

Impaired ACE2 glycosylation and protease activity lowers COVID-19 susceptibility in Gitelman's and Bartter's syndromes

Dear Editor,

The SARS-CoV-2 pandemic (COVID-19) has focused attention on the renin angiotensin system (RAS), specifically angiotensin-converting enzyme 2 (ACE2) as it serves as the entry point of the SARS-CoV-2 virus [1]. The SARS-CoV-2 virus attaches to its target cell via its surface spike (S) protein binding to ACE2 [2], which is then followed by subsequent fusion of the viral envelop with the host cell membrane through the action of specific proteases, such as cathepsin (Cat)-L [3]. ACE2, part of the RAS counter-regulatory system, opposes the activity of the regulatory RAS ACE/angiotensin II/AT1R axis by inducing endothelial-dependent vasodilation, and anti-proliferative and anti-inflammatory effects [4]. However, ACE2's role in the cellular entry of the virus raised clinical concerns regarding increased SARS-CoV-2 infection in patients treated with angiotensin receptor blockers or ACE inhibitors that increase ACE2 levels, although it is now clear that ACE2 upregulation has a protective impact on COVID-19 morbidity and mortality [5, 6].

Gitelman's and Bartter's syndromes (GS/BS), two rare genetic tubulopathies, present with hypokalemia and metabolic alkalosis, high Ang II levels and RAS activation yet normo-hypotension, protection from cardiovascular and renal remodeling, and—crucially—increased ACE2 and Ang 1–7 levels [7].

During the first Italian wave of the COVID-19 pandemic in early 2020, we assessed via telephone survey the impact of COVID-19 on our cohort of 128 GS/BS patients living in the main northern Italy COVID-19 hotspots. We found that none

of them had COVID-19 symptoms compared to the adjusted northern Italian general population's COVID-19 prevalence ($p < 0.008$), which again suggests that increased risk of COVID-19 due to increased ACE2 is unlikely [8]. A second survey on the same cohort 1 year later found that only eight patients tested positive for COVID-19, of which four were asymptomatic and four had very mild symptoms. Based on this and considering GS/BS patients' increased ACE2 levels, we sought to investigate the possible factors that render GS/BS patients at a minimum resistant to COVID-19. Given that blocking ACE2/viral S protein interaction is effective against SARS-CoV-2 infection and that increased pH, a feature of GS/BS, has been shown to interfere with ACE2 glycosylation (Refs. 1 and 2 in the Supporting Information), we recruited 20 GS/BS patients from the previous survey (13 females, 7 males, 32–68 years), with either GS ($n = 19$) or BS ($n = 1$) and 15 healthy controls (seven females, eight males, 29–52 years) and assessed the levels of mononuclear ACE2 and its glycosylation alongside plasma Cat-L activity (Supporting Information Methods).

GS/BS patients had higher nonglycosylated ACE2 levels (0.82 ± 0.19 d.u. vs. 0.67 ± 0.13 $p = 0.01$) and lower Cat-L activity (3.91 ± 1.13 r.f.u. vs. 5.31 ± 0.8 $p < 0.001$) (Fig. 1A,B) compared to healthy subjects. In addition, GS/BS's Cat-L activity inversely correlated ($p < 0.001$, $r = 0.78$) (Fig. 1C) with blood bicarbonate (HCO_3^-), while a negative correlation between ACE2 glycosylated isoform and HCO_3^- approaches statistical significance ($p = 0.08$).

The genetic defects of GS/BS inducing metabolic alkalosis alter chloride transport. Chloride anion (Cl^-) is a key factor in cellular homeostasis as changes in intracellular Cl^- concentration drive gene and protein expression, post-translational modification, and intracellular/extracellular pH [9]. Endo-lysosomal pH plays a critical role for the

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[Correction included on 01 August 2022 after original online publication: The copyright and legal statement has been updated.]

