The effect of coronaviruses on olfaction: systematic review*

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Abstract

Background: Unlike other respiratory viruses, SARS-CoV-2 causes anosmia without sinonasal inflammation. Here we systematically review the effects of the 7 known human coronaviruses on olfaction to determine if SARS-CoV-2 distinctly affects the olfactory system.

Method: PubMed, EMBASE, Web of Science, bioRxiv, medRxiv and DOAJ were searched for studies describing pathophysiological, immunohistochemical, cytological and clinical data.

Results: 49 studies were included. Common cold coronaviruses lead to sinonasal inflammation which can cause transient and chronic loss of smell. MERS-CoV entry receptors were not found in the nasal mucosa and it did not impair olfaction. SARS-CoV-1 had low affinity for its receptor ACE2, limiting olfactory effects. Anosmia is frequent in SARS-CoV-2 infections. SARS-CoV-2's entry factors ACE2 and TMPRSS2 are expressed in the nasal respiratory epithelium and olfactory supporting cells. SARS-CoV-2 appeared to target the olfactory cleft while diffuse nasal inflammation was not observed. Damage of the olfactory epithelium was observed in animal models. Alternative receptors such as furin and neuropilin-1 and the similarity of viral proteins to odourant receptors could amplify olfactory impairment in SARS-CoV-2 infection.

Conclusions: The pathophysiology of anosmia in SARS-CoV-2 infection is distinct from other coronaviruses due to preferentially targeting olfactory supporting cells. However, SARS-CoV-2 does not cause sinonasal inflammation in spite of preferred entry factor expression in the nasal respiratory epithelium. This raises doubts about the attention given to ACE2. Alternative receptors, odourant receptor mimicry and other as yet unknown mechanisms may be crucial in the pathogenesis of anosmia in SARS-CoV-2 infection. Further studies are warranted to investigate infection mechanisms beyond ACE2.

Key words: Coronaviruses, COVID-19, pathophysiology, olfaction disorders, systematic review

Introduction

Anosmia is a presenting symptom of coronavirus disease 2019 (COVID-19) [(1)]. COVID-19 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and can range from asymptomatic to lethal respiratory disease [(2)]. Olfactory dysfunction is reported by 43% of COVID-19 cases [(1)]. There is wide geographic variation, with a prevalence of 18% in Asia and 58% in Europe [(1)]. A recent meta-analysis revealed that anosmia might be underreported, as studies using smell testing report a prevalence of 77%, compared to 45% when anosmia was self-reported [(3)]. Anosmia occurs as the only complaint in 17% to 20% of cases and is predominant in mild disease [(4–6)]. Olfaction usually returns in 10 to 20 days but 6% of patients in a recent study reported persistent loss at least 60 days after onset [(7)]. Parosmia is reported by 32% of patients [(6)].

Anosmia in SARS-CoV-2 does not seem associated with nasal obstruction and rhinorrhoea [(8)]. This is different from other respiratory viruses, which can cause transient loss of smell by inflammation of the nasal respiratory epithelium, lining most of the nasal cavity [(9,10)]. The olfactory cleft, a small region at the top of the nasal cavity, is lined with olfactory epithelium [(10)]. The olfactory epithelium contains olfactory sensory neurons

Table 1. Characteristics of human coronaviruses[21,24,36,68,91,92].

Genus	Species	Disease	Entry receptor	Protease
Alpha	HCoV-NL63	Common cold	ACE2	-
	HCoV-229E	Common cold	ANPEP	TMPRSS2, CTSL[a]
Beta	HCoV-OC43	Common cold	Sialic acid	-
	HCoV-HKU1	Common cold	Sialic acid	-
	MERS-CoV	MERS	DPP4, Sialic acid[a], GRP78[a]	TMPRSS2, CTSL[a], Furin[a]
	SARS-COV-1	SARS	ACE2, DC-SIGN[a]	TMPRSS2, CTSL, CTSB[a], ADAM17, TMPRSS11D[a]
	SARS-COV-2	COVID-19	ACE2, DC-SIGN, L-SIGN, Basigin[a], Neuropilin-1[a]	TMPRSS2, CTSL[a], CTSB[a], Furin[a]

[a] Possible alternative entry factor. ACE2, Angiotensin-converting enzyme 2; ADAM17: ADAM metallopeptidase domain 17; TMPRSS, Transmembrane protease, serine; ANPEP, Alanine aminopeptidase; CTSL, Cathepsin L1; CTSB, Cathepsin B; COVID-19, Coronavirus disease 2019; DC-SIGN, Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin; GRP78, 78-kDa glucose-regulated protein; HCoV, Human coronavirus; L-SIGN, Liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin; MERS-CoV: Middle East respiratory syndrome-related coronavirus; SARS-CoV: Severe acute respiratory syndrome coronavirus.

(OSNs) and supporting cells, which include Bowman's glands and sustentacular, microvillar and basal cells [11]. Supporting cells are critical to olfaction, and stabilize and repair the olfactory epithelium [11].

