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Viewpoint

Chemosensory Dysfunction in COVID-19: Integration of Genetic and Epidemiological Data Points to D614G Spike Protein Variant as a Contributing Factor

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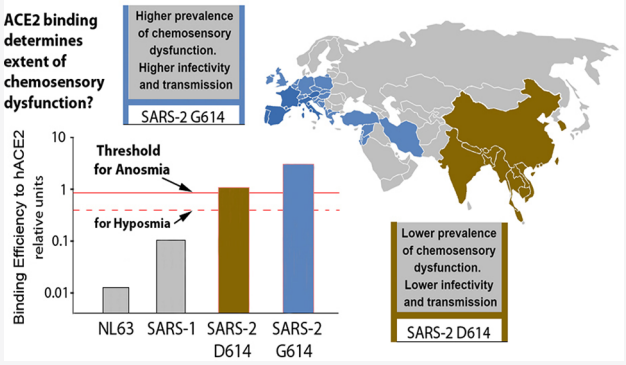
ABSTRACT: After several months of rapid pandemic expansion, it is now apparent that the SARS-CoV-2 coronavirus interferes with smell and taste sensation in a substantial proportion of COVID-19 patients. Recent epidemiological data documented intriguing differences in prevalence of chemosensory dysfunctions between different world regions. Viral genetic factors as well as host genetic factors appear to be relevant; however, it is not yet known which mutations or polymorphisms actually contribute to such phenotypic differences between populations. Here, we discuss recent genetic and epidemiological data on the D614G spike protein variant and assess whether current evidence is consistent with the notion that this single nucleotide polymorphism augments chemosensory impairments in COVID-19 patients. We hypothesize that this spike variant is an important viral genetic factor that facilitates infection of chemosensory epithelia, possibly acting together with yet to be identified host factors, and thereby increases smell and taste impairment. We suggest that the prevalence of chemosensory deficits may reflect the pandemic potential for transmissibility and spread which differs between populations.



KEYWORDS: *Chemosensory dysfunction, olfactory dysfunction, COVID-19, D614G variant, D614G mutation, ACE2*

INTRODUCTION

The rapid spread of the novel human SARS-CoV-2 coronavirus across the globe is associated with an unexpectedly high incidence of chemosensory deficits in infected patients. These neurological symptoms were rarely observed during the early phase of the pandemic in China; however, they were widely noticed in March, when the pandemic had reached Western countries.[1] In the last few months, numerous studies have confirmed that chemosensory dysfunction is one of the cardinal symptoms of COVID-19. Since the beginning of the pandemic, there has been a trend toward an increase in the incidence of chemosensory dysfunctions, especially between February and the end of March.[2] The latest and most extensive meta-analysis based on over 38 000 patients showed a statistically significant 3-fold higher prevalence of chemosensory dysfunction in Europe and America compared to Asia.[1] Multiple investigators have proposed that both viral and host genetic factors contribute to this phenomenon (reviewed by von Bartheld et al.).[1] Initial suggestions focused on the host factors such as ACE2 polymorphism and differences in expression levels of ACE2 variants between populations. However, no direct evidence has been shown so far, and results of genetic analysis of expression quantitative trait loci (eQTL) indicated that ACE2 tends to have higher expression levels in East Asian populations than in Caucasians, but these data were from lung tissue, not from the nasal olfactory epithelium.[3] Nevertheless, it is likely that the host genotype contributes to the magnitude of chemosensory deficits, since a classical twin study found heritability for anosmia in COVID-19 at 47%.[4] Viral genetic factors were initially considered to be less likely to play a role, since the SARS-CoV-2 mutation rate is relatively slow as compared to influenza viruses and even to SARS-CoV-1 coronavirus. This conclusion had to be revised recently with new insights based on the tracking of one particular mutation in the SARS-CoV-2 virus.