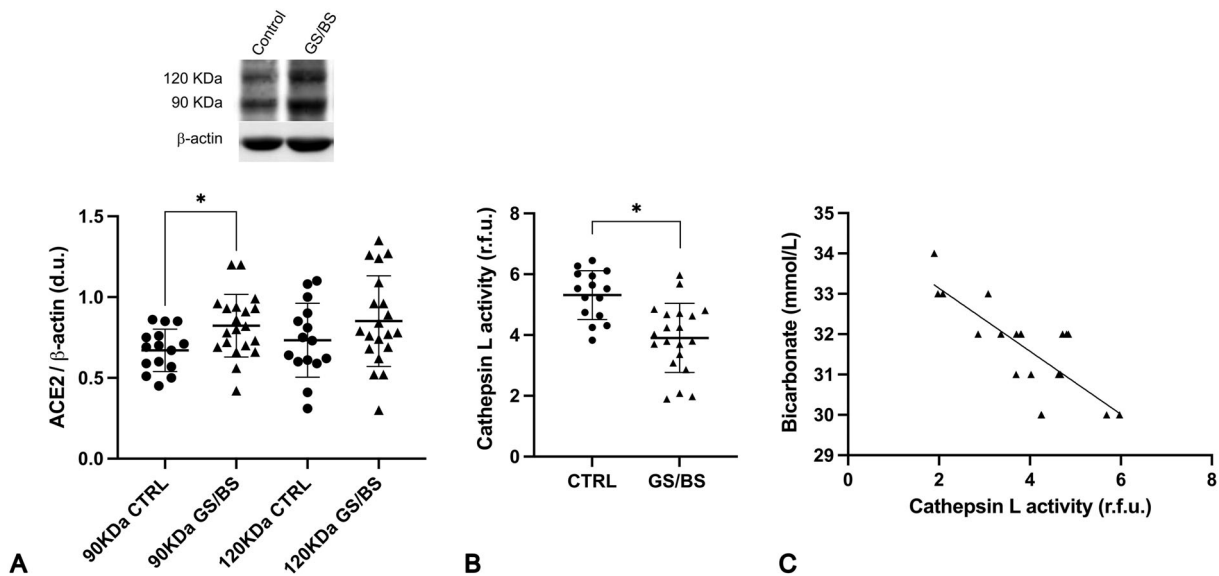


Fig. 1 (A) Protein expression of glycosylated (120 kDa) and nonglycosylated (90 kDa) isoforms of ACE2 in Gitelman's and Bartter's syndromes (GS/BS) and healthy control subjects (CTRL). Densitometric analysis of western blot products shows higher expression of nonglycosylated form in GS/BS, compared to controls. Data are shown as mean \pm SD. * $p = 0.01$. (B) Cat-L levels in GS/BS and CTRL. Cat-L activity is significantly lower in GS/BS compared to CTRL. Data are shown as mean \pm SD. * $p < 0.001$. (C) Metabolic alkalosis and its relationship with Cat-L activity. Correlation between blood HCO_3^- and Cat-L activity showing a significant inverse correlation ($p < 0.001$, $r = 0.78$). GS/B: black triangles; CTRL: black dots.

endocytic uptake of SARS-CoV-2. Increased intracellular organelle pH, in fact, interferes with both ACE2 glycosylation and the binding via S protein as observed in experiments with chloroquine (CQ)/hydroxychloroquine (HCQ) (Ref. 1 in the Supporting Information). The inverse correlation in GS/BS between blood HCO_3^- and Cat-L activity—alongside the trend toward a negative correlation between blood HCO_3^- and the glycosylated isoform of ACE2—also suggests that GS/BS patients' metabolic alkalosis underlies these effects.

GS/BS's higher level of nonglycosylated ACE2 alongside the reduced Cat-L activity, which has also been shown to be pH dependent, suggests that the endosomal processing system in GS/BS patients is impaired.

Both glycosylated ACE2 and Cat-L activity are critical for SARS-CoV-2 binding and infection [8]. The increased nonglycosylated ACE2 and decreased Cat-L activity found in GS/BS patients provide a mechanistic explanation for the near absence of COVID-19, and the very small number of GS/BS SARS-CoV-2 positives found either asymptomatic or with minimal symptoms. In addition, our find-

ings provide a rationale for pursuing the identification and/or synthesis of new drugs that specifically target ACE2 glycosylation and/or proteases involved in SARS-CoV-2 infection that avoid the potentially deleterious heart rhythm effects of hydroxychloroquine and chloroquine.

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Ethical Statement

The second survey was a telephone survey as well and the informed consent was asked as reported for the first survey (see ref [8]).

Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Supplementary Material and Methods

Supplementary References 