REVIEW

Molecular mechanisms involved in anosmia induced by SARS-CoV-2, with a focus on the transmembrane serine protease TMPRSS2

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Abstract

Since 2020, SARS-CoV-2 has caused a pandemic virus that has posed many challenges worldwide. Infection with this virus can result in a number of symptoms, one of which is anosmia. Olfactory dysfunction can be a temporary or long-term viral complication caused by a disorder of the olfactory neuroepithelium. Processes such as inflammation, apoptosis, and neuronal damage are involved in the development of SARS-CoV-2-induced anosmia. One of the receptors that play a key role in the entry of SARS-CoV-2 into the host cell is the transmembrane serine protease TMPRSS2, which facilitates this process by cleaving the viral S protein. The gene encoding TMPRSS2 is located on chromosome 21. It contains 15 exons and has many genetic variations, some of which increase the risk of disease. Delta strains have been shown to be more dependent on TMPRSS2 for cell entry than Omicron strains. Blockade of this receptor by serine protease inhibitors such as camostat and nafamostat can be helpful for treating SARS-CoV-2 symptoms, including anosmia. Proper understanding of the different functional aspects of this serine protease can help to overcome the therapeutic challenges of SARS-CoV-2 symptoms, including anosmia. In this review, we describe the cellular and molecular events involved in anosmia induced by SARS-CoV-2 with a focus on the function of the TMPRSS2 receptor.

Introduction

The outbreak of SARS-CoV-2 (COVID-19) originated in Wuhan, China, in December 2019 and spread rapidly across the world, causing a global pandemic [1]. More than 3,000,000 COVID-19 cases were reported worldwide by May 1, 2020. SARS-CoV-2-related olfactory dysfunction has been increasingly recognized as an isolated symptom or in association with other respiratory symptoms [2]. In a recent study, olfactory dysfunction was reported in 45% of COVID-19 patients [3]. Olfactory dysfunction is often transient, with sudden onset in the majority of cases, and

average time to recovery is between one and three weeks [4]. There is no significant relationship with sinonasal symptoms, indicating that the pathogenesis of COVID-19-related anosmia might be different from obstructive olfactory dysfunctions that are observed in the setting of other viral upper respiratory tract infections [2, 5]. Although the pathogenesis of COVID-19-related anosmia is not understood, various mechanisms have been proposed. Four different fundamental mechanisms have been proposed regarding smell dysfunction during COVID-19 infection, including infiltration of olfactory centers in the brain, rhinorrhea and nasal congestion and obstruction, damage of olfactory receptor neurons, and injury of supporting cells in the epithelium of the olfactory system. Various viral infections are able to induce nasal congestion, obstruction, and rhinorrhea, which subsequently impede access of odorants to the sensory epithelium and prevent their binding to olfactory receptors [6].

Physical nasal obstruction as a cause of anosmia in COVID-19 [7] has been ruled out due to the absence of nasal obstruction, congestion, and rhinorrhea in a large proportion of the patients with anosmia, who exhibit no mucosal swelling of sinuses or nasal clefts in radiographic imaging [8, 9]. Although sensorineural olfactory loss is considered a possible mechanism for anosmia [10], three major inconsistencies

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warrant attention, including the absence of viral protein expression, a lack of correlation of cell regeneration with clinical recovery, and a lack of virus in the neurons of the olfactory system. Replacement of olfactory receptor neurons requires 8 to 10 days, plus around five days for maturation of cilia following their death [11], while smell recovery after COVID-19 infection often requires less than seven days [4, 12]. Therefore, functional recovery of anosmia is faster than cilia maturation, neuronal replacement, and growth of neo-axons [6, 13]. Sudden smell loss followed by its rapid recovery is inconsistent with viral infiltration from nose into the olfactory centers of the brain [14, 15]. Currently, there is no evidence available regarding viral access to the brain through the olfactory route during the acute phase of anosmia [2, 16]. Indeed, data derived from genetically modified mouse models are contradictory regarding brain infiltration [17]. The hypothesis of virus-induced damage to support cells in the olfactory epithelium is partly supported by the observation of abundantly expressed entry proteins in sustentacular cells of the olfactory epithelium [18, 19]. However, sustentacular cell death is not necessarily an indication of neural death of olfactory receptors. Rapid smell recovery occurs in parallel with rapid replenishment of sustentacular cells, which is most often observed clinically [6].

The mechanism by which SARS-CoV-2 enters cells has similarities to those used by influenza virus and human immunodeficiency virus. These viruses have a spike protein (S protein) that belongs to the family of type I viral fusion proteins. The N-terminal subunit of the spike protein (S1) possesses the receptor-binding domain, which binds to the receptor angiotensin-converting enzyme 2 (ACE2) on the host cell, resulting in a conformational change in the S protein, and this is followed by enzymatic cleavage of the S protein by the transmembrane serine protease TMPRSS2 [20, 21]. The C-terminal domain of the spike protein (S2) possesses the heptad repeat domains HR1 and HR2, which facilitate viral entry by forming a six-helix bundle fusion core structure that helps to drive fusion of the viral membrane with the cell membrane [22]. TMPRSS2-induced priming of the S protein and interaction with the ACE2 receptor on the host cell surface are therefore important for viral entry [5, 23].

RNAseq data have implied that the *TMPRSS2* gene is expressed in non-neuronal and neuronal cells of the olfactory epithelium [24, 25]. However, in one investigation, no *TMPRSS2* expression was observed in the olfactory epithelium [26]. TMPRSS2 expression seems to be higher in non-neuronal olfactory epithelial cells than in olfactory receptor neurons [25, 27]. However, different levels of *TMPRSS2* expression are observed in various subpopulations of mature olfactory receptor neurons [25]. Such a mosaic expression pattern in major olfactory receptor neuron genes is not a typical observation. RNAseq profiling has demonstrated that

TMPRSS2 is expressed at higher levels in the murine olfactory epithelium than *ACE2* [18]. Data from expression profiling of the murine olfactory epithelium indicate that *ACE2* is expressed only in non-neuronal cells, whereas *TMPRSS2* expression is detected in both non-neuronal and neuronal cells, with possibly higher expression levels in non-neuronal cells [28].