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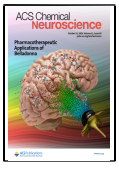

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GEOGRAPHICAL PREVALENCE OF D614G VARIANT AND PREVALENCE OF CHEMOSENSORY DEFICITS

The recent work on viral genetics done by Korber and colleagues showed that, early in the pandemic, SARS-CoV-2 spike protein had amino acid D (aspartic acid) at position 614. As the pandemic progressed, variant G614 with glycine at position 614 increased rapidly in frequency and it is now the dominant form globally.[5] When the pandemic arrived in Western countries, the G614 variant was already dominant in many European countries (55−85% by the end of April, 2020)[6] and now is almost exclusive in this region. In East Asia, the shift from D to G was slower and approximately 90−97% viral samples from Chinese patients showed D614 by the end of April.[6] However, even there the G614 variant now has become dominant. Thus, the world map showing higher D614 frequency in the first few months of the pandemic resembles the world map visualizing low prevalence of anosmia/ageusia as shown by the latest and most extensive meta-analyses.[1,2] Accordingly, the higher prevalence of the G614 variant in Europe/America correlates with a consistently higher prevalence of chemosensory dysfunction from this region.[1] In addition to data from China, also data obtained early in the pandemic from India and Australia showed relatively lower prevalence of anosmia/ageusia.[1] As Indian and Australian populations have different genetic backgrounds than Chinese populations, this suggests that, in addition to host genetic factors, the D614G spike variant may also contribute to the frequency of chemosensory dysfunction. On the other hand, a much higher frequency of chemosensory dysfunction was reported already in some of the first European patients diagnosed in late January and early February,[1,2] and at this time the G614 variant already largely dominated over D614 in most European countries.[5] Taken together, the latest genetic and epidemiology data suggest that the D614G spike variant may be, in part, responsible for the increased frequency of the chemosensory dysfunction during the current pandemic.

VIRUS BINDING TO HACE2, A CRITICAL STEP LEADING TO ANOSMIA, IS LIKELY AFFECTED BY THE D614G SPIKE VARIANT

The similarities between the geographic frequency of G614 and the geographic prevalence of chemosensory dysfunction are suggestive, but they are not sufficient to unequivocally support the hypothesis presented above. However, there are additional lines of evidence that strengthen this view.

The occurrence and incidence of olfactory deficits appears to depend on the affinity of the RBD (receptor binding domain), present in the spike protein at the viral surface, to the ACE2 host receptor. It is known that the SARS-CoV-1 virus responsible for the SARS pandemic of 2003 did not cause chemosensory dysfunction, even though it binds to the same human ACE2 receptor (hACE2). The binding affinity of the RBD in the spike protein of SARS-CoV-1 to hACE2 is about 1 order of magnitude lower than that of SARS-CoV-2.[7] Thus, SARS-CoV-1 binds hACE2 with an affinity that is likely not high enough to readily infect cells in the upper respiratory tract, including sustentacular cells in the olfactory epithelium which are crucial for olfaction.[8] Additionally, for the HCoV-NL63 virus, which is the third human coronavirus that also uses ACE2 as the host receptor, the affinity of S1-RBD of its spike protein for hACE2 was estimated to be several times lower than that of SARS-CoV-1.[9] Consistent with this low affinity to hACE2 binding, the NL63 virus was not reported to cause anosmia. For the above reasons, we propose that variants of the spike protein with higher RBD affinity to hACE2 and/or higher RBD exposure on the virion surface are strong candidates that may enhance SARS-CoV-2 infection in the olfactory epithelium and thereby increase the probability of chemosensory deficits (Figure 1). It must be emphasized that

