The MERS-CoV receptor gene is among COVID-19 risk factors inherited from Neandertals

Authors
Hugo Zeberg¹,²* and Svante Pääbo¹,³*

Affiliations
¹ Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, D-04103 Leipzig, Germany.
² Department of Neuroscience, Karolinska Institutet, SE-17177 Stockholm, Sweden.
³ Okinawa Institute of Science and Technology, Onna-son, Okinawa 904-0495, Japan.

*Corresponding authors: hugo.zeberg@ki.se, paabo@eva.mpg.de
Abstract
In the current SARS-CoV-2 pandemic, two genetic regions derived from Neandertals have been shown to increase and decrease, respectively, the risk of falling severely ill upon infection. Here, we show that 2-8% of people in Eurasia carry a variant promoter region of the DPP4 gene inherited from Neandertals. This gene encodes an enzyme that serves as a receptor for the coronavirus MERS-CoV and is currently not believed to be a receptor for SARS-CoV-2. However, the Neandertal DPP4 variant doubles the risk to become critically ill in COVID-19.

Main text
It was recently shown that the major genetic risk factor for falling severely ill when infected with the novel SARS-CoV-2 virus is inherited from Neandertals (Zeberg and Pääbo, 2020a). Furthermore, a protective genetic variant for severe COVID-19 located on chromosome 12 has been shown to be inherited from Neandertals (Zeberg and Pääbo, 2020b). In light of these findings, Neandertals haplotypes might be of particular importance in attempts to reveal the genetic factors that predispose individuals to severe COVID-19.

Early in the pandemic, the membrane-bound enzyme ACE2 was identified as a receptor for SARS-CoV-2 (Hoffmann et al. 2020). ACE2 has previously been shown to be a functional receptor for other coronaviruses, including HCoV-NL63 and SARS-CoV-1. The facts that males are more at risk for severe COVID-19 and that ACE2 is located on the X chromosome suggest that the ACE2 would emerge as a risk locus for severe COVID-19 in genetic association studies. However, to date we are not aware of that any association between ACE2 and severity of COVID-19 have been described. MERS-CoV, a coronavirus that was discovered in 2012 and have caused outbreaks in several countries (de Groot et al. 2013), utilizes DPP4 as a receptor (Wang et al. 2013; Stalin Raj et al. 2013). DPP4 is a membrane-bound enzyme that degrades a number of biologically active peptides. It is involved in several physiological systems, including the regulation of glucose metabolism (Mentlein, 1999). Inhibitors of DPP4 are used to treat diabetes and have been suggested to affect COVID-19 outcomes in preliminary studies (reviewed in Lim et al. 2021).

We looked for single nucleotide polymorphisms (SNPs) that carry Neandertal-like alleles at the ACE2 and DPP4 loci in the 1000 Genomes Project (Auton et al. 2015) (Supplementary Material). In a region encompassing the entire ACE2 gene and 50,000 base pairs up- and down-stream (chrX:15529156-15670192, hg19) we find two such SNPs. However, these SNPs are not in linkage disequilibrium (LD) ($r^2 = 4e-4$) and thus do not form a contiguous haplotype. For DPP4, we find 39 such SNPs in the corresponding region (chr2:162798751-162981052, hg19). They form several haplotypes ($r^2>0.9$) in and around the DPP4 gene (Fig. 1A).

To investigate if the Neandertal haplotypes in DPP4 are associated with severe COVID-19 we used the latest release of the COVID-19 Host Genetics initiative (HGI) (The COVID-19 HGI,
We find that under the rare disease assumption, the Neandertal-like alleles are associated with ~80% increased risk per allele of being hospitalized upon infection with SARS-CoV-2 (Supplementary Table S1). The most strongly associated SNP (rs117888248) has an odds ratio of 1.84 (95% CI: 1.41-2.41, p = 7.7e-6). The risk for carriers of this allele of requiring mechanical ventilation is increased by ~109% (OR = 2.09, 95% CI: 1.44-3.03, p = 1.2e-4). The Neandertal-like alleles form a ~26.3 kb-haplotype (r²>0.8). Of the 15 SNP defining the haplotype (Supplementary Table S1), 14 carry alleles seen in hetero- or homozygous forms in a Neandertal genome (Prüfer et al. 2017). This haplotype is derived from Neandertals (p = 0.023) according to a published formula (Huerta-Sanchez et al. 2014) and using parameters as previously described (Zeberg and Pääbo, 2020a) (Supplementary Materials). It covers the extended promoter region of DPP4.