There are numerous candidate drugs with the ability to inhibit SARS-CoV-2 replication and subsequent infection. Among these are inhibitors of ACE2 and inhibitors of TMPRSS2 serine protease. TMPRSS2 blockage might prevent SARS-CoV-2 entry into the cells [29]. Nafamostat mesylate and camostat mesylate are synthetic serine protease inhibitors with the ability to impair viral entry. In a clinical trial involving newly diagnosed outpatients with mild infection, oral camostat mesylate was shown to reduce the viral load of respiratory SARS-CoV-2, leading to rapid resolution of symptoms related to COVID-19 and amelioration of loss of smell and taste [30]. Thus, precise information about the functions of the TMPRSS2 receptor could aid in the development of therapeutic strategies against COVID-19 and its related complications, including anosmia. Here, we describe the molecular and cellular events related to anosmia-induced by SARS-CoV-2, focusing on the function of the TMPRSS2 receptor.

COVID-19 and olfactory disorder

Some of the key symptoms associated with COVID-19, such as fever, chills, cough, difficulty breathing or shortness of breath, sore throat, muscle pain, and sudden loss of taste or smell have also been highlighted previously [31]. Olfactory dysfunction (OD) is characterized by diminished or impaired smell ability while eating (retronasal olfaction) or sniffing (orthonasal olfaction) and is frequently observed in COVID-19 patients with mild symptoms, and even in otherwise asymptomatic carriers. Thus, OD can be considered a possible disease marker, especially in asymptomatic or minimally symptomatic cases [32, 33]. The available data about SARS-CoV-2-related OD indicate a sudden onset of olfactory damage that can occur with or without other signs. Anecdotal reports and unpublished data suggest that olfactory symptoms often resolve after about two weeks, but due to the lack of long-term follow-ups, it is not yet known what percentage of patients experience persistent postinfectious olfactory dysfunction. Coronaviruses are among the pathogens that are known to induce postinfectious OD. Indeed, cells of the nasal epithelium exhibit comparatively high levels of expression of the specific receptors essential for SARS-CoV-2 entry [34, 35]. Disturbance of cell function in the neuroepithelium of olfactory bulbs might lead to inflammatory alterations that impair the functions of olfactory

receptor neurons, resulting in impaired neurogenesis and/or improper functioning of these neurons, and these alterations could cause temporary or long-lasting OD [36].

In addition to the functional receptor ACE2, uptake of SARS-CoV-2 is facilitated by transmembrane serine protease 2 (TMPRSS2) [18, 37]. This protein is present in various organs, including heart, lungs, skeletal muscle, oral mucosa, respiratory cells, kidneys, and the central nervous system (CNS). This implies the possibility of multisystem disorders induced by COVID-19 affecting multiple organs simultaneously [14, 38]. Respiratory epithelial cells are the primary site of the attachment of SARS-CoV-2, and it is not particularly surprising that infection with this virus affects the sense of smell and taste [14, 39]. Several possible mechanisms have been proposed for COVID-19-associated anosmia, based on early studies and hospital observations.

The role of inflammation in the development of olfactory disorders induced by SARS-CoV-2

Over time, olfactory neurons begin to die due to ongoing overproduction of cytokines, leading to histological alterations of the neuroepithelium. Notably, when production of inflammatory cytokines is stopped, this is associated with the ability of stem cells in the basal epithelium to regenerate into new olfactory neuronal cells, helping in the recovery of the function of the olfactory system [40]. This suggests that inflammation could result in the development of sensorineural anosmia and that novel therapeutic strategies to eliminate certain inflammatory mediators might be effective in treating SARS-CoV-2-related anosmia [41].

Amongst the cytokines that contribute to inflammatory disorders of the nasal mucosa, tumor necrosis factor (TNF)-α is the most relevant, and it is a powerful mediator of inflammation in various cell types [42, 43]. Although the impact of TNF-α on olfactory neuronal functions is well established, the specific underlying mechanisms of action are not yet well understood. The expression of both TNF-α receptors and mRNA for TNF-α itself in normal epithelial cells has been demonstrated [44], and exposure of olfactory tissue to TNF-α has been associated with specific histopathological alterations. The development of inflammatory infiltration in the setting of local TNF-α expression has been shown to result in a notable expansion of the olfactory submucosa [40].

SARS-CoV-2 infection of the lower or upper respiratory tract causes mild to severe forms of acute respiratory syndrome, which are associated with the release of proinflammatory cytokines such as IL-1β. The binding of SARS-CoV-2 to Toll-like receptors (TLRs) is accompanied by release of pro-IL-1β. Cleavage of pro-IL-1β by caspase-1

leads to activation of inflammasomes and generation of mature IL-1β, which, in turn, is involved in fever, inflammation of lung tissue, and fibrosis. Notably, blocking of members of proinflammatory interleukin-1 family has been shown to be associated with therapeutic benefits with respect to various inflammatory disorders, including viral infections [45]. Torabi et al. [38] demonstrated an increase in the levels of IL-1β in the olfactory epithelium and suggested that little damage to the central nervous system occurred in cases of anosmia because virus was not detected in cerebrospinal fluid in their study. Although different hypotheses have been proposed by different authors regarding the underlying mechanisms involved in anosmia related to COVID-19 [10, 46–49], the exact mechanism is not known. The data from the above-mentioned study are in line with prior investigations on IL-1β and TNF-α that could be considered when developing treatment and control strategies for SARS-CoV-2 [38].

SARS-CoV-2-infection-associated apoptosis of olfactory cells

Early apoptosis of olfactory cells during viral infection has been studied in animal models. Evidence of apoptosis has been observed in mice with anosmia following intranasal inoculation with some other viruses. Apoptosis and reduced proliferation of olfactory epithelial cells have been demonstrated after intranasal inoculation with Sendai virus (strain 52), which is a mouse counterpart to parainfluenza virus in humans. Another investigation on influenza virus strain R404BP showed apoptosis of neuronal cells in the olfactory system [50]. Inhibited anterograde migration of the virus to the CNS and olfactory bulb has been observed following apoptosis in olfactory cells, and this prevented prolonged olfactory disturbance. This process might be an inherent reaction that prevents a significant course of infection secondary to the regenerative capacity of the neurons of the olfactory system. Viruses with the ability to delay or inhibit apoptosis of olfactory sensory neurons (OSNs) are more prone to enter the brain and olfactory nerve [51, 52].

