cardiovascular disease, with mortality rising to above 60% (3).

A well-established pathway by which the CoV-2 or the original SARS-CoV virus gains entry into cells includes membrane attachment by binding to angiotensin converting enzyme 2 (ACE2) (10,11), fusion of viral and cell surface membranes through the recruitment of host proteases (12–14), and virus internalization followed by assembly of cytoplasmic membranous structures into replication vesicles (15). In addition, there are other possible mechanisms of virus internalization such as binding to integrins (16,17), some of which also bind to ACE2 (18,19). Although CoV-2 or CoV cell internalization has been investigated in model systems (10–15) and is beginning to be evaluated in the human heart (20), it is unclear whether the virus enters human cardiac myocytes and whether the necessary biologic constituents are expressed in the heart. Of particular importance is the role of ACE2; in human ventricular myocardium, ACE2 is a highly functional enzyme present in cardiac myocytes (20) that breaks down angiotensin II to the counter-regulatory peptide angiotensin-(1-7) (21,22). In explanted human heart preparations from patients with end-stage heart failure with reduced (<0.50) left ventricular ejection fraction (HFrEF), ACE2 enzyme activity (22) as well as gene expression at the messenger ribonucleic acid (mRNA) (20,23) and protein (20,22) levels are up-regulated compared with organ donor control subjects. This is potentially important because up-regulated ACE2 might be a mechanism by which CoV-2 myocardial involvement is more prominent in patients with underlying heart muscle disease (20).

However, it is unclear whether ACE2 is up-regulated in intact hearts with less severe pathologic

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

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TABLE 1 Baseline Characteristics

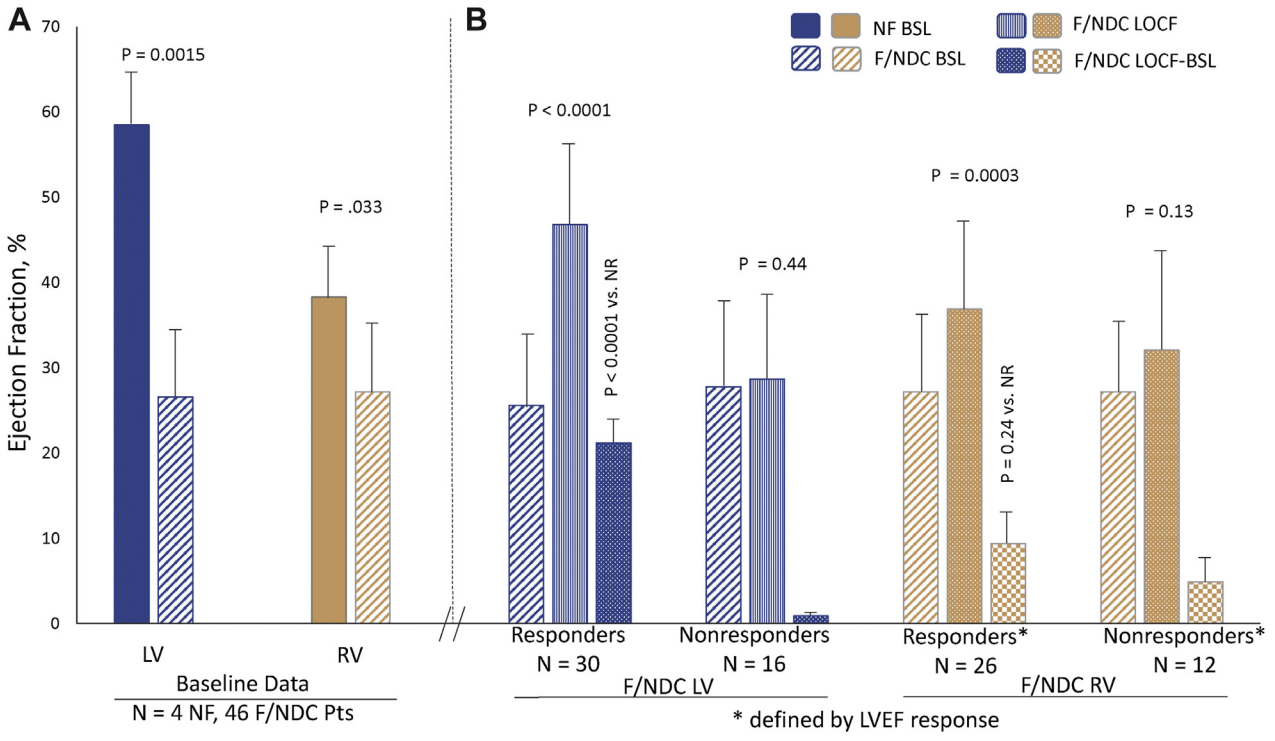
	NF (n = 4)	F/NDC (n = 46)	p Value NF vs. F/NDC	F/NDC R (n = 30)	F/NDC NR (n= 16)	p Value R vs. NR
Age, yrs	41.0 ± 17.1	45.6 ± 13.2	0.63	43.5 ± 13.2	49.7 ± 12.3	0.12
Sex			0.94			0.72
Male	3	33		21	12	
Female	1	13		9	4	
Race, ethnicity			0.15			0.35
White	4	30		21	9	
Black		6		4	2	
Hispanic		7		4	3	
Asian		2			1	
Native American		1		1	1	
Diabetes	0 (0)	8 (17)	0.36	5 (17)	3 (19)	0.86
Hypertension history	2 (50)	18 (39)	0.43	15 (50)	3 (19)	0.039
BSA, m^2	2.20 ± 0.42	1.97 ± 0.22	0.58	1.97 ± 0.22	1.96 ± 0.21	0.89
BMI, kg/m^2	30.9 ± 8.1	28.9 ± 5.7	0.67	29.0 ± 6.3	28.9 ± 4.8	0.99
Creatine clearance, mg/dl	87.7 ± 7.9	80.2 ± 22.5	0.18	85.3 ± 18.8	71.0 ± 26.2	0.066
HF failure duration, months	–	23.9 ± 45.9	–	7.0 ± 10.0	55.7 ± 67.0	0.011
Atrial fibrillation	1 (25)	10 (22)	0.88	4 (13)	6 (38)	0.058
NHYA functional class			0.87			0.55
I	2					
II	2	25		16	10	
III		21		14	6	
Heart rate, beats/min	77 ± 9	84 ± 21	0.24	87 ± 21	79 ± 20	0.21
SBP, mm Hg	118 ± 29	107 ± 14	0.51	106 ± 13	109 ± 17	0.51
LVEF, %	58.8 ± 7.4	26.6 ± 8.7	0.002	25.6 ± 8.2	27.8 ± 10.0	0.51
RVEF, %	38.3 ± 4.7	27.2 ± 9.0	0.032	27.2 ± 8.8	27.2 ± 9.7	0.99
LV end-diastolic volume, ml	–	232 ± 93	–	220 ± 83	255 ± 111	0.38
PWP, mm Hg	7 ± 6	12 ± 8	0.12	11.3 ± 8.7	14.5 ± 7.7	0.21
PAP, mm Hg	20 ± 2	24 ± 11	0.13	22.4 ± 9.9	27.1 ± 11.5	0.18
Cardiac index, $l/min/m^2$	2.8 ± 0.6	2.2 ± 0.6	0.10	2.3 ± 0.7	2.2 ± 0.6	0.74
Mixed venous NE, pg/ml	372 ± 152	492 ± 331	0.44	452 ± 267	574 ± 438	0.39
Mixed venous BNP, pg/ml	90 ± 46	234 ± 234	0.035	203 ± 240	334 ± 200	0.21
On ACE inhibitors,	4	44	0.59	27	14	0.75
Doses (enalapril equivalents), mg	6.6 ± 9.0	9.3 ± 6.1		8.8 ± 5.0	8.2 ± 5.0	
On ARBs		1, 1 missing		3	2	0.15
Doses (losartan equivalents), mg		50		46.9 ± 38.7	87.5 ± 17.7	
On beta-blockers						
Carvedilol	–	16	–	9	7	0.63
Doses, mg/day		75 ± 24		78 ± 23	71 ± 27	
Metoprolol succinate	–	30	–	21	9	0.33
Doses, mg/day		169 ± 55		176 ± 52	153 ± 62	

