JIM Research Letter

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Impaired ACE2 glycosylation and protease activity in Fabry disease protects from COVID-19

Dear Editor,

Angiotensin-converting enzyme 2 (ACE2) has attracted widespread research attention given its role as a receptor for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) responsible for the COVID-19 pandemic [Supplementary ref. 1]. SARS-CoV-2 is able to recognize and bind the host receptor ACE2 at its extracellular domain via its spike (S) glycoproteins. The S-ACE2 complex then produces conformational changes that favor the action of furin-like proteases as TMPRSS2 and Cathepsin (Cat)-L resulting in membrane fusion and viral entry into the host cell [1].

Aside from its role as a viral receptor, ACE2 is well known to play a critical role in the renin-angiotensin system, modulating the detrimental effects of angiotensin (Ang) II via conversion of Ang II into Ang 1–7, which has opposite effects to Ang II.

ACE2 is a type I transmembrane protein with a mw of 120 kDa in its most common glycosylated form [2]. It has six potential N-glycosylation sites, as conferred by the presence of the Asn-X-Ser/Thr motif (at positions Asn53, Asn90, Asn103, Asn322, Asn432, and Asn546) in its primary structure. The de-glycosylated form has a molecular weight of ~90 kDa [3]. Mutation at ACE2 glycosylation sites has been reported to impact SARS-CoV-2 binding [4].

Cat-L is a critical protease involved in S protein processing and thereby enhances SARS-CoV-2 viral entry. As it was already demonstrated for SARS-CoV-1 [Supplementary ref. 2,3], Cat-L was found to be highly correlated also with SARS-CoV-2 infection and associated with the severity of COVID-19 and its inhibition has been suggested as a possible therapeutic approach [5].

Fabry disease (FD), one of the most prevalent lysosomal storage disorder (LSD), is a monogenic inherited X-linked disease caused by mutations in the alpha galactosidase gene which lead to accumulation of glycosphingolipids, mainly globotriaosylceramide (Gb3) and lyso-Gb3, in the lysosomes, producing a multisystemic storage disorder [Supplementary ref. 4]. FD patients have an impaired intracellular biochemistry characterized by oxidative stress, mitochondrial dysfunction, impaired autophagy, and endolysosomal maturation that contribute to FD adverse outcomes [Supplementary ref. 5].

During the COVID-19 pandemic, a low rate of SARS-CoV-2 infection, with mild disease manifestations were reported in FD patients [6]. We also found a low rate of SARS-CoV-2 infection upon surveying our cohort of 40 ERT-treated FD patients, where only one patient tested positive for COVID-19 with only minor symptoms [7].

We have previously reported that patients affected by two rare genetic tubulopathies, Gitelman's and Bartter's syndromes (GS/BS), are protected from COVID-19 [8]. Both syndromes are characterized by metabolic alkalosis, which altering the lysosomal acidic pH required for normal endosomal processing, particularly glycosylation, likely impairs ACE2 glycosylation. This altered endosomal processing provides a mechanistic rationale for the higher non-glycosylated ACE2 levels and lower Cat-L activity found in GS/BS patients compared to healthy subjects, which likely underpin the reduced susceptibility to SARS-CoV-2 infection reported in these patients [8].

FD patients also have impaired endo-lysosomal functions [Supplementary ref. 5]. Given our findings in GS/BS patients, we tested whether similar effects might be responsible for them being less prone to SARS-CoV-2 infection and severe clinical manifestations of COVID-19 [6, 7]. This was assessed by measuring the levels of mononuclear ACE2 and its glycosylation alongside plasma Cat-L activity [Supplementary Methods] in 15 FD patients (10 females, 5 males, age range 25–72 y.o), in 1 patient affected by another LSD, Gaucher