Levine et al. have commented that neuronal apoptosis is a thorny dilemma, since many important neuronal cells are not renewable during the lifetime of the individual. This limits the advantage of large-scale apoptosis for each kind of respiratory viral infection [53]. This would be a different situation if neurons had self-renewal capacity. Olfactory receptor neurons (ORNs) are a very special exception because of their continual self-renewal every 30-120 days throughout life [54]. Thus, lifelong controlled programmed cell death of ORNs is a usual turnover event. People do not perceive any alteration in the olfactory system when permanent regulated apoptosis is occurring, but intense apoptosis

of ORNs can result in sudden loss of the sense of smell. Continuous regeneration of parts of the cranial nerves would make sense teleologically for natural restoration of the sense of smell [55].

It has been demonstrated within last decade that cells of the immune system and cytokines also participate in the regulation of neurogenesis and apoptosis in the neuroepithelium of the olfactory system. It was shown in mice that secretion of growth factors and cytokines and upregulation of ORN regeneration occurred in the presence of activated macrophages, while reduced neurogenesis was seen in their absence [56]. In late 2019, samples obtained from the olfactory mucosa of adult subjects were found to contain numerous immature neuronal cells as well as leukocytes, indicating that neurogenesis in the olfactory system occurs in adults and that immune cells play a fundamental role in homeostasis of the olfactory epithelium [28, 55].

Destruction of olfactory epithelial cells in COVID-19-induced anosmia

Viral infection can lead to partial or total destruction of the nasal olfactory epithelium (OE), including OSNs. In these cases, "postviral anosmia" or "post-upper respiratory infection (URI) anosmia" persists after clearance of rhinitis and associated symptoms of URI for weeks to months until injured regions in the OE of nasal tissue are regenerated. The underlying pathophysiological condition of "postviral anosmia" and related histological examinations have been described in the literature, particularly after infection by rhinoviruses [13, 57]. Neutrophilic inflammation ensues following infection of the nasal respiratory tract and OE, leading to rhinorrhea and mucosal edema. The conductive loss of smell is usually related to the underlying nasal congestion, although in these cases, histological evaluation of the OE revealed the absence of cilia and a loss of some OSNs, which were altered in the metaplastic squamous epithelium, suggesting a sensorineural contribution [5, 58].

Postviral anosmia after human coronavirus 2296 (HCoV-229E) infection has been reported to be associated with olfactory dysfunction lasting more than 6 months [59]. HCoV-229E uses human aminopeptidase N as a receptor for entry into host cells, unlike SARS-CoV-2 and SARS-CoV, which use ACE2 [23, 60]. In SARS-CoV-infected patients, overexpression of *ACE2* in the nasal respiratory epithelium has been detected [61], especially on ciliated cells, which is consistent with intranasal entry of the virus [62]. However, these data are in contrast with data from another study in which *ACE2* expression was detected in the basal part of the nasal epithelium [63]. Subsequent investigations revealed high expression of *ACE2* in goblet cells of the nasal respiratory epithelium

[34, 64]. Although goblet cells are absent in the OE [65], recent preliminary studies have found more-prominent *ACE2* expression in nonneuronal cells, such as stem cells, supporting cells, and perivascular cells [19]. Specific OE cells co-expressing *TMPRSS2* and *ACE2* have been identified using a single-cell RNA-seq approach. Preliminary data from a study performed by Fodoulian et al. demonstrated a critical role of sustentacular cells, which face the nasal cavity, in maintenance of the neuroepithelium and as primary cellular targets for entry of SARS-CoV-2 [66]. The high predisposition of nasal tissues to being infected by coronaviruses suggests that some smell loss could be partly attributed to damage to the local environment. COVID-19 patients with slower recovery of olfactory function may have suffered greater intranasal injury. The correlation of long-term olfactory impairment with the severity of clinical signs is not yet clear [5].

Involvement of the nervous system in olfactory impairment caused by COVID-19

Most neurons of the peripheral nervous system (PNS) are involved in the olfactory disorder associated with COVID-19. Signs and symptoms of PNS involvement in SARS-CoV-2 infection, including muscle pain, hypogeusia/ageusia, hyposmia/anosmia, and Guillain-Barre syndrome (GBS), are less severe than those involving the CNS [67]. Ageusia and anosmia are the most common PNS manifestations of SARS-CoV-2 infection, and they have also been observed in infections with other coronaviruses. These sudden-onset symptoms often occur in association with fewer nasal symptoms, including excessive nasal secretion or nasal obstruction [9]. Ageusia and anosmia are often observed in asymptomatic patients or as the initial disease manifestation, with no other symptoms [68]. Thus, it has been suggested by some researchers that individuals with such symptoms could be possible carriers and should be isolated. The sense of taste and smell is gradually regained in most patients after recovery from SARS-CoV-2 infection [69]. An animal study has demonstrated trans-neuronal dissemination of SARS-CoV-2 into the brain through olfactory pathways and its invasion of the olfactory neuroepithelium through the expression of *ACE2* and *TMPRSS2* in sustentacular cells [70, 71]. Consequently, anosmia occurs due to the disruption of the integrity of the olfactory neuroepithelium. However, anosmia is believed by some authors to be partly due to inflammation in olfactory neurons rather than structural damage to the receptors [72]. Nevertheless, administration of nasal corticosteroids is not yet strongly recommended because of the uncertainty of their benefits [73].

Receptors that affect the sense of smell during SARS-CoV-2 infection

When SARS-CoV-2 enters the olfactory pathway, there is a chance of subsequent brain infection [74]. Thus, it might be expected that olfactory epithelial cells express proteins that facilitate the entry of SARS-CoV-2. Presently, this is unknown, but if certain tissues are found to have a high viral load, this might be useful for diagnosis of infection in individuals with no symptoms [75]. Invasion of human cells by SARS-CoV-2 virus via ACE2 as its obligatory receptor facilitates further viral uptake by TMPRSS2, a priming protease [23]. Cells with high expression level of *TMPRSS2* and *ACE2* demonstrate strong viral attachment affinity and are more prone to infection [76], and identification of the relevant cell types is important [75].