Values are mean ± SD, n (%), or n. All doses are in mg/day. For definitions of responder, nonresponder see the Methods section.

ACE = angiotensin converting enzyme; ARB = angiotensin receptor blocker; BMI = body mass index; BNP = B-type natriuretic peptide; BSA = body surface area; F/NDC = nonischemic dilated cardiomyopathy with heart failure group; HF = heart failure; LV = left ventricle; LVEF = left ventricular ejection fraction; NE = norepinephrine; NF = nonfailing group; NR = nonresponder; NYHA = New York Heart Association; PAP = pulmonary artery pressure; PWP = pulmonary wedge pressure; R = responder; RVEF = right ventricular ejection fraction; SBP = systolic blood pressure.

remodeling/dysfunction than in explanted hearts from cardiac transplant recipients, who in contrast to most organ donor control subjects have been extensively treated with renin-angiotensin system (RAS) inhibitors that may affect *ACE2* expression (24,25). In addition, it is not clear whether the proteases that prime and facilitate membrane fusion are expressed or regulated in the remodeled, failing human heart. Finally, there is limited information on the status of integrins, particularly those that can bind to ACE2 or to the CoV-2 virus itself, to potentially effect virus-cell internalization (16). To obtain information on these mechanisms in nonfailing (NF) and remodeled intact human hearts, we analyzed data from a serial analysis of myocardial gene expression cohort study (26), where reverse remodeled left ventricles (LVs)

FIGURE 1 LVEF and RVEF Data



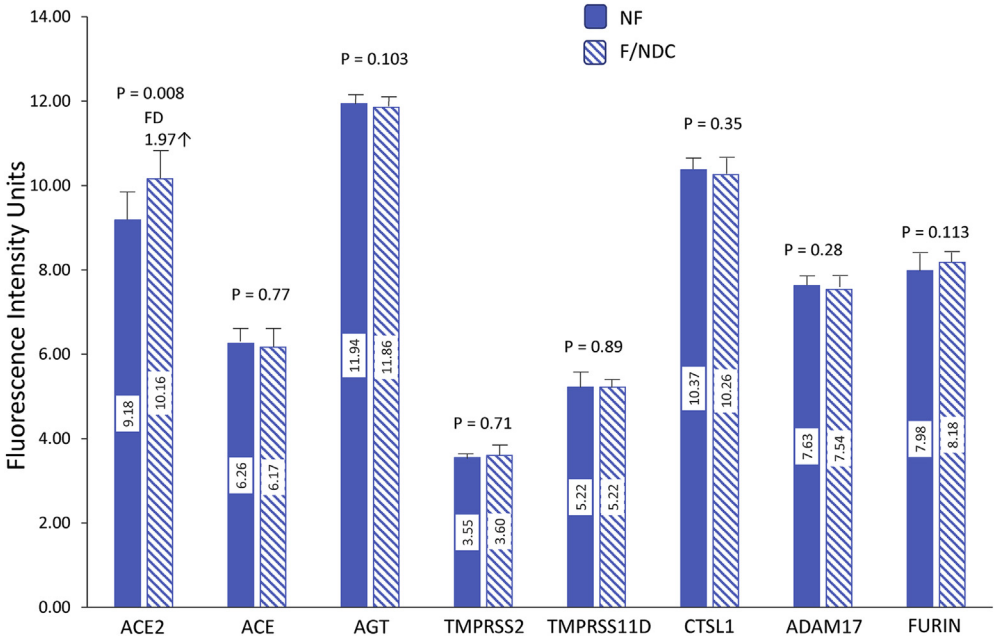
(A) Baseline (BSL) data in nonfailing (NF) control subjects and in patients (Pts) with heart failure from nonischemic dilated cardiomyopathy (F/NDC). **(B)** Data for patients with F/NDC who were treated for 3 or 12 months with beta-blocking agents to effect reverse remodeling in 30 responders and 16 nonresponders (NRs). Left ventricular ejection fraction (LVEF) and right ventricular ejection fraction (RVEF) are given in absolute percentages. Values are plotted as baseline values, last observation carried forward (LOCF) (n = 8 at 3 months, n = 38 at 12 months) and the LOCF-BSL difference calculated for each patient and averaged. Responders and nonresponders are defined by LVEF response as described in the Methods section.

were compared with unchanged ventricles exposed to the same pharmacologic regimen.

