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Research Letter

Proteomic analysis reveals upregulation of ACE2, the putative SARS-CoV-2 receptor in pressure- but not volume-overloaded human hearts

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Cardiovascular co-morbidities remain to be one of the most prominent risk factors for a poor clinical outcome of SARS-CoV-2 infection. In addition to the respiratory tract, the myocardium is also often affected by SARS-CoV-2.[1] Though the virus has been detected in myocardial tissue, it remains unclear if cardiac pathologies are caused directly by the virus or by other e.g. immunological factors.

Human angiotensin-converting enzyme 2 (ACE2) is recognized as the main receptor for SARS-CoV-2. It is expressed in many organs, including the respiratory tract, kidney and heart. Thus, some reports suggest that ACE2 play a role in cardiac SARS-CoV-2 infection.[2, 3] ACE2 is the rate limiting enzyme in the degradation of the fibrogenic and pro-inflammatory angiotensin II (AngII) peptide and therefore a major player in the pathophysiology of heart disease. A recent single cell RNA sequencing study showed increased ACE2 expression in cardiomyocytes of a very small cohort of patients with aortic stenosis (AS) and with heart failure.[3] To better understand the pathophysiological circumstances in which ACE2 is upregulated in the heart on protein level, we took advantage of a well-established proteomic approach and compared proteomic characteristics of n=75 human myocardial samples from n=41 patients with severe AS, n=17 patients with severe mitral valve regurgitation (MR), and n=17 controls. Myocardial samples were collected from the left ventricular septum during valve surgery and were frozen at -80°C. Both patient groups had cardiac hypertrophy but no significant reduced left ventricular ejection fraction (Figure 1A). Controls were healthy organ donors without cardiovascular diseases but whose hearts could not be transplanted due to non-medical reasons. Protein abundances were determined by label-free shot-gun mass spectrometry on a Thermo Orbitrap instrument and analyzed by MaxQuant with 1% FDR. The study was approved by the local ethics committee.

In patients with AS, ACE2 protein was 4.76-fold upregulated compared to controls (adj.p<0.0001) and 4.04-fold compared to patients with MR (adj.p<0.001). In patients with MR, ACE2 abundance did not show any significant differences when compared to controls (Figure 1B). To confirm the validity of these results, protein abundance was compared with available cardiac transcriptome data of 17 AS patients and 6 controls from the same cohort. Likewise to the proteomic results, ACE2 was significantly (adj. p<0.05) upregulated in AS compared to controls (Figure 1D). Moreover, there was a significant correlation between ACE2 protein abundance and mRNA expression levels (R=0.6, p<0.01) suggesting a direct link between cardiac ACE2 transcription levels and the amount of generated ACE2.

Furthermore, a logistic regression model (Pseudo-R2 0.40, p<0.001, AUC 0.89), adjusted for age, sex, antihypertensive medication revealed that allocation to the pressure-overloaded AS group (OR 44.2, 95% CI 1.5–1331.4, p=0.029) was the most relevant factor for ACE2 protein levels exceeding the inter-group median. ACE-inhibition (ACE-I) was another independent factor for ACE2 levels exceeding the inter-group median (OR 10.3, 95% CI 1.2-91.6, p=0.036) with 95% CIs above 1. Relevant effects were not found for AngII receptor blockers (ARB)s (OR 2.3, 95% CI 0.3-21.4, p=0.453). No relevant effects of age were found on quantitative ACE2 upregulation.

Additionally to a relevant pressure gradient across the valve, AS patients had higher blood pressures than MR and controls. Whereas both effects remain difficult to distinguish, the pathophysiological reason for an increased ACE2 expression in pressure-overload hearts might be a compensatory mechanism that mediates the well described anti-hypertrophic and anti-fibrotic actions of ACE2 in the heart.[4] This assumption is further supported by the fact that the intensity of ACE2 abundance positively correlates with the pressure gradient in AS (Coef. 0.028, 95% CI 0.003-0.054, p=0.029). Despite covering a large range of proteins, ACE abundance was below the detection limit and thus could not be quantified robustly. However,

ACE mRNA was detected, and expression levels were non-significantly (adj. p=0.06) increased in AS compared to controls.