Although transcriptomic investigations have evaluated gene expression profiles in the epithelium of the olfactory system in different species, expression of *TMPRSS2* and *ACE2* in neurons and nonneuronal cells present in the epithelium and their age-dependent expression level remain controversial [25, 27, 77]. In an *in vivo* animal model, high expression levels of *TMPRSS2* and *ACE2* were observed in sustentacular cells using *in situ* hybridization, RT-PCR, RNAseq, immunocytochemistry, and western blot, suggesting these cells as possible targets of SARS-CoV-2 in the epithelium of the human olfactory system [18]. Since sustentacular cells play a fundamental role in supportive metabolism of olfactory neurons and the sense of smell [78], COVID-19-induced damage to sustentacular cells has been proposed to result in olfactory damage in COVID-19 patients [18].

The TMPRSS2 and its role in the olfactory system

TMPRSS2 gene is conserved in frogs, rhesus monkeys, chimpanzees, cows, dogs, rats, mice, chickens, zebrafish, and *C. elegans*. In humans, it is located at 21q22.3 and possesses an open reading frame with 15 exons encoding 492 residues. It has a scavenger receptor cysteine-rich (SRCR) domain, which contributes to its attachment to extracellular molecules or other cell surfaces, an LDL receptor class A (LDLRA) domain, which is a calcium-binding site, a type II transmembrane domain, and a serine protease domain of the S1 family, which cuts at lysine or arginine residues. Furthermore, *TMPRSS2* contains an androgen response element upstream of the coding region. Dihydrotestosterone and testosterone are potent transcriptional regulators of this gene via the androgen receptor [79].

A disulfide bond links the membrane-bound portion of this protein with the catalytic domain, which is positioned in the extracellular region. The N-terminal domain is located in the intracellular region, and it is followed by a stem region, a transmembrane region, and a protease domain containing the catalytic triad of serine, histidine, and aspartate necessary for cleavage activity [80]. Interaction of the intracellular domain with components of the cytoskeleton and signaling molecules is important for intracellular peptide trafficking. The stem region, which contains the SRCR and LDLRA domains, facilitates protein-protein interactions. The enzymatic region is able to cleave receptors of the cell membrane, growth factors, cytokines, and extracellular matrix components. Following autocleavage, the serine protease domain is secreted into the epithelium and subsequently interacts with extracellular matrix proteins on the cell surface and proteins of neighboring cells [81].

The isoform of TMPRSS2 containing 492 amino acids is called isoform 2. Alternative mRNA splicing results in formation of isoform 1, which is similar to isoform 2 with the difference that it has an extra N-terminal cytoplasmic domain and has been observed to be expressed in lung-related tissues. Both of these molecules are activated autocatalytically, indicating that cleavage of the proenzyme occurs between the carboxyl-end catalytic region and the rest of the molecule. This leads to conformational changes in the protease domain that are essential for conversion to its active form. The generation of alternative isoforms results in circulating and membrane-bound forms. The presence of a single N-terminal fragment and two fragments are characteristic of isoforms 2 and 1, respectively, suggesting potential differences in cleavage specificity and, possibly, intracellular localization [82]. The major fraction of the mature protease after autocatalytic cleavage is membrane-bound; however, a portion is also present in the extracellular matrix. Isoform 1 is colocalized with the viral haemagglutinin at the site where it is activated by proteolytic cleavage. Isoform 1 also activates the SARS-CoV S protein, allowing the virus to enter target cells through a cathepsin-L-independent pathway [83].

Among members of the type II TTSP family, TMPRSS2 is unusual because of its participation in various complexes. Most of the TMPRSS2 receptor is released from zymogen complexes, which do not appear to be stable despite the presence of endogenous protease inhibitors. Therefore, complexes containing TMPRSS2 do not seem to be by-products of specific mechanisms for prevention of zymogen activation. TMPRSS2 contains 22 cysteine residues, and its serine protease domain includes eight cysteines in its conserved regions. Cys140 in the serine protease domain is unpaired and therefore has the potential to participate in the formation of disulfide-linked complexes. This is a potentially important observation suggesting a novel mechanism by which

protease activity can be regulated by complex formation [80, 84, 85].

Binding of ACE2 to the receptor-binding domain (RBD) of the S protein initiates viral infection. It has been hypothesized that the high rate of infection and transmissibility of SARS-CoV-2 compared to other coronaviruses such as SARS-CoV might be attributed to high affinity of the S protein for the ACE2 receptor [86–88]. However, the binding of the RBD domain of SARS-CoV-2 to ACE2 is less efficient than that of SARS-CoV [89], suggesting the involvement of other factors. Thus, the virus might have undergone natural selection to overcome its low affinity for ACE2 [90], as coronaviruses are able to specifically adapt to a host due to their high genomic plasticity [91]. The S protein of SARS-CoV-2 is cleaved by TMPRSS2 after it binds to the ACE2 receptor. Cleavage at the S1/S2 and S2 sites is important for fusion of the viral membrane with the membrane of the host cell [23, 92]. In the absence of TMPRSS2 receptors, SARS-CoV-2 appears to use other proteases, including cathepsin B/L, which is present in lung tissue but is not able to substitute for TMPRSS2 in the case of MERS-CoV and SARS-CoV [93].