METHODS

PATIENT MATERIAL AND PROTOCOL. Control subjects were 4 individuals with left ventricular ejection fractions (LVEFs) ≥50% (mean: 59 ± 7%) who had endomyocardial biopsies (EmBx) to rule out myocarditis or other infiltrative processes and had no histopathologic abnormalities. Patients with HFrEF and pathologic eccentric remodeling consisted of 47 patients with LVEFs ≤40% and New York Heart Association functional class II or III heart failure from nonischemic dilated cardiomyopathy with heart failure (F/NDC) of uncertain etiology. These patients had right ventricular (RV) mid-distal interventricular septum EmBx performed at baseline for diagnostic and research purposes, and then after 3 and 12 months of beta-blocker therapy for research material only, as previously described (26,27) in the BORG (Effect of Beta-blockers on Structural Remodeling and Gene Expression in the Failing Human Heart) study (NCT01798992). The 4 subjects with normal LV function and 46 of the 47 patients with NDC (mean LVEF: 26.6 ± 8.7%) had technically adequate global gene expression measurements by microarray in extracted RNA, as previously described (26). Beta-blockers were initiated and up-titrated to target doses (26,27), reaching mean doses at the last EmBx and EF measurements of 75 ± 17 mg/day for 16 patients treated with carvedilol, and 169 ± 55 mg/day for 30 patients treated with metoprolol succinate. The clinical study was designed to detect differences in gene expression mediated through blockade of individual β_1-, α_{1A}- and β_2-adrenergic receptors, but no systematic changes between treatment groups were detected (27) and the 3 groups, who had in common

FIGURE 2 RAS and Protease mRNA Expression



Renin-angiotensin system (RAS) and protease messenger ribonucleic acid (mRNA) expression, by microarray in fluorescence units (a logarithmic signal) ± SD. Data supplied for 4 NF control subjects and 46 patients with F/NDC. FD = fold difference; other abbreviations as in Figure 1.

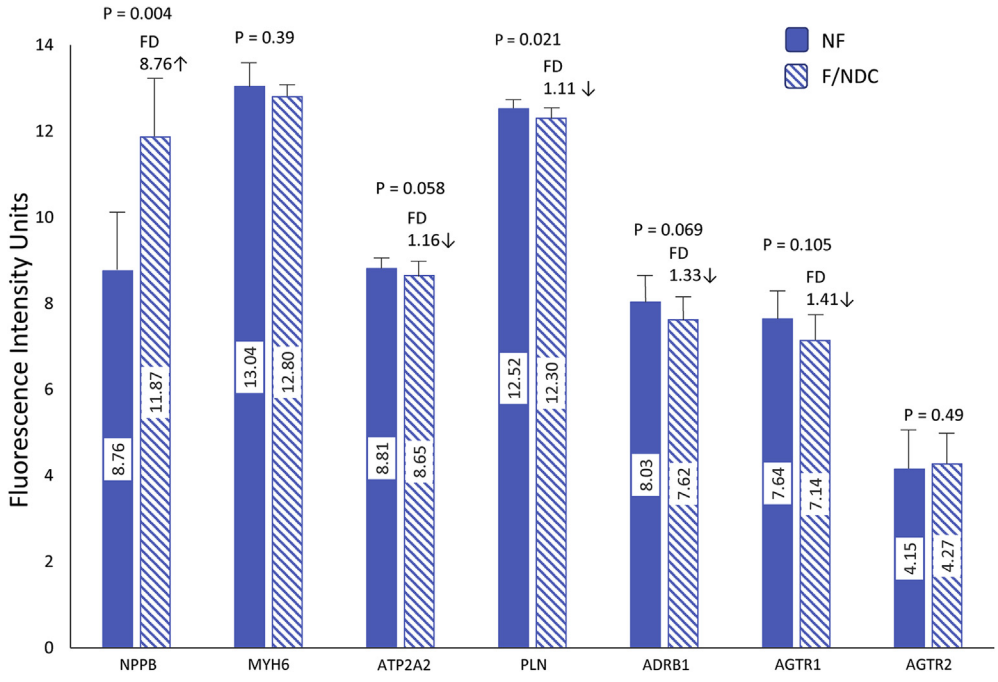
blockade of β_1- adrenergic receptors, were combined into 1 cohort.

Eight patients had only a 3-month EmBx (1 sudden cardiac death, 1 LV assist device placement, 1 traumatic injury unrelated to the study that precluded compliance with the protocol, 5 withdrawals before 12 months), and their results were grouped with the 12-month studies in a last observation carried forward (LOCF) analysis. In these 8 subjects in the LOCF analysis, the degree of reverse remodeling was not statistically significantly different from patients with 12-month measurements (respective improvements in LVEF of 16 ± 5% [given as absolute percentage] vs. 22 ± 4%; p = 0.28). In addition to microarray, global gene expression was also measured by ribonucleic acid sequencing (RNA-Seq), in 6 "super-responders" with LVEF increases of ≥10 absolute percent compared with 6 sex- and age-matched subjects with LVEF increases of <5 absolute percent (Supplemental Appendix and Supplemental Table S1) (26,28). LVEF was measured by radionuclide single-photon emission computed tomography imaging as previously described (27,29), and a reverse remodeling responder (R) in the entire 46-patient F/NDC cohort was defined as an LOCF increase in LVEF of ≥5 absolute percent at 3 months or ≥8% at 12 months (26,27). Nonresponders (NRs) were subjects who did not meet these LVEF change criteria. EmBx and right heart catheterization were performed as previously described (26,27), and there were no procedure-related complications.

All patients signed written consent for this multicenter study conducted at the University of Colorado Anschutz Medical Campus and the University of Utah Medical Center. The study included a data and safety monitoring board and was approved by the institutional review boards at both sites.

RNA EXTRACTION AND mRNA EXPRESSION MEASUREMENTS. RNA extraction was performed as previously described (26,27). All individual patient mRNA measurements were from the same RNA extraction, typically involving 2 to 5 separate EmBx samples per study. Extracted RNA was stored at −80°C until use, and microarray and RNA-Seq methodologies were as previously described (26). Microarray gene expression data were normalized by log-scale robust multi-microarray analysis to yield output in fluorescence intensity on an exponential scale. RNA-Seq data transcript levels were quantified

FIGURE 3 **mRNA Expression of Genes Associated With Remodeling**



The mRNA expression of genes associated with remodeling is presented. See **Figure 2** for further description. Abbreviations as in **Figures 1 and 2**.

as fragments per kilobase of exon per million mapped reads, as previously described (26). Fold change or fold difference was calculated by $\log_2$ transformation as described (26).