Although, care should be taken when interpreting results in terms of causality due to the observational design of the present study, ACE-I intake was another independent factor for ACE2 levels exceeding the inter-group median (Fig. 1B). Whether this fact is relevant in patients with SARS-CoV-2 is still speculative. Until now, there is no evidence that ACE-I or ARBs are associated with a more severe outcome in patients infected with SARS-CoV-2.

In conclusion, the present study found evidence that ACE2 is differently regulated in pressure- or volume-overload hearts due to valve disease. Further research is needed to investigate whether other pressure load conditions such as arterial hypertension, also lead to increased ACE2 expression and whether such conditions can enhance ACE2 expression also in tissues of initial SARS CoV-2 infection, such as the upper airways.

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Figures legend:

Figure 1: Distinct ACE2 expression of the putative SARS-CoV-2 receptor in heart disease. T(A) Table with patients' and controls' characteristics. Difference between groups was tested by Welch's t-test (numerical) or chi-squared test (categorical). Significant differences between the patients' groups and controls are marked bold. ACE2 expression values from (B) protein measurements as log2 LFQ intensities (n = 41 AS, 17 MR, 17 CON) and (C) RNA sequencing as log2 counts per million (n = 17 AS, 6 CON). Individual values per patient are plotted as dots. Missing values are not shown but were down-shift imputed for statistical testing. (D) Sample-wise derivation of cardiac ACE2 protein abundance from the median log2 expression value (set to 0). Each bar represents one sample, while annotation columns below denote selected baseline characteristics. Statistical analysis was performed using R including limmas moderated t-test and BH multiple testing correction. AS = aortic stenosis, MR=mitral regurgitation, CON=control, ARB=angiotensin II receptor blocker, ACE=angiotensin converting enzyme. Significance levels - ns: $p > 0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

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	AS n = 41	MR n = 17	Con n = 17
Age, years	**68 ± 9**	**60 ± 14**	44 ± 15
BMI, kg/m2	**28 ± 4**	27 ± 3	25 ± 5
Female gender, n (%)	21 (51)	6 (35)	8 (47)
Systolic blood pressure, mmHg	**140 ± 19**	131 ± 16	117 ± 21
Diastolic blood pressure, mmHg	74 ± 11	76 ± 14	74 ± 17
Serum Creatinine, mg/dl	0.9 ± 0.2	1.0 ± 0.2	1.0 ± 0.4
Hypertension, n (%)	**27 (69)**	**11 (65)**	0
Dyslipidemia, n (%)	**8 (21)**	3 (18)	0
Diabetes mellitus, n (%)	7 (17)	2 (12)	0
Coronary artery disease, n (%)	2 (5)	2 (12)	0
Atrial fibrillation paroxysmal	2 (5)	2 (12)	0
Atrial fibrillation permanent	0 (0)*	2 (12)*	0
Left ventricular end-diastolic volume, ml/m^2	73 ± 17.4*	108 ± 34.5*	-
Left ventricular myocardial mass, g/m2	71 ± 20	67 ± 15	-
Mean pressure gradient aortic valve, mmHg	56 ± 15*	4 ± 8*	-
Mitral valve regurgitation, grade (mild/ moderate/ severe)	(41/ 0/ 0)*	(0/ 10/ 7)*	-
Left ventricular ejection fraction, %	60 ± 7.4*	64 ± 6.2*	-
Medication			
ACE inhibitor, n (%)	**15 (37)**	**5 (29)**	0
Angiotensin receptor blocker, n (%)	**9 (22)**	**7 (41)**	0
Beta blocker, n (%)	**20 (49)**	**10 (59)**	0
Diuretics, n (%)	**12 (29)**	**5 (29)**	0