Anosmia is speculated to be associated with COVID-19-induced damage to the epithelium and its consequent inflammation and/or malfunction of neuronal receptors located in the olfactory structure. The latter is of great importance, since SARS-CoV has been shown to infect brain tissue of transgenic mice expressing human ACE2 protein following its initial impact on olfactory receptors [94]. If this also applies to ASRS-CoV-2, the olfactory epithelium requires *TMPRSS2-* and *ACE2*-expressing cells, facilitating viral infection [75, 76]. To investigate this, Bilinska et al. examined *TMPRSS2* and *ACE2* expression in mouse olfactory epithelial cells [18]. They found that expression of both enzymes could be observed in sustentacular OE cells. While *ACE2* expression was not detected in olfactory receptor neurons (ORNs), a low level of *TMPRSS2* was expressed in mature ORNs. The preferred targeting of sustentacular cells by SARS-CoV-2 results in buildup of infected cells, which interferes with their metabolism. The improper functioning of sustentacular cells could partly explain the loss of olfaction due to the importance of these cells for olfaction by endocytosis of olfactory binding protein-odorant complexes and secretion of odor-binding proteins [95]. However, this could not explain the ability of SARS-CoV-2 to target the cells of brain tissue. Additional investigation is required to determine if SARS-CoV-2 is able to infect ORNs on the way to subsequently infecting the brain [18]. Murine OE cells have been reported by Bilinska et al. [18] to contain larger amounts of ACE2 proteins than respiratory epithelial cells, making OE cells more prone to being infected by SARS-CoV-2 than the cells in the respiratory epithelium. This effect can be simulated in human OE and respiratory epithelial cells to compare levels of *TMPRSS2* and *ACE2*. If

this observation also applies to human OE cells, it might be more appropriate to test for SARS-CoV-2 in the OE than in the respiratory epithelium, potentially reducing the likelihood of obtaining a false-negative test result for COVID-19 [96].

Interaction of different strains of SARS-CoV-2 with the TMPRSS2 receptor

The increased transmission efficiency of the Omicron variant of SARS-CoV-2 has caused great concern. Zhao et al. showed that the replication and fusion activity of the Delta variant is significantly increased in VeroE6/TMPRSS2 cells, while the fusion and replication of the Omicron variant are much less dependent on TMPRSS2 [97]. For fusion of the cellular and viral membranes, a conformational change in the spike protein is required [98]. After cleavage at the S1/S2 junction by furin, the S2' site can be cleaved by either endosomal cathepsin B/L or cell-surface TMPRSS2. High *TMPRSS2* expression was demonstrated in lung alveolar cells through single-cell sequencing [34]. TMPRSS2-enhanced replication of the Delta strain is correlated with earlier *in vivo* investigations indicating more-extensive involvement of alveolar pneumocytes in infection by the Delta variant [99]. Zhao et al. demonstrated a higher dependence of the Omicron variant on TMPRSS2 in comparison to the Delta variant and suggested possible poorer replication of the Omicron variant in the lungs than the Delta variant [97].

Preliminary epidemiological studies have suggested that the Omicron variant causes milder disease [97]. The Delta and Omicron variants both use the same S2' cleavage site, but there is nevertheless a marked difference in TMPRSS2 dependence for viral replication, which might be attributed to a difference in the cleavage site for furin, which is P681H for Omicron and P681R for Delta. Using a pseudovirus system, Peacock et al. demonstrated a much higher efficiency of TMPRSS2-mediated entry of pseudoviruses carrying a polybasic furin cleavage site at the S1/S2 junction than of pseudoviruses with a deletion of this cleavage site [100]. The Omicron variant has been shown by Zhao et al. to be much less fusogenic than the Delta variant. This feature could be due to the difference in the furin cleavage site at the S1/S2 junction as well as the difference in TMPRSS2 dependence. The Delta variant was shown previously to be more fusogenic than the wild-type or Alpha variant containing the P681H mutation [97, 99, 101, 102]. The observation of syncytia in postmortem lung samples of expired SARS-CoV-2 patients has suggested an association of the fusion acuity of virus with the severity of disease [103]. In addition to cleaving the spike protein for its activation, TMPRSS2 plays role as an interferon antagonist, and *TMPRSS2* overexpression

is able to reverse restriction of replication of SARS-CoV-2 by NCOA7, which is an interferon-stimulated gene [104].

Further investigations evaluating how viral replication is affected by TMPRSS2 in the context of the innate immune response will be of great interest. Although a higher level of expression of TMRPSS2 has been observed in both Calu3 and VeroE6/TMPRSS2 cells, the Omicron variant exhibits different replication efficiency in these cell lines. While Omicron can replicate to the same level as the Delta variant in VeroE6/TMPRSS2 cells at 48 and 72 hpi, it reaches a significantly lower level than the Delta variant in Calu3 cells at 48 and 72 hpi. This difference could be due to the fact that SARS-CoV-2 does not use endocytosis to enter Calu3 cells [97]. A study has demonstrated the inability of chloroquine, which is an inhibitor of endosomal acidification, to prevent replication of SARS-CoV-2 in Calu3 cells [105], whereas another study showed inhibition by chloroquine in VeroE6/TMPRSS2 cells, indicating viral entry through the endosomal pathway [97]. Further studies using animal models are needed to determine whether differences in *TMPRSS2* dependence result in different tissue tropism or disease severity [97].

Risk of COVID-19 and *TMPRSS2* gene polymorphisms

There is a non-uniform impact of the COVID-19 crisis across ethnic groups, with certain groups affected disproportionately [106]. Discrepancies in the rate of infection and case fatality rate could be attributed to multiple causes, including underlying comorbidities, differences in access to medical care, social distancing policies, population age structure and coverage, and reliability of epidemiological data demonstrating higher mortality in the elderly population and individuals with underlying comorbidities [107, 108]. However, there have been many deaths of young and healthy individuals due to rapid cytokine storms [109]. Although this is not the whole story, and not all of the disparities among groups can be explained by these factors, data from countries that apply strict standards for gathering and presenting epidemiological data suggest that variations in human genetic makeup might explain differences in susceptibility to and severity of disease in different populations [106], and there is evidence supporting a role of variations in the *TMPRSS2* and *ACE2* genes in susceptibility to COVID-19 among different populations [110, 111].

Within the human *TMPRSS2* gene, numerous single-nucleotide polymorphisms (SNPs) have been identified in a recent analysis using computational modeling (dbSNP, NCBI). Of these, just 21 variations with minor allele frequency of 0.01-0.95 were found to affect enzyme function [112], and only two of those are missense variations (rs75603675 and rs12329760). In several investigations, the rs12329760 polymorphism, which is referred to as the p.Val160Met variant, has been demonstrated to be associated with the risk of prostate cancer. This could confirm the clinical consequences related to this genetic variant [113–117]. This genetic variation is located on exon 6 and could alter the function of the gene (Figs. 1 and 2). Although it affects gene expression, it has no effect on the mRNA structure or function of *TMPRSS2* (Fig. 2). Various effects of *TMPRSS2* gene polymorphisms have been presented in our previous publications [118–126], but additional SNPs have been found to have an impact on the risk of COVID-19. These *TMPRSS2* gene polymorphisms and their association with the risk of COVID-19 are listed in Table 1.