DATA ANALYSIS AND STATISTICAL TESTS. We used both baseline comparisons between NF control subjects and patients with F/NDC plus changes from baseline in patients with F/NDC to construct an ordered classification of 4 degrees of remodeling association (Supplemental Appendix, Supplemental Table S2). For baseline studies, where only microarray data were available, alpha was set at <0.05. As described in the Supplemental Appendix (Supplemental Table S2), in serially evaluated patients with F/NDC these results were then linked to the reverse remodeling mRNA results measured by both the microarray and RNA-Seq platforms. The estimated alpha levels based on achieving $p < 0.05$ in more than 1 condition including directionality requirements are given in the Supplemental Appendix (Supplemental Table S2): *unequivocal evidence,* 0.0001; *evidence*, 0.002; and *possible evidence*, 0.08. The statistical significance of all gene expression data was analyzed in the same way, by nonparametric methods using Wilcoxon rank sum or signed rank tests. Correlation analysis was by Spearman rho LVEF data for which evidence of non-normal distribution was absent (26), and baseline characteristics were analyzed by Student's *t*-tests or contingency table analysis.

Because several analyzed gene expression groups had small counts (NF, n = 4; RNA-Seq, n = 6 R and 6 NR; LOCF 3-month F/NDC, n = 5 Rs and 3 NRs) we used mean ± SD or, for change values, SEM as estimates of central tendency and dispersion, in both the small count groups and larger groups to which these data were compared. For microarray data (30 R and 16 NR), group size was sufficient for data to be also presented as median (interquartile range [IQR]). Thus in gene expression analyses conducted exclusively in groups where sample sizes were >6 nonparametric statistics and median (IQR) data are presented, but when smaller gene expression group sizes were analyzed or compared, nonparametric significance tests plus mean ± SD or SEM are used.

R (R Foundation, Vienna, Austria), GraphPad Prism (GraphPad Software, La Jolla, California) and XLSTAT/Excel (Microsoft, Redmond, Washington) were the statistical software packages used.

TABLE 2 Change From Baseline Data, Microarray, and RNA-Seq

	Microarray						RNA-Seq						
	R		NR		R vs. NR		R		NR		R vs. NR		Level of Evidence Remodeling Association*
Gene	FC	p Value	FC	p Value	FC	p Value	FC	p Value	FC	p Value	FC	p Value	
RAS													
ACE2	0.675	<0.0001	1.144	0.60	0.591	0.0006	0.402	0.031	1.216	0.69	0.331	0.004	UE
ACE	1.040	0.65	1.02	0.67	1.016	0.95	1.065	0.69	1.087	0.44	0.980	0.44	NE
AGT	1.049	0.32	1.078	0.30	0.97	0.76	0.924	0.44	1.049	1.00	0.973	0.18	NE
AGTR1	1.479	0.003	1.253	0.13	1.181	0.44	1.814	0.031	1.404	0.031	1.293	0.18	PE
AGTR2	0.964	0.21	1.181	0.23	0.816	0.060	0.900	0.31	1.703	0.44	0.528	0.24	NE
Proteases													
TMPRSS2	1.000	0.84	1.000	0.63	1.000	0.62			Transcript not detected				NE
TMPRSS11D	0.943	0.044	1.017	0.99	0.928	0.23	0.977	1.00	2.149	0.59	0.452	0.84	NE
CTSL1	1.180	0.006	1.085	0.013	0.918	0.92	1.100	0.16	0.999	0.69	1.101	0.18	NE
ADAM17	1.018	0.56	1.025	0.67	0.993	0.92	0.865	0.094	1.042	0.44	0.830	0.65	NE
FURIN	1.043	0.82	0.952	0.22	1.10	0.55	0.912	0.31	9.938	0.31	0.972	0.48	NE
Remodeling													
NPPB	0.522	<0.0001	1.783	0.38	0.295	0.0015	0.078	0.031	1.404	0.84	0.055	0.009	UE
MYH6	1.171	0.052	0.821	0.002	1.426	0.0002	2.426	0.062	0.885	0.56	2.742	0.015	E
ATP2A2	1.050	0.077	0.989	0.60	1.062	0.056	1.388	0.031	1.005	1.00	1.044	0.31	PE
PLN	1.108	0.001	0.986	0.43	1.123	0.006	1.281	0.22	1.034	0.44	1.239	0.24	E
ADRB1	1.335	0.001	1.124	0.53	1.187	0.16	1.596	0.031	1.084	0.84	1.473	0.041	E
Reference													
GAPDH	1.026	0.99	0.983	0.30	1.044	0.69	0.994	0.84	1.071	0.44	0.928	0.48	NE

FC values are calculated from mean values as described in the methods; values <1.0 indicate a decrease in expression and >1.0 an increase. The p values are calculated by Wilcoxon rank sum of messenger ribonucleic acid readouts as described in the methods. *GAPDH* baseline NF and F/NDC were, respectively, 13.45 ± 0.08 and 13.40 ± 0.13 fluorescence units; p = 0.69. *Level of evidence of remodeling association is as described in the Supplemental Appendix and Supplemental Table S2.

E = *evidence*; FC = $\log_2$ fold change; NE = no evidence; PE = possible evidence; RAS = renin-angiotensin system; RNA-Seq = ribonucleic acid sequencing; UE = unequivocal evidence; other abbreviations as in Table 1.