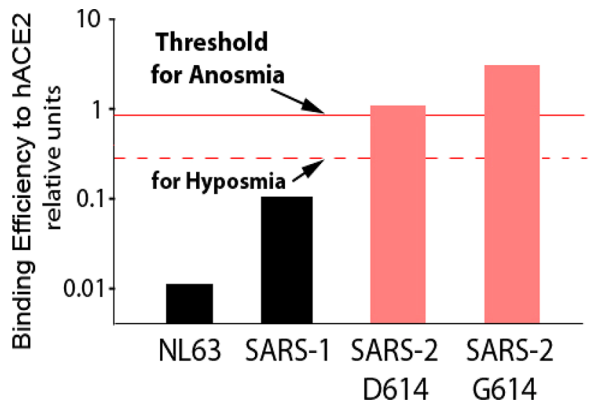

Figure 1. Schematic representation of the key role of the coronavirus spike protein-RBD binding efficiency to human ACE2 in the development of olfactory dysfunction. NL63, SARS-CoV-1, and SARS-CoV-2 are three human coronaviruses that use hACE2 to enter host cells. The RBD of spike proteins in NL63 and SARS-CoV-1 have lower affinity to hACE2 as compared to SARS-CoV-2. The D614G substitution, even though it is not located within the RBD (so it will not change the affinity of pure RBD to hACE2), changes the interprotomer spike energetics and enhances RBD exposure, thus favoring the likelihood of binding of the G614-spike protein to hACE2 as compared with the D614 variant.[11] Convincing alternative explanations have also been proposed, indicating that G614 results in spike protein stabilization and increased spike protein incorporation into pseudovirions, thus creating more ACE2-binding sites on the virion surface.[12] We propose that hyposmia and anosmia require virus binding in the olfactory epithelium above a certain threshold, and for this reason chemosensory dysfunctions did not occur with infections of the SARS-CoV-1 or NL63 viruses. Thresholds and binding efficiencies are approximations based on the literature for different cell lines and are not yet known for cells in the olfactory epithelium. RBD, receptor binding domain of the spike protein; hACE2, human ACE2 receptor, NL63, human coronavirus HCoV-NL63.

the binding values estimated in Figure 1 are from data obtained for respiratory tract cells and cell lines, since no such data for chemosensory epithelia are yet available.

The above considerations prompt a mechanistic assessment of the impact of the D614G mutation on efficiency of virus binding and entry to ACE2-expressing cells in the olfactory epithelium. Amino acid position 614 is located within the spike protein near the carboxyl terminus of the S1 subunit, and thus adjacent to, but outside of the RBD domain. Molecular modeling data shows that D614G is located on the surface of the spike protomer, and recent studies report that the amino acid substitution D614 to G614 favors the open conformational state of the spike protein. Microsecond all-atom simulations suggest that this open conformation called "1-up" results in an enhanced RBD exposure on the virion surface and thus increases the probability of binding to the ACE2 host receptor.[10] Therefore, the G614 spike variant is not truly affecting the affinity of the "isolated" RBD to hACE2; however, it increases the efficiency of virus binding to host cells *in vivo* as

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compared to D614. As a result, it makes the G614 variant more capable of infecting cells.[10] Using single cycle spike-pseudotyped virus, the binding probability and the resulting transduction efficiency of the G614 variant to ACE2 was predicted to be approximately 2−6 times higher as compared to D614. This supports our hypothesis that the G614-containing SARS-CoV-2 strain may contribute to the higher incidence of chemosensory deficits observed in Western countries.

The structural and functional consequences of the D614G mutation are not yet clear and are a topic of ongoing debate. There are plausible alternative explanations for molecular consequences of this mutation. Zhang and colleagues showed that the G614 variant correlates with less spike protein subunit S1 shedding, reduced cleavage, and more efficient incorporation of the spike protein into the viral particles.[11] The reduced cleavage and increased spike protein stability may be due to the D614G mutation's impact on the adjacent furin-cleavage site (FCS) located at the position near the 682−686 amino acids. An FCS is present in SARS-CoV-2 but not in SARS-CoV-1 and NL63 coronaviruses, and it was shown to be essential for virus cell entry and infectivity.[12] Cryo-electron microscopy data suggest that host protease cleavage within the FCS determines the adoption of the open conformation by the spike protein that is required to bind to hACE2.[13] Homology modeling of the spike protein region spanning the FCS revealed that the D614G mutation affects the secondary structure at the FCS region and thus possibly modulates spike protein cleavage and in consequence protein stability and binding to hACE2.[6] Another structural analysis corroborates with experimental data conclusions of Zhang and colleagues, showing that the D614G substitution promotes S1−S2 spike subunit association. This stabilizes the spike protein, but without direct interaction with the FCS site.[14] Both of the above proposed mechanisms ultimately lead to an increase in G614-virus binding and cell entry to the host cells. This may be due to the enhanced exposure of the RBD domain on the surface of the virus (mechanism 1)[10] and/or the higher amount of the more stable spike protein present in the envelope of the virus (mechanism 2).[11,14]

