Do naturally occuring SARS-CoV-2 receptor-binding domain substitutions in mink pose a risk factor for humoral escape in humans?

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To the editor: In their recent rapid communication "SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020", Oreshkova *et al.* describe the infection and spread of SARS-CoV-2 on two mink farms in North Brabant, The Netherlands [1]. On both farms, workers experienced coronavirus related symptoms prior to the outbreaks, leading to the assumption that the workers introduced the virus within these farms. Sequencing suggested one worker to have contracted the virus from mink. In supplementary table 2 of their publication, the authors described the identified substitutions in detail, including an Y462F substitution in 5 out of 6 mink and a F495L substitution in 1 out of 6 mink (numbering according to Wuhan-1: EPI_ISL_402125). In a more recent comprehensive description of the SARS-CoV-2 infections on Dutch mink farms, a total of 66 infected farm workers (of the 97 tested) were identified on a total of 16 different mink farms [2]. A detailed phylogenetic analysis was performed and the authors noted the existence of 3 big transmission clusters, divided in 5 separate clusters (A-E) displayed in a phylogenetic tree containing all Dutch SARS-CoV-2 sequences to date. In figure 5, the authors describe the same Y462F substitution as an Y453F substitution (numbering according to Wuhan-1: NC_045512.2) and the F495L as a F486L substitution. As both reference sequences are identical in length, it is unclear where this naming discrepancy comes from. In addition, L452M and N501T substitutions are identified. In both papers the authors refrained from highlighting the importance of these substitutions, while these positions have been identified as key positions involved in the binding to the ACE-2 receptor by SARS-CoV-2 [3].
Currently, in a total of 63 of 143 Dutch mink SARS-CoV-2 sequences that have currently been deposited to GISAID, several substitutions were observed in or near key receptor binding residues for SARS-CoV-2 (see table 1). A combination of similar substitutions was observed in 6 GISAID strains obtained from human cases probably connected to the mink farms (NB-EMC-265, NB-EMC-266, NB-EMC-267, NB-EMC-270, NB-EMC-271 and NB-EMC 277).

Substitution	Farms
Y453F	1/21 NB01+NB-EMC-1, 11/13 NB02+NB-EMC-2, 5/5 NB-EMC-15, 5/5 NB-EMC-16, 5/5 NB-EMC-18, 5/5 NB-EMC-19, 2/2 NB-EMC-25,
F486L	2/21 NB01+NB-EMC-1, 1/13 NB02+NB-EMC-2, 6/9 NB-

	EMC-6, 7/7 NB-EMC-23, 2/2 NB-EMC-26
L452M and F486L	5/5 NB-EMC-17, 7/7 NB-EMC-23, 2/2 NB-EMC-26
N501T	2/21 NB01+NB-EMC-1, 1/1 NB-EMC-5, 1/9 NB-EMC-6, 1/6 NB-EMC-8

Table 1 Overview of key SARS-CoV-2 ACE-2 receptor binding residues and names of the mink farms where the strains were identified (according to Wuhan-1: NC_045512.2 numbering)

As mentioned, positions Y453, F486 and N501 have been identified as key contact residues in the SARS-CoV-2 S protein with L452 being directly adjacent to contact residue Y453, highlighting it's potential relevance [3]. In addition, a recent analysis comparing the human ACE-2 receptor to the mink ACE-2 receptor showed a G354H amino acid substitution in the key five amino acid residues (353-KGDFR-357) binding region in ACE-2 in mink [4]. The assumption is that the virus might need to change its ACE-2 binding properties to enhance its binding affinity to the mink ACE2-receptor. Combined, these findings indicate that more efficient mink ACE-2 usage could be an evolutionary driving force explaining the current SARS-CoV-2 evolutionary pattern in mink. While such naturally occurring evolution could be expected after a zoonotic event, the observed mutations in mink farm-related SARS-CoV-2 strains might also have direct consequences for the protective immunity in humans. As the receptor binding domain region is also an important immunological region that normally elicits strong protective antibody responses [5]. Antibodies that are elicited after a natural occurring SARS-CoV-2 infection in humans are mainly targeted at the S-protein, in particular the receptor binding domain [6]. Therefore, it is not unreasonable to assume that the substitutions occurring in the receptor binding domain of mink-associated SARS-CoV-2 viruses, might also alter the potential level of protection provided by antibodies directed at this region in humans post-infection or vaccination. It is therefore of the utmost importance to determine whether or not the neutralizing capability of human seroconvalescent sera remains effective against viral variants with these identified substitutions. With the currently ongoing transmission of SARS-CoV-2 in minks (and maybe other mustelid species) in several countries, it is of uttermost importance that the emergence and spread of virus strains with mink-related receptor binding domain substitutions is closely monitored and communicated.

In summary, mink-related SARS-CoV-2 strains demonstrate substitutions in areas of the genome crucial to ACE-2 receptor binding and effectiveness of the neutralizing antibody responses. As the putatuive implications of

these observations to our opinion have not been clearly addressed in current literature on mink farm-related SARS-COV-2 infections, we wish to raise awareness and urge for close monitoring of and the expedited release of SARS-CoV-2 sequencing data in mink and other mustelid species across the world. [1] N. Oreshkova *et al.*, "SARS-CoV-2 infection in farmed minks , the," *Euro Surveill.*, vol. 25 (23), no. May, pp. 1–7, 2020.

[2] B. B. Oude Munnink *et al.*, "Jumping back and forth: anthropozoonotic and zoonotic transmission of SARS-CoV-2 on mink farms," *bioRxiv*, p. 2020.09.01.277152, 2020.

[3] J. Lan *et al.*, "Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor," *Nature*, vol. 581, no. 7807, pp. 215–220, 2020.

[4] T. Hayashi, K. Abiko, M. Mandai, N. Yaegashi, and I. Konishi, "Highly conserved binding region of ACE2 as a receptor for SARS-CoV-2 between humans and mammals," *Vet. Q.*, vol. 451, no. 2, pp. 1–8, Sep. 2020.

[5] W. Tai *et al.*, "Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine," *Cell. (10)(2e). Immunol.*, vol. 17, no. 6, pp. 613–620, 2020.

[6] P. J. M. Brouwer *et al.*, "Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability," *Science (80-.).*, vol. 369, no. 6504, pp. 643–650, 2020.

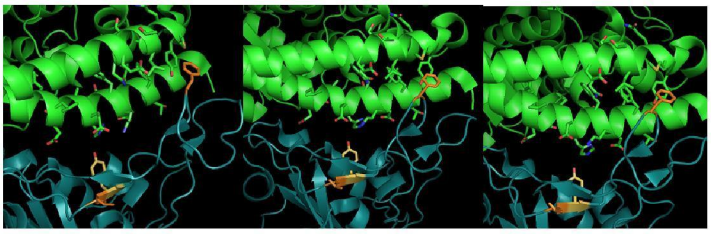

Figure 1. Positions Y435 and F468 are in close proximity and form strong hydrogen bonds indicative for interacting position between SARS-CoV-2 S protein receptor binding domain and human ACE-2 receptor. [3] Picture produced by PyMol (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC) on structure PDB 6MOJ.