RESULTS

BASELINE CHARACTERISTICS. Baseline characteristics for the 46 subjects with F/NDC and 4 NF control subjects are given in Table 1. At baseline, the F/NDC and NF characteristics were similar, except for measurements and biomarkers of ventricular dysfunction and remodeling. In patients with F/NDC, LVEF was more reduced than RVEF (Table 1, Figure 1A), but both were $p < 0.05$ versus NF. Right heart catheterization data trended abnormal in F/NDC but no measurement was $p < 0.05$ versus NF. BNP was elevated in patients with F/NDC compared with in NF control subjects ($p = 0.035$), but norepinephrine was not significantly different ($p = 0.44$). These data describe a relatively young (mean age 45.6 ± 13.2 years), well-compensated HFrEF population with moderate LV dysfunction and remodeling (LVEF: 27.2 ± 9.0%, LV end-diastolic volume: 232 ± 93 ml). The baseline characteristics of the R and NR groups are also given in Supplemental Table S3. A markedly greater duration of heart failure in NRs versus Rs (55.7 ± 67.0 months vs. 7.0 ± 10 months, respectively; $p = 0.011$) was the only difference.

The protocol mandated angiotensin converting enzyme (ACE) inhibitor background therapy and diuretic agents as needed. Spironolactone was administered as tolerated, and an angiotensin receptor blocker (ARB) could be substituted in patients who are ACE inhibitor-intolerant. Table 1 gives the ACE inhibitor and ARB doses in enalapril and losartan equivalents (30), at both baseline and the average dose during the 12-month study. All 4 of the NF control subjects were on ACE inhibitors for suspected and ultimately unproved heart muscle disease, and 44 of the 46 patients were on an ACE inhibitor at baseline. There is no difference in ACE inhibitor dose (in mg) between patients with F/NDC and NF control patients, and no difference between reverse remodeling Rs and NRs in average dose of ACE inhibitors or ARBs.

REVERSE REMODELING AS MEASURED BY LVEF AND RVEF OVER 3 OR 12 MONTHS. Figure 1B plots baseline, LOCF (8 patients at 3 months and 38 at 12 months), and the LOCF – baseline change for both LVEF and RVEF, by R and NR groups in the F/NDC cohort. For Rs versus NRs, there is a marked increase

in LVEF (by 21.2 ± 1.8 [SEM absolute percent] vs. 0.9 ± 0.8%; $p < 0.0001$) and a lesser, nonsignificant increase in RVEF (respectively, 9.4 ± 2.1% vs. 4.9 ± 3.1%; $p = 0.24$). Remodeling data for the selected super-responder subcohort and control subjects are in the Supplemental Appendix (Supplemental Table S1), and follow the same pattern, except that the LVEF change from baseline was larger than in the entire cohort (30.7 ± 4.2% vs. 21.2 ± 1.8%; $p = 0.035$).

BASELINE CHANGES IN mRNA EXPRESSION IN PATIENTS WITH NDC VERSUS NF CONTROL SUBJECTS. ACE2, other RASs, and proteases. Figure 2 contains F/NDC versus NF baseline mRNA abundance data, for *ACE2*, 2 associated RAS genes (*AGT* and *ACE*), and 5 proteases that have been implicated in CoV-2 or CoV-2–cell membrane priming and fusion (12–14,31,32). Compared with NF control subjects, *ACE2* is substantially up-regulated in F/NDC, by 1.97-fold ($p = 0.008$). *ACE* expression is unchanged from control, as is angiotensinogen. The angiotensin II type 1 and type 2 receptors (*AGTR1*, *AGTR2*) are plotted in **Figure 3** and are not different between NF and F/NDC. None of the proteases shown in **Figure 2** exhibit differences in mRNA expression in F/NDC versus NF, and only 1 (down-regulation of cathepsin L-like 3) of 11 additional proteases (Supplemental Appendix and Supplemental Table S3) exhibited any change in the F/NDC group.

Remodeling-associated genes. At baseline, *NPPB*, *PLN*, and *ATP2A2* exhibited changes similar to those in previously reported reverse transcription polymerase chain reaction (PCR) data (27). *NPPB* was up-regulated by 8.8-fold in F/NDC, and its expression versus that of *ACE2* was significantly related (Spearman rho = 0.73; $p < 0.0001$). Unlike in previous studies using reverse transcriptase PCR (26,27,33,34), *MYH6* was unchanged between NF and F/NDC but was markedly up-regulated on reverse remodeling (**Table 2**) consistent with previously reported data (26,27,34).

Integrins. **Table 3** contains NF versus F/NDC baseline data for 10 integrins previously reported to bind to ACE2 (18,19), facilitate viral internalization (17), or be associated with LV remodeling (35) or cardiac myocyte injury protection (36). Only 1, the laminin binding integrin ITGA7, has not been reported to be involved in pathogenic virus cell internalization. Five integrins in **Table 3** bind to the arginine-glycine-aspartic acid (RGD) motif, recently identified in the CoV-2 spike protein binding domain (16) and used by multiple viruses for binding to integrins. Of the ACE2 binding integrins, *ITGA5* expression was 1.28-fold higher in patients with F/NDC compared with NF control subjects ($p = 0.039$), whereas *ITGB1* and *ITGA2* were not different. *ITGA5* has also been associated with virus internalization (17) and LV remodeling where it decreases with LV assist device treatment (35). Of the 6 non-ACE2 binding integrins listed in **Table 3** that have been associated with virus internalization, 4 reached a p value of <0.05 for differences between patients with F/NDC and NF control subjects, 2 up-regulated (*ITGB3* and *ITGA4*) in patients with F/NDC and 2 down-regulated (*ITGB6* and *ITGA6*). For the 3 genes previously associated with remodeling other than *ITGA5*, 2 (*ITGA6* and *ITGB6*) were down-regulated in patients with F/NDC consistent with the decreased expression of *ITGA6* in human LVs with dilated cardiomyopathies (35), or the up-regulation previously reported with LV assist device treatment (35). The laminin binding integrin ITGA7 was up-regulated in patients with F/NDC.