Therapeutic strategies targeting the TMPRSS2 receptor

Multiple mechanisms involved in the replication and infectivity of SARS-CoV-2 provide potential targets for pharmacological interventions. Infection of macrophages, pneumocytes, and pulmonary mast cells requires the viral S protein. The entry pathway involving binding of the S protein to the ACE2 cell receptor is mediated through host-cell-derived TMPRSS2 serine protease [23]. Viral RNA release, replication, and translation occur after viral entry into the host cell. Translated viral polyproteins are eventually cleaved by viral proteases to form mature effector proteins [133]. Viral infection is initiated by the interaction of viral S protein with ACE2 on cytoplasmic membrane of the host cell. Numerous candidate drugs have the potential to inhibit SARS-CoV-2 replication and subsequent rounds of infection. Such drugs included ACE2 inhibitors and inhibitors of TMPRSS2 serine protease. Blocking of TMPRSS2, which is essential for priming of the S protein, and inhibition of ACE2, which is the host cell receptor of SARS-CoV-2, might prevent cellular entry of SARS-CoV-2 [29].

Shen et al. demonstrated the importance of a guanine-rich tract in the human *TMPRSS2* promoter for formation of an intramolecular G-quadruplex structure in the presence of K^+ for gene transcriptional activity. In order to stabilize G-quadruplexes, seven novel benzoselenoxanthene analogues have been introduced. Compounds that downregulate *TMPRSS2* gene expression have been shown to suppress propagation of influenza A virus *in vitro* [134]. Thus, small molecules that target the TMPRSS2 gene G-quadruplex and subsequently inhibit TMPRSS2 expression provide a novel strategy against influenza A virus. This could also be a potential anti-SARS-CoV-2 target [134].

Numerous viruses employ host cell proteases to activate their envelope glycoproteins, including MERS coronavirus (MERS-CoV), Ebola virus, influenza virus, and SARS
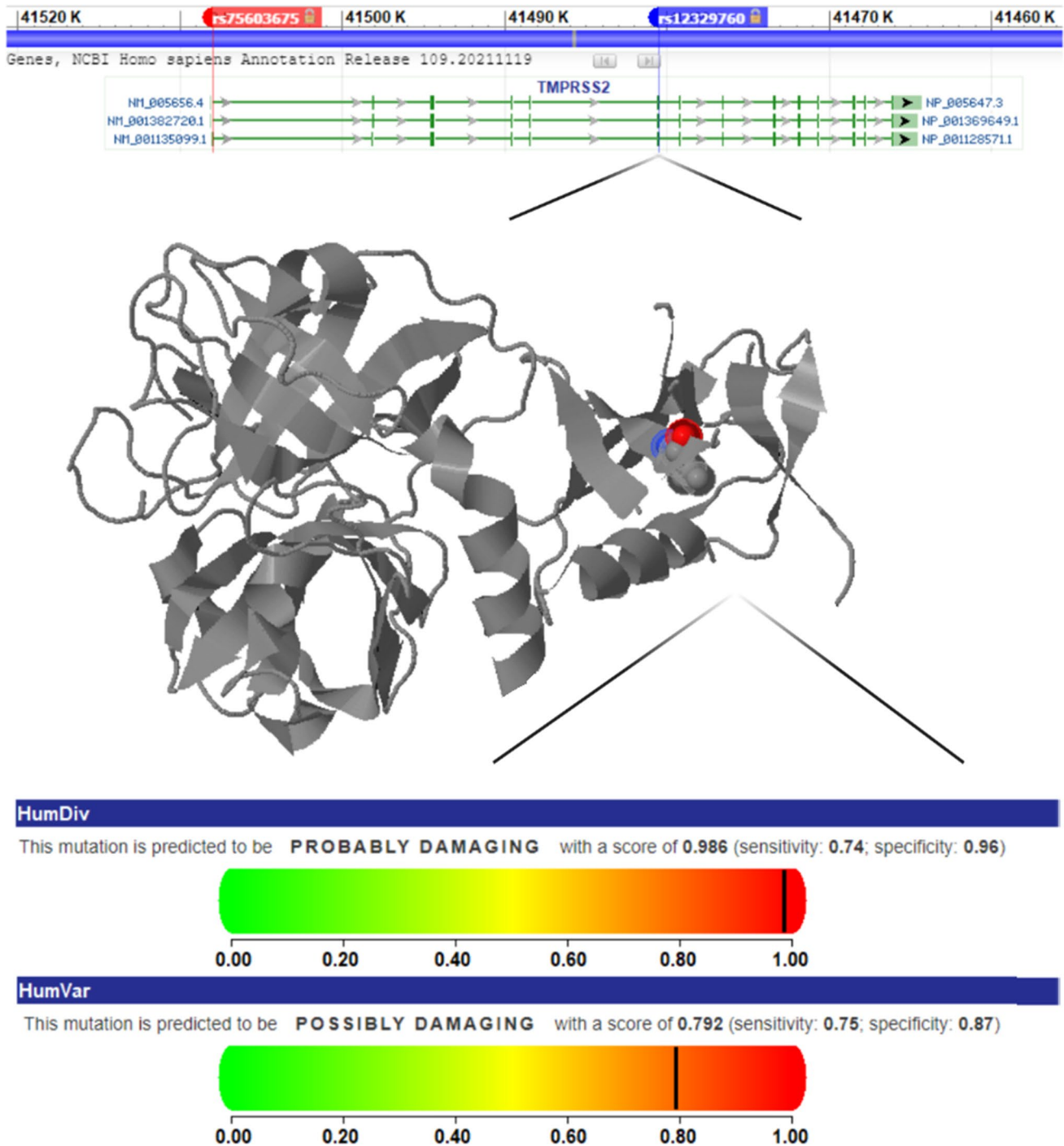
Fig. 1 *TMPRSS2* gene map and features of two common gene variations, rs12329760 and rs75603675. The *TMPRSS2* gene contains 15 exons, and rs12329760 and rs75603675 are located on exons 1 and 6, respectively. The position of the rs12329760 polymorphism in the three-dimensional structure of the protein suggests an effect on enzyme function.