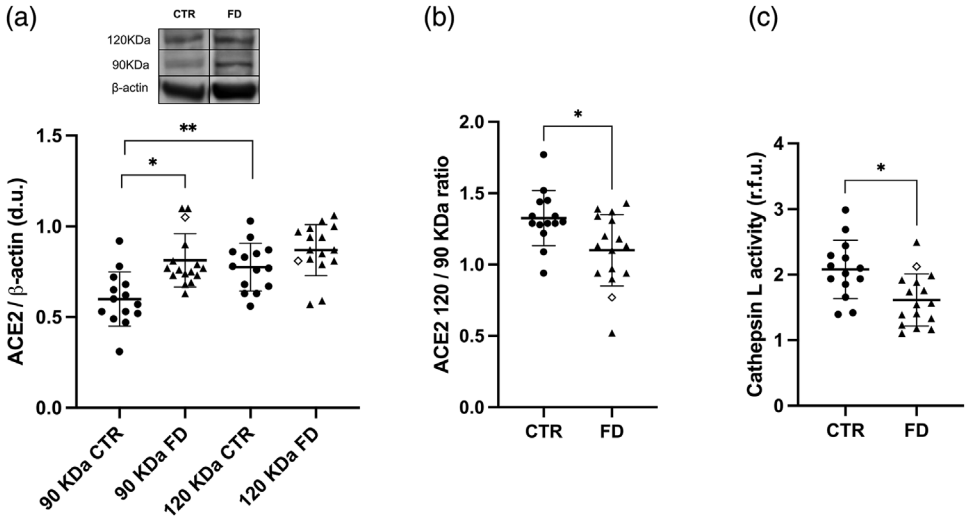

Fig. 1 *(a) Protein expression of glycosylated (120 kDa) and non-glycosylated (90 kDa) isoforms of angiotensin-converting enzyme 2 (ACE2) in Fabry disease (FD) and healthy subjects (CTR). Densitometric analysis of Western blot products shows higher expression of non-glycosylated isoform in FD, compared to CTR. CTRs have higher glycosylated ACE2 isoform. Data are shown as mean ± SD. *p < 0.001, **p < 0.0001. (b) Glycosylated/non-glycosylated ACE2 ratio in FD and CTR. Data are shown as mean ± SD. *p = 0.011. (c) Cat-L levels in FD and CTR. Cat-L activity is significantly lower in FD compared to CTR. Data are shown as mean ± SD. *p = 0.005. CTRL: black dots; FD: black triangles; GD: white diamond.*

disease (55 y.o.) [Supplementary ref. 6], and in 14 healthy subjects (6 females, 8 males, age range 25–59 y.o.).

FD patients had higher non-glycosylated ACE2 levels (0.81 ± 0.15 d.u. vs. 0.60 ± 0.15, $p < 0.001$) and lower Cat-L activity (1.61 ± 0.39 r.f.u. vs. 2.08 ± 0.44, $p = 0.005$) (Fig. 1a,c) compared to healthy subjects. In addition, healthy subjects had significantly increased glycosylated ACE2 compared to the non-glycosylated form (0.77 ± 0.13 d.u. vs. 0.60 ± 0.15, $p < 0.0001$), (Fig. 1a). Moreover, glycosylated/non-glycosylated ACE2 ratio was lower in FD patients compared to healthy subjects (1.33 ± 0.19 vs. 1.10 ± 0.25, $p = 0.011$), (Fig. 1b), further supporting the difference in the balance between the two ACE2 isoforms in FD and healthy subjects.

The results of this study parallel those reported in GS/BS patients [8] and again provide a mechanistic explanation for the protection from SARS-CoV-2 infection and severe manifestations of COVID-19 observed in FD patients. The endosomal disruption in FD patients, as evidenced by their higher non-glycosylated ACE2 levels, likely negatively affects viral entry and infection. The effect of altered glycosylation underpins the mode of chloroquine (CQ) antiviral role against SARS-CoV and SARS-CoV-2 [9]. CQ blocking the necessary acidification of trans-Golgi network (TGN)/post-Golgi increases endosomal pH, thereby interfering with terminal glycosylation of ACE2.

As Cat-L upregulation is a severe enhancer of SARS-CoV-2 entry and infection [5], the altered endosomal function-dependent reduced Cat-L activity observed in FD patients suggests that S priming can be severely affected, providing an important protective mechanism. Moreover, the altered endosomal processing provides a robust mechanistic rationale for the effects of the combination of nirmatrelvir-ritonavir (Paxlovid), a new antiviral drug, which exerts its effect via inhibition of proteins involved in lysosomal processes key for SARS-CoV-2 cell entry and replication–transcription [7].

In summary, the findings in FD disease and those coming from our previous study in GS/BS highlight the essential role of endosomal–lysosomal functions in SARS-CoV-2 infection. The observed protection from SARS-CoV-2 infection in GS/BS patients could be the result of the impaired lysosomal functions due to their chronic metabolic alkalosis and in FD patients the result of impaired

lysosomal functions due to the LSD [7]. Moreover, FD and GS/BS patients' physiology provides an "in vivo" human model where the effects of endosomal pH, ACE2 glycosylation status, and Cat-L activity alter SARS-CoV-2 infection rate and severity and point to these as targets to fight COVID-19.

Author contributions

Conceptualization; data curation; formal analysis; investigation; methodology; writing—original draft: Ilaria Caputo. *Conceptualization; formal analysis; investigation; methodology; writing—original draft*: Giovanni Bertoldi. *Data curation; formal analysis; investigation; methodology*: Giulia Driussi. *Data curation; investigation; resources; writing—original draft*: Luca Sgarabotto. *Data curation; resources; validation*: Gianni Carraro. *Data curation; investigation; resources; writing—original draft*: Lucia Federica Stefanelli. *Conceptualization; writing—review and editing*: Paul A. Davis. *Conceptualization; funding acquisition; supervision; writing—original draft; writing—review and editing*: Lorenzo A Calò.

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Conflict of interest statement

The authors have no conflict of interest to declare.

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