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editing efficiency can be significantly increased to 65–100% if the gRNAs are fused to a mobile RNA sequence, such as truncated *FLOWERING LOCUS T (FT)* [10]. This suggests that a mobile RNA sequence allows gRNAs to move between cells, thus enabling targeting of genomic sequences in meristem and germline cells. It may therefore be possible to fuse the Cas9/gRNA system with a mobile RNA sequence, and then use the corresponding engineered SYN virus to infect plants, thus achieving genome editing completely independently of tissue culture (Figure 1). With these improvements, one can envisage editing a trait of choice in virtually any plant, be it in the field or elsewhere, to obtain transgene-free genome editing for biotechnological improvement of crops.

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Forum

Host Polymorphisms May Impact SARS-CoV-2 Infectivity

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Based on a broad public database compilation, we support the hypothesis that germinal polymorphisms may regulate the expression of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cellular target itself and proteases controlling the process of its shedding or, conversely, its internalization. Consequently, a genetic influence on individual susceptibility to coronavirus disease 2019 (COVID-19) infection is strongly suspected.

General Background

In addition to the need for virus detection, evaluation of individual serological response [1], and biological analytical tools to manage COVID-19 on a population level, there is an urgent need to obtain objective information to identify at-risk individuals and to understand the marked variability in the severity of the disease in

Glossary

Expression quantitative trait locus (eQTL): a genomic locus that explains the variation in gene expression of nearby genes.

Insertion/deletion (Indel): an insertion or deletion of bases in the genome.

Minor allele frequency (MAF): frequency at which the second most common allele occurs in a given population.

general, as well as in given populations. A current hypothesis is that SARS-CoV-2 clinical manifestations are governed by human genetics [2]. Thus, in this context, here we develop two complementary themes: (i) a more thorough examination of the membrane shedding of angiotensin-converting enzyme 2 (ACE2), the SARS-CoV-2 cellular target, and its potential repercussion on virus propagation; and (ii) a description of the interindividual variability of the genes (SNPs) involved in ACE2 processing and their potential impact on the risk of contracting COVID-19.

ACE2 Expression and COVID-19

Chen *et al.* recently examined a large Genotype–Tissue Expression (GTEx) database and investigated the expression of ACE2 in different human tissues [3]. The authors stressed that, counterintuitively, expression of the SARS-CoV-2 target was inversely related to certain risk factors, showing higher levels in Asian females compared with Asian males and a significant decrease in patients with type 2 diabetes mellitus. Globally, at a population level, there was a negative correlation between ACE2 expression and COVID-19 severity. Recent data provide evidence that ACE2 is effectively shed from membranes, a process that is fine-tuned at different levels [4] involving two cell membrane proteases: disintegrin and metalloproteinase domain-containing protein 17 (ADAM17) and transmembrane protease serine 2 (TMPRSS2) [4]. More precisely, ADAM17 acts directly on ACE2

ACE2 shedding > Viral load
Virus kept at distance from sensitive tissues of the host

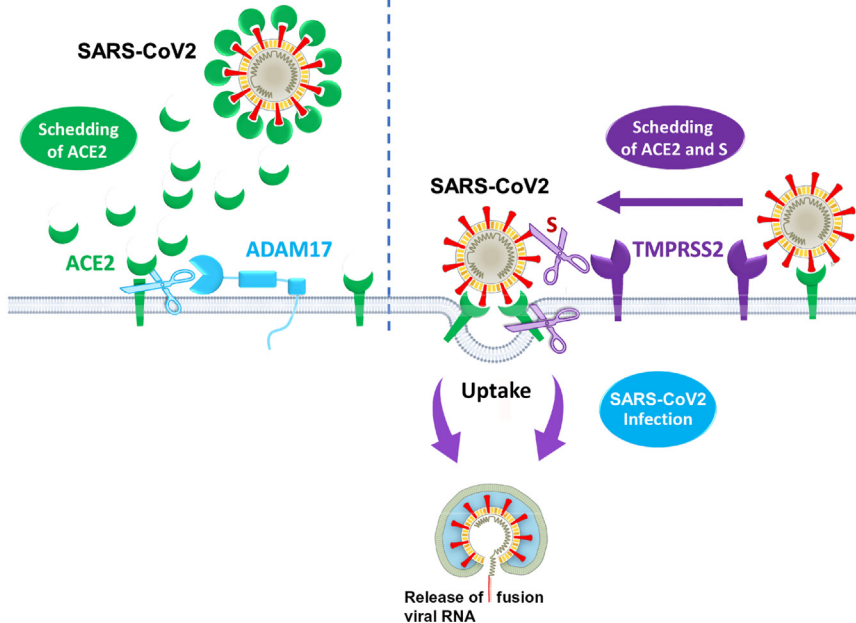


Figure 1. Graphic Support for the Hypothesis of Angiotensin-Converting Enzyme 2 (ACE2)-Related Virus Neutralization at Distance due to the Presence of Enzymatic Membrane Mechanisms Regulating ACE2 Shedding and Virus Entry. Abbreviations: ADAM17, disintegrin and metalloproteinase domain-containing protein 17; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane protease serine 2.

and leads to ACE2 shedding into the extracellular cellular space, while TMPRSS2 cleaves not only ACE2, but also the S protein of SARS-CoV-2, thus leading to membrane fusion and cellular uptake of the virus. Consequently, while ADAM17 and TMPRSS2 both act on ACE2, they may have opposite effects on net ACE2 shedding (Figure 1). When the respective proteolytic activities of ADAM17 and TMPRSS2 result in more ACE2 shedding than internalization, it follows that this situation may constitute a natural barrier to infection. This could be due to the interaction between soluble ACE2 with the virus at a distance from sensitive tissues. Subsequently, on this basis, one can hypothesize that, when the viral load is high, the shedding barrier effect is

overwhelmed, thus facilitating subsequent infection.