CHANGES IN mRNA EXPRESSION ON REVERSE REMODELING. ACE2, other RASs, and proteases. Changes in R or NR fold changes based on mean values are given in **Table 2**, whereas median (IQR) values for microarray data are presented in Supplemental Tables S3 and S4. In microarray measurements, the near 2-fold up-regulated *ACE2* at baseline was down-regulated as LV remodeling improved, declining to 0.675-fold (an approximate 1.5-fold decrease; $p < 0.0001$). Three other RAS genes (*ACE*, *AGT*, and *AGTR2*) and only 1 of the proteases shown in **Table 2** exhibited changed expression on reverse remodeling. The protease that was down-regulated in patients with F/NDC (cathepsin L-like gene 3) was not changed with reverse remodeling (fold change from baseline: 1.03; $p = 0.62$) (Supplemental Table S3). Although in the R versus NR comparison, *PRSS1* was down-regulated on reverse remodeling (fold change: 0.90; $p = 0.042$) (Supplemental Table S3) the change was related to an up-regulation in the NR group rather than a change in the R group. *AGTR1* met criteria for *possible evidence* ($p < 0.08$) for an association with remodeling, with R and not NR up-regulation on microarray measurements. RNA-Seq data exhibited a decrease in *ACE2* expression on reverse remodeling ($p = 0.004$), with a greater fold change of 0.40-fold (approximate 2.5-fold decline) than in microarray data. Thus, *ACE2* met criteria for *unequivocal evidence* ($p < 0.0001$) of a remodeling association. RNA-Seq data for all other RAS and protease genes (**Table 2**, Supplemental Table S4) were similar to microarray measurements.

TABLE 3 Analysis of Baseline and Serial/Reverse Remodeling Integrins Gene Expression Data

	Baseline mRNA Expression (Array Fluorescence Units)				Array Change From Baseline						RNA-Seq Change From Baseline						
					R		NR		R vs. NR		R		NR		R vs. NR		
Gene	NF (Mean ± SEM)	F/NDC (Mean ± SEM)	FD, F/NF	p Value	FC	p Value	FC	p Value	FC	p Value	FC	p Value	FC	p Value	FC	p Value	Level of Evidence Remodeling Association*
ACE2 binding, virus internalization																	
ITGB1†	8.09 ± 0.05	7.90 ± 0.23	0.88	0.13	0.973	0.18	1.020	0.82	0.954	0.29	0.769	0.031	1.055	0.44	0.729	0.15	NE
ITGA2	5.16 ±0.86	5.40 ± 0.57	1.18	0.46	1.273	0.15	1.082	0.60	1.198	0.52	1.595	0.22	1.069	0.84	1.492	0.31	NE
ACE2 binding, virus internalization, LV remodeling																	
ITGA5†	10.14 ± 0.21	10.49 ± 0.37	1.28	0.039	0.880	0.004	1.001	0.53	0.880	0.11	0.827	0.16	0.953	0.84	0.867	0.48	E
Virus internalization																	
ITGB3†	3.84 ± 0.04	4.00 ± 0.16	1.11	0.026	0.944	0.015	1.080	0.034	0.873	0.0007	Transcript not detected						E
ITGA4	3.50 ± 0.05	3.73 ± 0.22	1.18	0.030	0.985	0.73	1.032	0.98	0.954	0.74	1.188	0.84	1.176	0.44	1.010	1.00	PE
ITGA9	7.20 ± 0.13	7.27 ± 0.14	1.05	0.39	0.970	0.13	0.992	0.74	0.978	0.58	1.016	0.69	1.029	0.56	0.988	0.82	NE
ITGAV†	11.91 ± 0.13	11.95 ± 0.13	1.03	0.60	0.979	0.56	0.977	0.32	1.002	0.95	0.670	0.031	0.961	1.00	0.698	0.009	PE
Virus internalization, LV remodeling																	
ITGA6	7.86 ± 0.03	7.58 ± 0.21	0.83	0.009	1.033	0.67	0.988	0.98	1.046	0.43	1.019	1.00	1.154	0.22	0.883	0.39	PE
ITGB6†	5.62 ± 0.39	5.11 ± 0.25	0.70	0.034	1.292	<0.0001	1.070	0.53	1.208	0.002	1.295	0.44	1.020	0.56	1.270	0.39	E
Laminin binding, LV remodeling																	
ITGA7	9.75 ± 0.18	10.07 ± 0.24	1.24	0.044	0.913	0.015	1.032	0.53	0.885	0.026	0.749	0.031	1.013	0.84	0.740	0.065	UE

See Table 2 and the methods for description of how FC or FD are calculated, based on $\log_2$ transformation. The p values are from Wilcoxon rank sum tests of NF versus F/NDC microarray (array) fluorescence intensity or Wilcoxon signed rank tests of baseline versus 3- or 12-month last observed carried forward values (change from baseline). Responders and nonresponders are defined by LVEF response. *Level of evidence of remodeling association is as described in the Supplemental Appendix and Supplemental Table S2. †When dimerized, the gene contains binding motif for an arginine-glycine-aspartic acid sequence, a domain contained in the CoV-2 spike protein and in other viruses.

FD = fold difference; mRNA = messenger RNA; other abbreviations as in Tables 1 and 2.

Remodeling-associated genes. As expected, these genes generally changed their expression in directions opposite to their baseline levels (Table 2 and Supplemental Table S4). *NPPB*, which encodes a precursor of a counter-regulatory peptide considered the premier biomarker of pathologically remodeled ventricular myocardium in tissue (27,34) or plasma (37), exhibited expression behavior very similar to *ACE2* including an *unequivocal evidence* rating for remodeling association. *MYH6, PLN*, and *ADRB1* all had remodeling associated ratings of *evidence* ($p < 0.002$), whereas *ATP2A2* rated *possible evidence*.

Integrins. Reverse remodeling changes in integrins are given in Table 2 and Supplemental Table S4. For the $p < 0.05$ baseline-changed genes, *ITGB3, ITGB6*, and *ITGA5* had at least 1 platform showing significant change on reverse remodeling and thus qualified for *evidence* of a remodeling association (Table 3). *ITGA4* and *ITGA6* expression did not significantly change on reverse remodeling, and their baseline changes therefore qualified as *possible evidence*. For *ITGA7*, both microarray and RNA-Seq data met criteria for dynamic down-regulation of the baseline up-regulated expression and a rating of *unequivocal evidence* of a remodeling association, with a pattern identical to that of *ACE2* and *NPPB*. Thus, of the 10 integrins examined, 5 had at least some evidence of an association with remodeling.