Thus, the risk of becoming severely ill in COVID-19 when carrying the Neandertal haplotype at the DPP4 locus is of similar magnitude as the ~100% increase in risk associated with the previously described Neandertal haplotype on chromosome 3 (Zeberg and Pääbo 2020a) (Fig. 1B). Both these risk haplotypes have stronger effect sizes than the protective Neandertal haplotype on chromosome 12, which decreases the risk of becoming severely ill by ~23% (Zeberg and Pääbo 2020b). The Neandertal DPP4 haplotype is present in ~1% of Europeans, ~2.5% in South Asians ~4% in East Asians, and ~0.7% in admixed Americans (Fig. 1C). It is absent among Africans south of the Sahara.

We calculated the statistical significance of the association between the Neandertal DPP4 haplotype and severe COVID-19 under the null-hypothesis that Neandertal haplotypes have no impact on COVID-19. Because only a fraction of the Neandertal genome is found among present-day humans, and because Neandertal haplotypes are on average longer than other haplotypes, the statistical power to detect associations with Neandertal haplotypes is better than for genome-wide analyses. When we consider Neandertal haplotypes that are present in a frequency of ≥1% among Europeans in the 1000 Genomes Project and are identified in previously published maps of Neandertal contributions, we find that the effective number of hypotheses is 5,761, yielding an 'introgression wide' significance threshold of 8.7e-6 (Supplementary Material). Thus, under the null-hypothesis that Neandertal gene variants has no impact on COVID-19, the association of the DPP4 haplotype with severe disease is significant.

It was recently shown that the spike protein of SARS-CoV-2 binds to DPP4 (Li et al. 2020) and that DPP4 is a functional receptor for HIV (Li et al. 2020). Moreover, DPP9, a homolog of DPP4, is associated with severe COVID-19 (Pairo-Castineira et al. 2020). However, one report suggest that SARS-CoV-2 does not use DPP4 as a receptor (Zhou et al. 2020). Nevertheless, the current findings suggest that the interaction of SARS-CoV-2 with membrane-bound and secreted forms of DPP4 deserves further investigation. Inhibitors of DPP4, which can be administered orally and are used in the treatment of diabetes mellitus, may also deserve attention with respect to possible effects on viral interactions with the its host cells, as recently pointed out (Scheen 2020, Lim et al. 2021).
It is striking that among eight genetic loci that affect the risk to contract severe COVID-19 when infected with SARS-CoV-2 (Païro-Castineira et al. 2020), three carry allelic variants derived from Neandertals. This suggests that the genetic inheritance from Neandertals may have a larger impact on this disease than would naïvely be expected. However, local adaptation to infectious diseases often differs among human populations that have been separated by a few tens of thousands of years (Rees et al. 2020). Neandertals evolved largely independently from modern humans for about half a million years (Prüfer et al. 2014), even if rare genetic contacts occurred (Kühlwilm et al. 2016; Meyer et al. 2016, Posth et al. 2017, Petr et al. 2020). Given the long time of separation, Neandertal adaptation to infectious diseases may therefore have differed drastically from that of modern humans.

The combination of large effect sizes and small number of Neandertal loci (and correspondingly smaller number of the multiple tests requiring correction) may allow associations with infection disease susceptibility to be detected in smaller cohorts than if all variants across the genome are considered. For the DPP4 locus, we estimate that approximately two times more patients than currently available in HGI will be needed to achieve a 80% probability to detect the association between DPP4 and severe COVID-19 with the standard genome-wide significance threshold (p<5e-8) (Supplementary Materials).

The three Neandertal genomes available to date, which vary in age between ~120,000 years and ~50,000 years and come from Europe and southern Siberia, are all homozygous for the risk variants on chromosome 2. Furthermore, the late Neandertal genome in Europe, which is most closely related to the Neandertals that mixed with modern humans, was homozygous also for the risk variants on chromosome 3 (Prüfer et al. 2017). It is thus likely that the risk variants at both these loci were at high frequency among late Neandertals. Barring other factors that may affect disease outcome, this means that, if alive today, a late Neandertal individual would have ~4-16 times higher risk of becoming critically ill if infected by SARS-CoV-2. This may support speculations that epidemic diseases could have played a role in the demise of Neandertals.