ACE2, TMPRSS2, and ADAM17 Gene Polymorphisms

Cao *et al.* [5] compiled a database analysis of all 1700 variants in the region of the *ACE2* gene located on the X chromosome. They identified 15 unique **expression quantitative trait loci** (eQTLs; see **Glossary**) variants [14 SNPs and 1 **insertion/deletion** (INDEL)] with higher **minor allele frequencies** (MAF) in the Asian population than in a European population (MAF of 0.05 versus 0.35–0.48 for the top six most common variants). Interestingly, their data showed that the 11 most common variants (MAF >0.05) were associated with increased expression

of *ACE2* in tissues, suggesting, according to the authors, a different sensitivity to SARS-CoV-2 infectivity. However, the functional basis of the influence of SNP on ACE2 expression remains to be established.

In this context, we performed a complementary *in silico* study including SNPs regulating gene expression not only for *ACE2*, but also for *ADAM17* and *TMPRSS2* (Table S1 in the supplemental information online). Overall, and based on the ACE2 expression-associated MAF between ethnic populations, it appears that Asians express a higher level of ACE2 than Caucasians, while Africans show an intermediary level of ACE2 expression. This is consistent with the findings previously reported by Cao *et al.* [5]. It is still debatable whether these differences should be taken into consideration in epidemiological studies on COVID-19 covering ethnic associations with disease occurrence [6]. Importantly, the diseases associated with a high level of SARS-Cov-2 infection (hypertension and diabetes) were found to be related to a lower expression of ACE2, in relation to the respective allelic distribution. This relationship concurs well with the study by Chen *et al.* pointing towards a negative correlation between ACE2 expression and COVID-19 severity [3].

It has been reported that subjects with rs383510/T and rs2070788/G genotypes of *TMPRSS2* located on chromosome 21q22.3 are more prone to develop a severe form of A (H1N1) influenza and acute respiratory distress syndrome [7]. Of note, males have been shown to be more likely to develop a severe form of H1N1 influenza and there is evidence that androgens are positive regulators of *TMPRSS2* [8]. Importantly, the alleles at risk (T for rs383510 and G for rs2070788) are linked to increased gene expression (Table S1 in the supplemental information online), logically supporting the hypothesis of a higher level of viral cell entry. It is

tempting to extrapolate this SNP influence to SARS-CoV-2 infectivity.

The *ADAM17* locus on chromosome 2p25.1 presents two clusters and three unique SNPs that induce strong differences in terms of allelic profiles between Asian and European populations that are associated with hypertension [9] and/or sepsis [10]. Of note, most of these SNPs are located in the promoter region of *ADAM17* and are associated with either positive or negative eQTL, depending on the SNP and the tissue (Table S1 in the supplemental information online). Therefore, there is a strong possibility that genetic polymorphisms influencing *ADAM17* expression also contribute to the modulation of ACE2 shedding intensity.

Practical Consequences

Taken together, the above-discussed data advocate in favor of a multifactorial genetic impact on the risk of SARS-Cov-2 infectivity and possible disease severity. A relatively simple and easy-to-perform test, such as quantitative PCR [11] or MASSarray [12], would allow large-scale individual SNP profiling for *ACE2*, *ADAM17*, and *TMPRSS2* to identify possible at-risk populations vulnerable to viral infection. On this basis, a 'multiSNPs risk score' could be established that would be applicable to large populations and, thus, it might then be possible to identify subjects carrying a combination of favorable alleles for *ACE2*, *ADAM17*, and *TMPRSS2* conferring a lesser risk of contracting SARS-Cov-2 infection, and vice versa. Such an analytical strategy was recently developed based on patient genetic characteristics for immunogenetic profiling designed to

personalized immunotherapy [12]. A similar supervised genetic approach to COVID-19 risk assessment could complement the current unsupervised GWAS investigations, which require large population studies exploring the whole patient genome for DNA variations in an attempt to explain interindividual differences in COVID-19 severity [e.g., the Howard Hughes Medical Institute (HHMI) genetic project and the COVID-19 Human Genetic Effort [2]]. Ideally, in a final step, a multifactorial predictive index could be established incorporating SNP analysis and other, more established, risk factors.

In summary, until now, genetic influences on COVID-19 interindividual susceptibility have been largely underestimated; thus, we hope that the discussion might fill this gap and will pave the way for confirmatory investigations at experimental and clinical levels.

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Supplemental Information

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Resources

ⁱwww.hhmi.org/news/patients-with-severe-forms-of-coronavirus-disease-could-offer-clues-to-treatment

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