coronavirus (SARS-CoV) [61, 135, 136]. Cleavage of the viral spike protein and its subsequent activation are required for membrane fusion and entry into the host cell, and this is facilitated by TMPRSS2 [21, 23, 137, 138]. Figure 3 shows how the administration of two serine protease inhibitors, camostat and nafamostat, can prevent the virus from entering the cell. Camostat mesylate, a commercial serine protease inhibitor is able to partially block HCoV-NL63 and SARS-CoV entry in *TMPRSS2*- and *ACE2*-expressing HeLa cells [139]. Indeed, inhibition of TMPRSS2 in human lung Calu-3

(A)

(B)

(C)
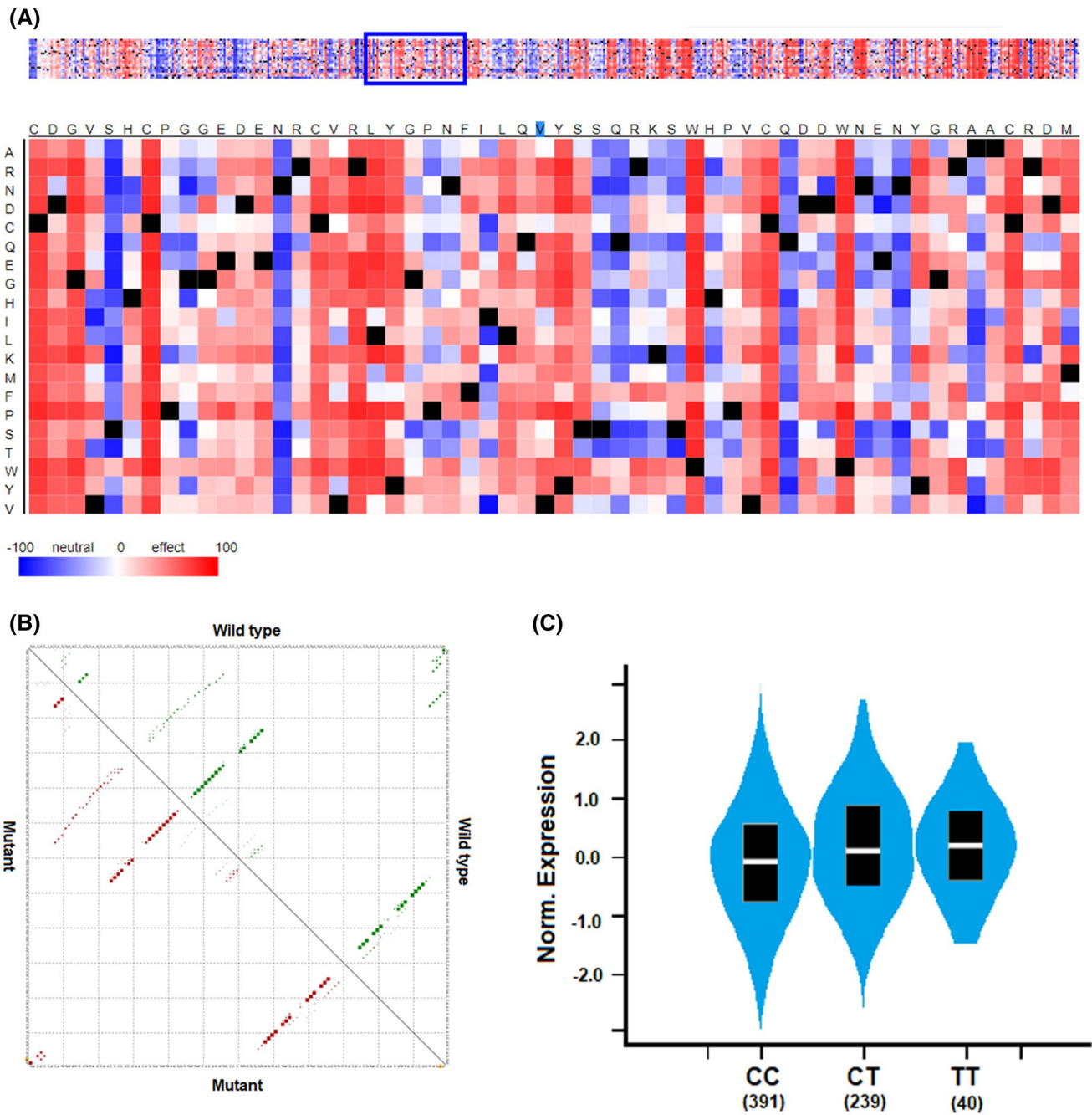
Fig. 2 Effects of the rs12329760 polymorphism on protein and mRNA function and gene expression. **A** Potential deleterious impact of rs12329760 on the TMPRSS2 protein. **B** RNAsnp analysis show-ing no impact on mRNA structure. **C** Effect of this genetic variation on *TMPRSS2* expression.

cells using camostat mesylate was shown to be associated with significantly diminished SARS-CoV-2 infection [23].