D614G SPIKE VARIANT AS THE FIRST PUTATIVE VIRAL FACTOR AFFECTING INCIDENCE OF ANOSMIA

There are further similarities between the functional properties of the D614G spike variant and chemosensory dysfunction caused by the SARS-CoV-2 coronavirus. It has been shown that the switch to the G614 variant does not increase the severity of the disease.[5] Similarly, the chemosensory deficits correlate with the mild rather than severe COVID-19 form.[1] It is now established, by using molecular modeling and "wet" experiments, that the D614 to G614 switch increases viral infectivity *in vitro* and likely increases disease transmissibility.[5,11,15] It should be emphasized that currently no other mutation in the SARS-CoV-2 spike protein with similar functional characteristics and distribution frequency is known, despite the fact that many others were detected and examined.[15] Similarly, a suspected increase in SARS-CoV-2 infectivity and a faster interindividual spread outside of East Asia appears to be associated with an increase in the incidence of chemosensory dysfunction during the pandemic's progression.[2] Finally, recent studies show that both the G614 variant and a more frequent occurrence of chemosensory deficits correlate with a higher viral load in the upper respiratory tract.[5,16] Although the above correlations require further experimental confirmation, they do support the scenario that the switch from D614 to G614 in the SARS-CoV-2 spike protein may contribute to the increase in prevalence of chemosensory dysfunctions in the COVID-19 pandemic.

Taking into account the above considerations, we propose that viral switching from D614 to G614 and the increased incidence of chemosensory deficits are functionally linked and reflect the same current features of the COVID-19 pandemic, mainly increased infectivity and transmissibility. It must be emphasized that the D614G spike variant is not the only mechanism and that additional factors, such as the yet uncharacterized host genetic variants of ACE2 and TMPRSS2, may similarly contribute to the incidence of chemosensory deficits in COVID-19. The level of ACE2 expression may also be an important factor, as young children (which were shown to have lower ACE2 expression in the upper respiratory tract) have recently been reported to have lower anosmia prevalence.[17] Furthermore, incomplete X chromosome inactivation may lead to higher ACE2 expression in females, and females have a trend toward increased olfactory dysfunction in COVID-19 compared to males.[1] Further studies should establish the extent to which host genetic factors contribute to anosmia in COVID-19 as compared to viral factors such as the D614G variant. Other confounding factors are three additional mutations of the G clade, present in 5′UTR, nsp3, and RdRp protein, which are in linkage disequilibrium with G614, as they may also have an effect on chemosensory dysfunction, although not by affecting ACE2 binding. The hypothesis proposed here will have to be thoroughly examined when more molecular and epidemiological data have become available. Global monitoring of SARS-CoV-2 mutations based on sequencing is more accurate than data available on chemosensory deficits, as the latter have more variabilities and confounding factors. Thus, the usage of retrospective data and their reinterpretation in the context of the role of the D614G variant in chemosensory dysfunction is limited. This issue is further complicated by the fact that most currently circulating SARS-CoV-2 viruses contain the G614 mutation, implying that patients infected with the D614 variant are at present rare. An alternative approach is to utilize human olfactory epithelium obtained from biopsies and grown *in vitro* which can then be infected with different virus strains to quantify efficiency of cell entry and infectivity. The limitation of this approach is the lack of possibility to directly correlate infectivity with olfactory deficits as well as the risk of molecular changes in olfactory epithelium cultures as compared to the *in vivo* situation. The usage of appropriate animal models is another strategy to obtain insights about the role of the D614G variant in the incidence of chemosensory dysfunction.

CONCLUDING REMARKS

The spike protein mutation D614G became dominant in the SARS-CoV-2 virus during the COVID-19 pandemic. The exact impact of the mutation on the disease phenotype is a critical issue and remains to be precisely elucidated. Combined genetic, structural, and epidemiological data suggest that the D614G switch may cause increased prevalence of chemosensory deficits as observed during the pandemic progression from East Asia to Western countries. The increased binding of the G614 spike variant to the ACE2 host receptor and/or

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increased cell entry efficiency by spike protein stabilization and reduced cleavage may be the underlying mechanism. Therefore, efficiency of SARS-CoV-2 binding and entry into the olfactory epithelium above a certain threshold may cause olfactory dysfunction in COVID-19. To gain more insights, future studies will need to examine the binding and the entry of D614G SARS-CoV-2 variants as well as SARS-CoV-1/NL63 specifically in ACE2-expressing cells of the olfactory epithelium such as sustentacular cells. Currently available data mainly examined cells of the lower and upper respiratory tract, and cell-type specific differences likely exist.[8] Importantly, the prevalence of olfactory deficits may reflect the pandemic potential for transmissibility and spread which differs between populations.

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Conceptualization, R.B. and C.S.vB.; graphics, K.B. and C.S.vB.; writing, R.B., K.B., and C.S.vB.; final review and editing, R.B., K.B., and C.S.vB.

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Notes

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