DISCUSSION

IN PATIENTS WITH MILD-MODERATE F/NDC, MYOCARDIAL *ACE2* GENE EXPRESSION DYNAMICALLY REGULATES WITH LV REMODELING INDEPENDENT OF RAS INHIBITOR TREATMENT AND MAY EXPLAIN HEIGHTENED COVID-19 CLINICAL RISKS. Figure 4 illustrates the positioning and role of ACE2 within the renin-angiotensin-aldosterone system. In the human LV, this zinc-dependent carboxypeptidase catalyzes the conversion of the octapeptide angiotensin II to the counter-regulatory heptapeptide angiotensin-(1-7) with high activity and efficiency (21). ACE2 exerts a very important function in the heart, by reducing levels of angiotensin II as well as by generating a counter-regulatory peptide that activates MAS1 receptor pathway signaling through G_q and multiple downstream events including reducing extracellular signal related kinase 1/2 MAP kinase activation (38,39). We found that in a setting where RAS inhibitors were not a potentially confounding variable *ACE2* mRNA expression was substantially up-regulated in intact failing/remodeled ventricular myocardium, confirming and extending previous work in explanted human hearts (20,22,23). From its up-regulated expression in F/NDC at baseline, with reverse remodeling *ACE2* then down-regulated toward control values in measurements by both mRNA

platforms to yield an *unequivocal evidence* rating for remodeling association corresponding to a p value of <0.0001. In the current study, the source of starting material was interventricular septum that reflects molecular changes in either the RV (33) or LV (27), and the reverse remodeling changes were mostly confined to the LV. In addition, the mRNA expression of the 42% ACE2 homologous (40) enzyme ACE was not altered at baseline or on reverse remodeling. Angiotensinogen was similarly unchanged in failing/remodeled ventricular myocardium. The behavior of *ACE2* gene expression was analogous and substantially related (baseline values Spearman rho of 0.73; $p < 0.0001$) to that of *NPPB*, another counter-regulatory gene whose remodeling association rating was *unequivocal evidence*.

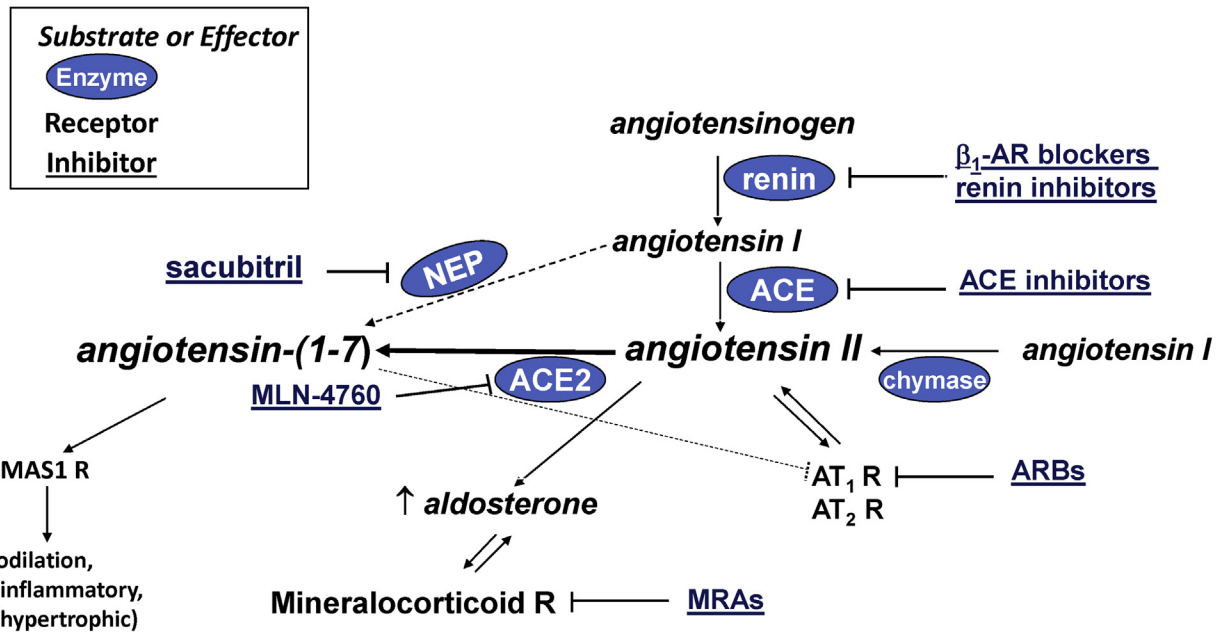
The up-regulation of *ACE2* in remodeled intact LV myocardium from subjects with only mild-moderate HFrEF indicates that this regulatory change is not confined to end-stage disease in patients who have been treated with RAS inhibitors (20,22,23), and suggests that increased expression of ACE2 in remodeled LVs could be the reason why patients infected with CoV-2 underlying heart muscle disorders are at heightened clinical risk (2,3).

PROTEASES THAT PARTICIPATE IN CoV-2–CELLULAR MEMBRANE PRIMING AND FUSION DO NOT EXHIBIT REGULATED EXPRESSION IN F/NDC. We also evaluated the expression of 5 proteases that participate in fusion of viral and cell membranes (12–14,31,32) when triggered by SARS coronavirus binding to ACE2 (41,42). Expression of *CTSL1* (13), *TMPRSS11D* (13,14), *ADAM17* (31), and *FURIN* (13,32) were detected by both microarray and RNA-Seq platforms. In contrast, TMPRSS2 (12–14), recently implicated in CoV-2–membrane fusion in model systems (12), was low abundance as measured by microarray and could not be detected by RNA-Seq. None of these proteases and only 1 (cathepsin L-like 3) of an additional panel of 11 others exhibited differences between NF control subjects and patients with F/NDC, and none changed expression in Rs with reverse remodeling. However, this does not exclude the possibility that protease protein abundance or enzyme activity may have changed with remodeling.

