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Letter to the Editor

Determinants of circulating angiotensin-converting enzyme 2 protein levels in the general population

Similar to SARS-CoV, the novel coronavirus (SARS-CoV-2) uses the angiotensin-converting enzyme 2 (ACE2) receptor to infiltrate target cells [1]. Previous experimental study provided the genetic proof that ACE2 is indeed a crucial for effective replication of infectious SARS-CoV [2]. Indeed, pathologic alterations in lungs of infected mice were reduced in ACE2 knockout mice compared to wild-type mice. It was suggested that higher ACE2 protein expression might be associated with a higher local viral load [2]. Of note, ACE2 may be upregulated in subjects with cardiovascular disease to counteract the adverse effects of Ang II. Available data from SARS-CoV-2 patients showed that the elderly, obese and patients with underlying diseases such as diabetes and cardiovascular disorders are susceptible to severe form of infection [3].

A soluble form of the catalytic ACE2 ectodomain can be released in the circulation by sheddases such as ADAM17. So far, the associations of circulating ACE2 levels with the anthropometric and clinical correlates in the general population are still not fully elucidated. Here, we explored the determinants of ACE2 concentration in a cohort of 544 participants randomly recruited from the general population free from clinically overt heart disease and diabetes [4].

Written informed consent was obtained. Serum ACE2 levels were measured using a multiplex Proximity Extension Immunoassay (Cardiovascular II panel, Olink Bioscience, Uppsala, Sweden). ACE2 levels were expressed in Normalized Protein eXpression (NPX) units, which were calculated from Ct values. Because NPX is expressed in a log2 scale, a 1 NPX difference means a doubling of protein concentration. We calculated the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) as the product of fasting glucose (in mmol/L) and serum insulin (in μmol/L) divided by 22.5. We searched for variables associated with the ACE2 level using stepwise linear regression. The continuous and categorical variables considered for entry in the stepwise models were sex, age, body mass index (BMI), HOMA-IR, hypertension (yes/no), systolic blood pressure, use of ACE inhibitors (n=27), angiotensin receptor blockers (n=23) or betablockers/diuretics (n=118). We set the *P* values for variables to enter and to stay in the stepwise regression models at 0.05. To further illustrate statistically significant associations, we reported multivariable-adjusted parameter estimates (±95%CI) for serum ACE2 levels by categories of variables selected in stepwise linear regression.

From the 544 participants (mean age, 57.0±10.4 years; 52.9% women), 274 (50.4%) were hypertensive of whom 164 (30.2%) were on antihypertensive drug treatment. In multivariable stepwise regression analyses, serum ACE2 levels independently and significantly increased with male sex, age and HOMA-IR analyzed continuously ($P<0.001$ for all). Of note, BMI and hypertension status lost their significance when HOMA-IR was added in the stepwise model ($P\geq0.13$). Indeed, HOMA-IR accounted for most of the explained variances in ACE2 levels (7.8%, <0.0001). Overall, the selected anthropometric covariables (sex and age) and HOMA-IR explained 17.4% of the total variance for ACE2. In support, Fig. 1 presents ACE2 levels by sex, age groups, and HOMA-IR quartiles. Subjects belonging to the fourth quartile of HOMA-IR distribution had significantly higher ACE2 levels as compared to the participants from other quartiles ($P\leq0.0079$).

Taken together, in the general population we observed a significantly higher ACE2 levels in males, in older subjects (>55 years old), and in those with insulin resistance. Our findings are in line with clinical reports on severity of SARS-CoV-2 in elderly subjects, males and patients with obesity and diabetes [3]. Therefore, we suggest that the link between anthropometric and metabolic traits and severe form of SARS-CoV-2 infection might be attributable in some degree to a higher ACE2 levels at baseline which might reflect higher ACE2 expression in tissues and, therefore, higher shedding of ACE2 from the cell membrane. Interestingly, in our cohort of middle-age participants, use of ACE inhibitors and angiotensin receptor blockers was not a significant predictor of circulating ACE2 levels, which is consistent with previous reports in a cohort of patients with symptomatic heart failure [5]. To the best of our knowledge, our study is the first to demonstrate the direct link between the circulating ACE2 and insulin resistance (prediabetes) in the general population. This is in line with previous experimental and clinical studies demonstrating that ACE2 plays of major role in glucose homeostasis. For instance, in non-obese diabetic mice, significant increases in ACE2 activity in serum, pancreas, and heart were observed compared to control mice [6]. With regard to protein expression, ACE2 were increased in lungs and heart at early stage of diabetes [6]. Moreover, associations of type 2 diabetes with increased ACE2 expression in lungs and plasma ACE2 levels were demonstrated in the recent GWAS analysis [7]. *Park* et al. demonstrated that the urinary ACE2 concentrations were associated with glucose intolerance and T2DM [8]. However, we acknowledge that there is no compelling evidence of a connection between baseline level of circulating ACE2 and the susceptibility to and severity of SARS-CoV-2 infection [9]. Further studies should also clarify the genetic variance in the ACE2 gene and environmental stimuli that might predispose to high levels of ACE2 expression and its concentration, and, therefore, influence the outcome of SARS-CoV-2 infection in the community.

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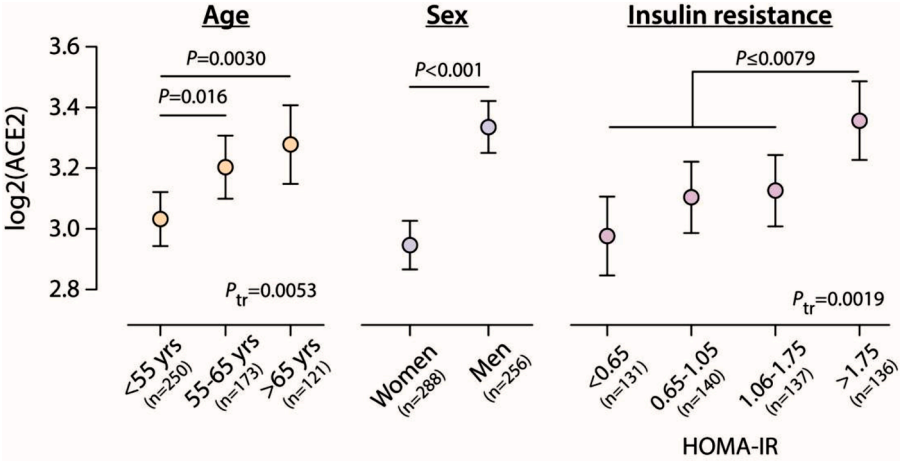

Fig. 1. Multivariable-adjusted parameter estimates (±95%CI) for serum ACE2 levels by age groups, sex and HOMA-IR quartiles. ACE2 indicates angiotensin converting enzyme 2, HOMA-IR, homeostatic model assessment of insulin resistance.

Declaration of Competing Interest

The authors declare they have no conflict of interest.

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