**Acknowledgments.** We are indebted to the COVID-19 Host Genetics Initiative (HGI) for making the GWAS data available, to Tomislav Maricic for valuable input, and to the Max Planck Society and the NOMIS Foundation for funding.
Figure 1. The Neandertal DPP4 locus. A) Linkage disequilibrium to the index variant rs117460501 in Europeans in 1000 Genomes data (Auton et al. 2015). Note the step-wise decay in linkage disequilibrium, representing recombination events of longer Neandertal haplotypes. The core haplotype ($r^2 > 0.8$) covers the extended promoter of DPP4 and has a length of 26.3 kb. B) Phenotypic impact of three Neandertal haplotypes on COVID-19 severity. The DPP4 haplotype is located on chromosome 2, whereas the previously described risk and protective haplotypes are situated on chromosomes 3 and 12, respectively. Severe diseases is defined as an infection requiring hospitalization, whereas very severe disease requires active respiratory support beyond supplemental oxygen therapy. C) Carrier frequencies of the DPP4 risk haplotype in continental populations. Data from the 1000 Genomes Project.
Supplementary Materials

Neandertal gene-flow at the DPP4 locus

To detect introgression we first looked at alleles homozygously found in three high-coverage Neandertal genomes (Prüfer et al. 2014, Prüfer et al. 2017, Mafessoni et al. 2020) and absent in 108 Yoruba individuals, yielding two SNPs at ACE2 and 39 and at DPP4. To further study the DPP4 locus, we investigated all SNPs in linkage disequilibrium with rs117888248 (Supplementary Table S1) in the Neandertal genome (https://bioinf.eva.mpg.de/jbrowse/) most closely related to the Neandertal population that contributed genetically to present-day human (Prüfer et al. 2017). We demonstrated gene flow from Neandertals at the DPP4 locus as previously described for the chromosome 3 locus (Zeberg and Pääbo, 2020a); using sites carrying alleles fixed among Neandertals and absent among Yoruba, phylogenetic trees, and the equation derived by Huerta-Sanchez et al. (2014). Previous genome-wide analyses have also found gene-flow at this locus (Vernot et al. 2014, Sankararam et al. 2012, Steinrücken et al. 2018, Chen et al. 2020, Skov et al. 2020).

Introgression-wide significance

The three hitherto described Neandertal genetic risk factors for severe COVID-19 are all present at allele frequencies of \( \geq 1\% \) in European genomes from the 1000 Genomes Project and the COVID-19 HGI cohort. None of them are present in the three African populations in the 1000 Genomes Project with lowest amount of Neandertal admixture (Chen et al. 2020), i.e., Mende in Sierra Leone (MSL), Esan in Nigeria (ESN) and Yoruba in Ibadan, Nigeria (YRI). All three haplotypes carry derived alleles homozygously present in the three high-coverage Neandertal genomes (Prüfer et al. 2014, Prüfer et al. 2017, Mafessoni et al. 2020). We filtered SNPs fulfilling these criteria using a set of high-confidence Neandertal haplotypes defined by the intersection of previously published genome-wide maps of gene-flow from Neandertals (Vernot et al. 2014, Sankararam et al. 2012, Steinrücken et al. 2018, Chen et al. 2020, Skov et al. 2020). To exclude Neandertal-like variants due to incomplete lineage sorting, we further required the resulting haplotypes to have a length of at least 10 kb. Using these criteria, we identify 40,055 SNPs. We use these SNPs to estimate the effective number of hypotheses in European genomes from the 1000 Genomes Project using the Genetic Type I Error Calculator (Li et al. 2011). This yields a significance threshold of 8.7e-6 and a suggestive threshold of 1.7e-4, for a Neandertal “introgression-wide association study” ("IWAS").