INTEGRINS, PARTICULARLY *ITGA5*, ARE REGULATED WITH REMODELING AND ARE CANDIDATES FOR CoV-2 BINDING AND CELL ENTRY. We considered that integrins could be a potential participant in CoV-2 cell entry and found *evidence* or *unequivocal evidence* of remodeling-associated up-regulation in 5 of the 10 investigated. *ITGA5*, rated *evidence* of an association with remodeling, and its protein product can bind to ACE2 (19) plus a motif in CoV-2 (16) and thus is a candidate for involvement in CoV-2 cell binding and internalization. The only integrin that achieved *unequivocal evidence* of remodeling association was the laminin binding cardiac and skeletal muscle integrin *ITGA7*, whose gene product has not been reported to bind to ACE2 or pathogenic viruses and which, like ACE2 and BNP, can be viewed in a counter-regulatory context (36). Five of the 10 integrins evaluated bind to an RGD motif, common in pathogenic viruses as a means of cell surface binding and virus internalization, and recently identified in CoV-2 (16). Of these, only *ITGA5* and *ITGB3* were up-regulated, meaning if Cov-2 did utilize RGD-integrin binding for internalization, these 2 monomers might predispose to greater cell entry. However, these integrins do not dimerize with each other (43), and the *ITGA5-ITGB1* or *ITGAV-ITGB3* protein product heterodimers $\alpha5\beta1$ or $\alpha V\beta3$, both commonly used by pathogenetic viruses for cell entry, would need to be formed. If RGD binding is disregarded (17), the other up-regulated integrin (rated as *possible evidence*) was *ITGA4*, which also does not dimerize with *ITGB3* (43). Alternatively, up-regulated integrins could affect virus replication in cardiac myocytes via interaction with integrin linked kinase (44), a pseudokinase adaptor molecule known to bind to *ITGB1* and *ITGB3* gene products (45).

CARDIAC MYOCYTE CELL ENTRY OF CoV-2, YET TO BE ESTABLISHED, IS POTENTIALLY POSSIBLE BASED ON BINDING AND INTERNALIZATION MECHANISMS BEING PRESENT IN HUMAN VENTRICULAR MYOCARDIUM. Despite substantial evidence that myocardial involvement is common and potentially devastating in COVID-19, identification of CoV-2 virus in cardiac myocytes has not been reported. Cardiac findings on autopsy of patients who had COVID-19 are limited to a single case of severe pulmonary involvement in which on tissue examination no myocardial pathologic findings were observed (46), and 2 pulmonary death cases in which postmortem myocardial needle biopsy findings were deemed likely to be secondary to pre-existing underlying conditions (47). One of the 2 needle biopsy subjects had a negative tissue block CoV-2 PCR (47). There is thus far only a single case report of an EmBx in a COVID-19 PCR-proven case, in a patient in cardiogenic shock (48). This patient had electron microscopy–imaged coronavirus in ventricular myocardial interstitial cells but not in cardiac

FIGURE 4 Position of ACE2 Within RAAS



Position of angiotensin converting enzyme 2 (ACE2) within the renin-angiotensin-aldosterone system (RAAS). Substrate or effector molecules are in **italics**; enzymes are within **ovals**; receptors (Rs) are **bolded and shadowed text**; and inhibitors are **underlined**. ARB = angiotensin receptor blocker; AT_1 R = angiotensin type 1 receptor; β_1-AR = beta 1-adrenergic receptor; MASR = MAS1 (also known as MAS) receptor; MRA = mineralocorticoid receptor antagonist; NEP = neutral endopeptidase.

myocytes, despite evidence of myofibril lysis (48). However, based on remodeling associated up-regulation in *ACE2* and the demonstration that all other myocardial cell entry constituents exist in human ventricular myocardium, it would be surprising if CoV-2 cannot bind to, enter, replicate, and damage human cardiac myocytes. Myocytes contribute approximately 70% of tissue volume in the human LV (49), and *ACE2* is definitely cardiac myocyte-expressed according to cell marker findings (20), single-cell RNA data (50), and the degree of enzyme activity in vivo (21) and in explanted hearts (22).Thus cardiac myocytes appear to be a vulnerable target for CoV-2, and this needs to be addressed by further histopathologic investigation in hearts exhibiting CoV-2-associated myocardial dysfunction.

ACE2 AS A POTENTIAL THERAPEUTIC TARGET IN CoV-2 INFECTION. As has been noted by others (51), ACE2 and its receptor for the spike protein binding domain are an attractive therapeutic target for treating or preventing CoV-2 infections. ACE2 small molecule inhibitors have been developed (52) and dramatically lower ACE2 activity in failing human LV preparations, at nanomolar concentrations (22). However, ACE2 is an important counter-regulatory enzyme in the heart, responsible for converting angiotensin II to angiotensin-(1-7) that is anti-proliferative, antifibrotic, and a vasorelaxant. Based on gene ablation, ACE2 is considered an "essential regulator of heart function" (53). It follows that any ACE2 inhibitor would need to block CoV-2 binding without decreasing enzyme activity, which may be possible through antibody inhibition (11) or the use of decoy receptors (54). Another possibility would be to deploy an ACE2 activator such as diminazene (55) if an ACE2 CoV-2 receptor inhibitor diminishes ACE2 activity. However, if any of these approaches are taken, it would be important to rule out other mechanisms of CoV-2 cell entry that would be uninhibited, such as virus-integrin binding.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: ACE2, a counter-regulatory enzyme robustly expressed in human LV and RV and the major pathway for breaking down angiotensin II into the antihypertrophic, antifibrotic, and vasorelaxant peptide angiotensin-(1-7), has been hijacked by severe acute respiratory syndrome coronaviruses as the binding site for initiation of cell entry. *ACE2* is up-regulated in eccentrically remodeled/failing LVs, and then decreases expression with reverse remodeling. This regulatory behavior was independent of RAS inhibitors as doses of ACE inhibitors or ARBs were not different in patients with reverse remodeling compared with in those without reverse remodeling. *ACE2*'s remodeling expression behavior was identical to *NPPB*, another counter-regulatory gene that leads to generation of the antiproliferative vasodilator B-type natriuretic peptide. Up-regulated ACE2 should be viewed as beneficial to a remodeled, failing heart, although it may increase the risk of CoV-2 cell invasion and cytopathology. It stands that any therapeutic approach involving ACE2 should focus on inhibiting CoV-2 binding, while maintaining enzyme activity.

TRANSLATIONAL OUTLOOK: The data presented are another example of reverse translation, in this case where an enzyme that was originally characterized and shown to be up-regulated in failing, pathologically remodeled human hearts was discovered to be the cell transducer for a worldwide pandemic. The precise biologic mechanisms involved in severe acute respiratory syndrome coronavirus cell entry and damage are now being investigated at the basic science level, and therapeutic strategies involving ACE2 inhibition or intervention at downstream events may result in forward translation back to the clinical setting.

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APPENDIX For supplemental explanations and tables, please see the online version of this paper.

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