Camostat and camostat mesylate are synthetic serine protease inhibitors that were developed decades ago to treat dystrophic epidermolysis [140], oral squamous cell carcinoma [141, 142], and chronic pancreatitis [143–145], to inhibit and exocrine pancreatic enzymes [146, 147]. Camostat mesylate (NI-03), prescribed three times per day at the recommended dose of 100–300 mg, has been manufactured by Nichi-Iko Pharmaceutical Co., Ltd. and Ono Pharmaceutical, Japan, as an oral drug [145]. A clinical trial on 95 patients who received 200 mg of camostat mesylate three times per day for 2 weeks for the treatment of dyspepsia associated with non-alcoholic mild pancreatic disease demonstrated only mild adverse effects [143], indicating that this drug is well tolerated

Table 1 Association of *TMPRSS2* genetic polymorphisms with the risk of COVID-19

References	Polymorphism ID	Variant type	Country	Sample size	Outcomes
Wulandari et al. [117]	rs12329760	Missense	Indonesia	95	The data showed an association between p.Val160Met genetic variation and SARS-CoV-2 infectivity and the outcome of COVID-19.
Torre-Fuentes et al. [127]	rs61735792 rs61735794	Synonymous Synonymous	Spain	120	These two synonymous polymorphisms displayed a true association with the infection.
Monticelli et al. [128]	rs12329760 rs2298659	Missense Synonymous	Italy	1177	The frequencies of two polymorphisms, rs12329760 and rs2298659, were associated with severity of COVID-19.
Curtis et al. [129]	rs35803318 rs41303171	Synonymous Missense	UK	488377	The frequency of neither of these two polymorphisms was significantly different between the case and control groups.
Schönfelder et al. [130]	rs2070788 rs383510 rs12329760	Intron Intron Missense	Germany	492	The rs383510 intron variant in the *TMPRSS2* gene is associated with increased risk of COVID-19.
Ravikanth et al. [131]	rs12329760	Missense	India	1030	There was a significant association between *TMPRSS2*-rs12329760 and decreased severity of COVID-19.
Akin et al. [132]	rs2070788	Intron	Netherlands	188	A protective role for the genotype of rs2070788-AA as a determinant of COVID-19 severity was observed.
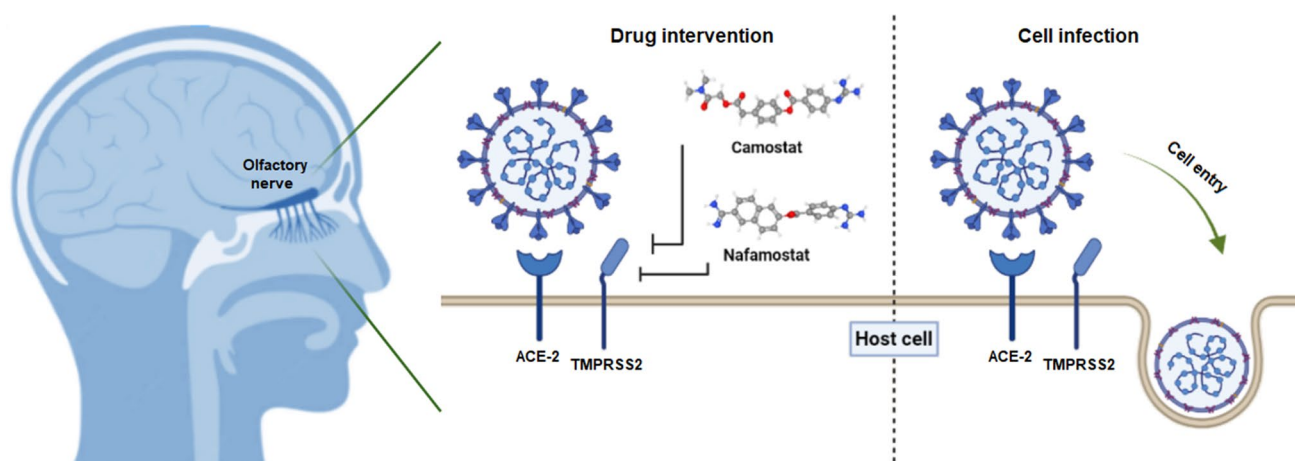
Fig. 3 Inhibitory effect of camostat and nafamostat on SARS-CoV-2 entry into the cell. Because TMPRSS2 plays a key role in virus entry, the serine protease inhibitors camostat and nafamostat can inhibit virus cell entry by blocking TMPRSS2.

[29]. A clinical trial demonstrated a more rapid resolution of COVID-19 symptoms and amelioration of lost taste and smell in outpatients with newly diagnosed mild COVID-19 infection who received oral camostat mesylate [30]. Nafamostat mesylate is a synthetic serine protease that is clinically approved in Japan to treat disseminated intravascular coagulation and acute pancreatitis. It is also prescribed as an anticoagulant for extracorporeal circulation [148–150]. In a study screening about 1100 FDA-approved drugs, nafamostat mesylate was found to prevent MERS-CoV S-protein-mediated viral membrane fusion with *TMPRSS2*-expressing lung Calu-3 host cells by inhibiting TMPRSS2 protease activity [151]. Since the S proteins of SARS-CoV-2 and MERS-Cov share considerable amino acid sequence similarity [152, 153], nafamostat mesylate could potentially inhibit cellular entry of SARS-CoV-2. In a cell culture study on simian Vero E6 cells infected with SARS-CoV-2, nafamostat mesylate was found to have inhibitor activity, with an EC_{50} of 22.50 μM [154], suggesting its ability to prevent SARS-CoV-2 infection. In a phase II, randomized, multicenter, open-label trial including 19 subjects with a severe form of acute pancreatitis, daily intravenous administration of nafamostat mesylate at a dose of 240 mg for five consecutive days showed benefits without significant adverse effects [150].

Conclusion

A significantly high proportion of patients with COVID-19 have symptoms of anosmia, but the underlying molecular and cellular mechanisms remain unclear. Upper respiratory infection manifests classically as nasal obstruction and rhinorrhea, leading to conductive olfactory loss. Postviral anosmia might ensue in a subacute form after resolution of acute symptoms of upper respiratory tract infection. However, preliminary data regarding COVID-19 cases have revealed a novel syndrome of acute-onset anosmia without nasal obstruction or rhinitis. New studies have revealed the identity of the cells responsible for viral entry into the olfactory neural system. Reviewing the literature on anosmia induced by viral infection allows specific mechanisms of anosmia to be postulated, but the exact mechanisms are not yet clear. Several hypotheses have been proposed for COVID-19-associated anosmia. According to an animal study, coronaviruses are able to disseminate transneuronally into the brain using olfactory pathways and invade the olfactory neuroepithelium in a manner dependent on the expression of *ACE2* and *TMPRSS2* in sustentacular cells. Because of its important role in viral entry, information about the function of *TMPRSS2* could suggest therapeutic strategies against COVID-19 and its complications, including anosmia.

Declarations

Conflict of interest None of the authors declare a conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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