Sample size needed to detect the DPP4 haplotype

As shown above, the power to detect a variant is improved if only introgressed Neandertal haplotypes are considered, although under different null-hypotheses. We calculated the sample size needed to achieve genome-wide significance (p<5e-8), using standard techniques (Pirinen et al. 2020). If there is a non-zero effect, i.e., \( \beta \neq 0 \), then the z-score is distributed as \( z \sim \mathcal{N}(\beta/SE, 1) \) and \( z^2 \sim \chi^2((\beta/SE)^2) \). The parameter \((\beta/SE)^2\) is known as the non-centrality parameter and scales linearly with sample size. The
non-central chi-squared distribution was used to calculated the probability of observing a sufficiently strong association, i.e. statistical power. To reach 80% power to detect the Neandertal \textit{DPP4} haplotype we find that we need approximately twice the sample size. For 99% detection probability the sample size needs to tripple.

\textit{Data availability}

The archaic genomes are availability at the server of the Max Planck Institute for Evolutionary Anthropology (http://cdna.eva.mpg.de/neandertal/Vindija/VCF/ and https://bioinf.eva.mpg.de/jbrowse/) and the modern human genomes at the 1000 Genomes Project server (http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/). GWAS summary statistics can be obtained from the COVID-19 Host Genetics Imitative (https://www.covid19hg.org/results/, round 4 release: A/B2\_ALL\_eur\_leave\_23andme).
Supplementary Table

Supplementary Table S1. SNPs in linkage disequilibrium with rs117888248 and the corresponding Neandertal alleles. LD data from the 1000 Genomes Project, “Vindija” refers to a Croatian Neandertal genome (https://bioinf.eva.mpg.de/jbrowse/).

| Chr | Pos   | rsid   | LD with rs117888248 | Ref | Alt/Risk | Vindija |
|-----|-------|--------|---------------------|-----|----------|---------|
| 2   | 162936216 | rs117888248 | 1.000              | C   | T        | T/T     |
| 2   | 162938711  | rs79624636   | 1.000              | G   | C        | G/C     |
| 2   | 162933386  | rs116906287   | 1.000              | A   | G        | A/G     |
| 2   | 162932814  | rs118098838   | 1.000              | G   | T        | T/T     |
| 2   | 162940017  | rs76135328    | 1.000              | C   | A        | A/A     |
| 2   | 162941569  | rs117460501   | 1.000              | A   | G        | G/G     |
| 2   | 162944795  | rs117965144   | 1.000              | C   | T        | T/T     |
| 2   | 162945153  | rs117516594   | 1.000              | G   | A        | G/A     |
| 2   | 162949980  | rs188765526   | 1.000              | G   | A        | G/G     |
| 2   | 162955612  | rs145259049   | 1.000              | C   | T        | C/T     |
| 2   | 162956598  | rs77914221    | 1.000              | T   | C        | T/C     |
| 2   | 162957351  | rs57355997    | 1.000              | T   | A        | A/A     |
| 2   | 162938141  | rs75778040    | 0.908              | A   | T        | T/T     |
| 2   | 162932254  | rs6709208     | 0.899              | C   | T        | T/T     |
| 2   | 162931074  | rs115498481   | 0.808              | G   | T        | T/T     |
References

1. Auton, A. et al. (2015) A global reference for human genetic variation. Nature. doi: 10.1038/nature15393
2. Chen, L. et al. (2020) Identifying and Interpreting Apparent Neanderthal Ancestry in African Individuals. Cell. doi: 10.1016/j.cell.2020.01.012
3. de Groot R.J. et al. (2013) Middle East respiratory syndrome coronavirus (MERS-CoV): announcement of the Coronavirus Study Group. Journal of Virology. doi: 10.1128/JVI.01244-13
4. Hoffmann, M. et al. (2020) SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. doi: 10.1016/j.cell.2020.02.052
5. Huerta-Sánchez, E. et al. (2014) Altitude adaptation in Tibetans caused by introgression of Denisovan-like DNA. Nature. doi: 10.1038/nature13408
6. Kuhlwilm, M. et al. (2016) Ancient gene flow from early modern humans into Eastern Neanderthals. Nature. doi: 10.1038/nature16544.
7. Li, M. et al. (2011) Evaluating the effective numbers of independent tests and significant p-value thresholds in commercial genotyping arrays and public imputation reference datasets. Human Genetics. doi: 10.1007/s00439-011-1118-2.
8. Li, Y. et al. (2020) The MERS-CoV Receptor DPP4 as a Candidate Binding Target of the SARS-CoV-2 Spike. iScience. doi: 10.1016/j.isci.2020.101160.
9. Lim, S. et al. (2021) COVID-19 and diabetes mellitus: from pathophysiology to clinical management. Nature Reviews. doi: 10.1038/s41574-020-00435-4.
10. Mafessoni, F. et al. (2020) A high-coverage Neandertal genome from Chagyrskaya Cave. Proceedings of the National Academy of Sciences. doi: 10.1073/pnas.2004944117
11. Mentlein, R. (1999). Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. Regulatory Peptides. doi: 10.1016/S0167-0115(99)00089-0.
12. Meyer, M. et al. (2016) Nuclear DNA sequences from the Middle Pleistocene Sima de los Huesos hominins. Nature. doi: 10.1038/nature17405
13. Païro-Castineira, E. et al. (2020) Genetic mechanisms of critical illness in Covid-19. medRxiv. doi: 10.1101/2020.09.24.20200048.
14. Petr, M. et al. (2020) The evolutionary history of Neanderthal and Denisovan Y chromosomes. Science. doi: 10.1126/science.abb6460
15. Posth, C. et al. (2017) Deeply divergent archaic mitochondrial genome provides lower time boundary for African gene flow into Neanderthals. Nature Comm. doi: 10.1038/ncomms16046
16. Pirinen, M. et al. (2020) GWAS 3: Statistical power. https://www.mv.helsinki.fi/home/mjxpirin/GWAS_course/material/GWAS3.html. Retrieved 2020-12-11.
17. Prüfer, K. et al. (2014) The complete genome sequence of a Neanderthal from the Altai Mountains. Nature. doi: 10.1038/nature12886.
18. Prüfer, K. et al. (2017) A high-coverage Neandertal genome from Vindija Cave in Croatia. Science. doi: 10.1126/science.aao1887
19. Rees, J.S. et al. (2020) The Genomics of Human Local Adaptation. Trends in Genetics. doi: 10.1016/j.tig.2020.03.006.
20. Sankararaman, S. et al. (2012) The date of interbreeding between Neandertals and modern humans. PLoS Genet. doi: 10.1371/journal.pgen.1002947.
21. Scheen, A.J. (2020) DPP-4 inhibition and COVID-19: From initial concerns to recent expectations. Diabetes and Metabolism. doi: 10.1016/j.diabet.2020.11.005.
22. Skov, L. et al. (2020) The nature of Neanderthal introgression revealed by 27,566 Icelandic genomes. Nature. doi: 10.1038/s41586-020-2225-9
23. Stalin Raj, V. et al. (2013) Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. Nature. doi: 10.1038/nature12005
24. Steinrücken, M. (2018) Model-based detection and analysis of introgressed Neanderthal ancestry in modern humans. Molecular Ecology. doi: 10.1111/mec.14565
25. The COVID-19 Host Genetics Initiative (2020) The COVID-19 Host Genetics Initiative, a global initiative to elucidate the role of host genetic factors in susceptibility and severity of the SARS-CoV-2 virus pandemic. Eur. J. Hum. Genet. doi: 10.1038/s41431-020-0636-6
26. Vankadari, N. and Wilce, JA (2020) Emerging WuHan (COVID-19) coronavirus: glycan shield and structure prediction of spike glycoprotein and its interaction with human CD26. Emerg Microbes Infect. doi: 10.10582/22221751.2020.173956
27. VanLiere, J.M. and Rosenberg, N.A. (2008) Mathematical properties of the $r^2$ measure of linkage disequilibrium. Theoretical Population Biology. doi:10.1016/j.tpb.2008.05.006
28. Vernot, B. and Akey, J.M. (2014) Resurrecting surviving Neandertal lineages from modern human genomes. Science. doi: 10.1126/science.1245938
29. Wang, N. et al., (2013) Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4. Cell Res. doi: 10.1038/cr.2013.92
30. Zeberg, H. and Pääbo, S. (2020a) The major genetic risk factor for severe COVID-19 is inherited from Neandertals. Nature. doi: 10.1038/s41586-020-2818-3
31. Zeberg, H. and Pääbo, S. (2020b) A genetic variant protective against severe COVID-19 is inherited from Neandertals. BioRxiv. doi: 10.1101/2020.10.05.327197
32. Zhou, P. et al. (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. doi: 10.1038/s41586-020-2012-7