When odourants reach the olfactory epithelium, they dissolve in mucous secreted by Bowman's glands [11]. The dissolved odourants bind to proteins made by sustentacular cells. This odourant–protein complex binds onto odourant receptors expressed on OSNs, which transduces signals to the brain via the olfactory bulb [10,11]. Basal cells ensure the continuous turnover of OSNs [11].

Loss of smell in the common cold has been described to be mediated by proinflammatory mediators such as tumour necrosis factor (TNF), interleukins (ILs) and interferons (IFNs) [12]. Inflammation in the nasal cavity presents as obstruction and rhinorrhoea, which can block airflow and odourants from reaching the olfactory epithelium [13]. Temporary conductive loss is rarely distressing, often goes unnoticed and usually recovers with viral clearance [13].

Transient viral loss of smell can become chronic postviral olfactory dysfunction (PVOD) [9]. The pathophysiology is unclear but scarring of the olfactory epithelium was observed in patients with PVOD [14,9]. Recovery tends to be slow and uncertain [15]. However, studies with long observation periods have shown that olfaction can improve long after the diagnosis. One study found improvement in a third of 246 patients followed for 2 years, while another found 19 of 21 patients improved olfaction after 3 years, with the likelihood and extent of recovery related to the severity of the initial olfactory loss [9,15]. Parosmia is reported by 56% to 65% of patients during PVOD, thought to arise from incomplete perception due to missing OSNs and the mismatched regeneration of nervous pathways [15-17]. Causative viruses are difficult to establish but rhinoviruses, parainfluenza viruses, Epstein-Barr viruses and coronaviruses were identified in patients with PVOD [18,19].

The seven coronaviruses known to infect humans are Human coronavirus (HCoV)-NL63, HCoV-229E, HCoV-HKU1, HCoV-OC43, Middle East respiratory syndrome coronavirus (MERS-CoV), SARS-CoV-1 and SARS-CoV-2 (Table 1) [20,21]. HCoVs are endemic and cause 10% to 30% of common colds [21]. SARS-CoV-1 and MERS-CoV cause SARS and MERS, respectively [21]. SARS-CoV-1 has not resurfaced since an early 2000s outbreak [21]. MERS-CoV circulates in the Middle East but is fortunately poorly transmitted [22].

Coronaviruses infect targets by attaching viral spike proteins to host cell receptors [23]. The spike protein is then cleaved by a host protease, exposing fusion peptides that enable infection [23]. Target cells need to co-express a coronavirus' preferred entry factors for successful infection [23]. Therefore, the distribution of entry factors governs tissue tropism and pathogenicity [24]. Table 1 lists the entry factors used by coronaviruses.

Olfactory and sinonasal symptoms were rarely reported in MERS-CoV and SARS-CoV-1 infections, suggesting restricted tropism for the nasal cavity [25]. In contrast, HCoVs are thought to often conductively impair olfaction in the common cold, indicating tropism for the nasal respiratory epithelium [25,26]. The unusual presentation of anosmia in SARS-CoV-2 infections suggests that the olfactory mucosa is preferentially targeted. In this systematic review we compare the effects of coronaviruses on olfaction to determine if SARS-CoV-2 distinctly affects the olfactory system.

Methods

Search

PubMed, EMBASE, Web of Science, bioRxiv, medRxiv and Directory of Open Access Journals (DOAJ) were searched on

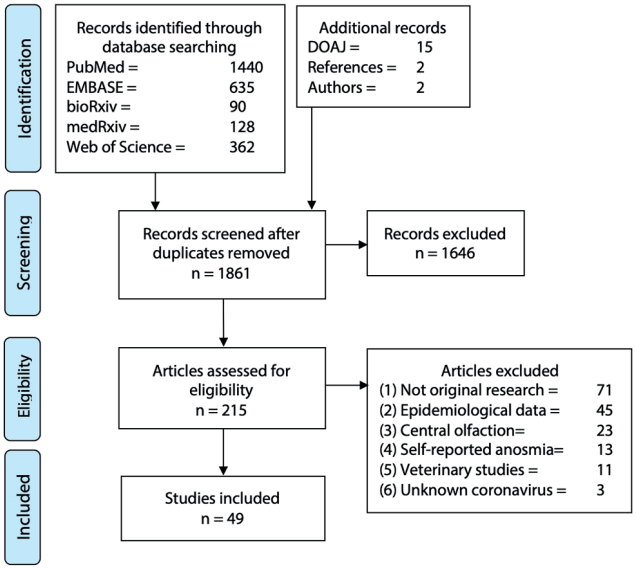

Figure 1. PRISMA selection workflow.

October 7, 2020. Synonyms were organized in three groups: virus, anosmia and pathophysiology. Full queries are shown in Supplement Table 1. References and authors were consulted for adjunct inclusions.

Study selection

Original studies on olfactory pathology in coronavirus infections with pathophysiological, immunohistochemical, cytological or clinical data were included.

The following studies were excluded: 1) not original research such as reviews and hypotheses, 2) epidemiological studies 3) studies restricted to the olfactory bulb, nerve and brain, 4) case reports on self-reported anosmia without clinical examination, 5) veterinary studies not intended to model human disease and 6) unspecified coronaviruses.

Data extraction

Key pathophysiological, immunohistochemical, cytological and clinical outcomes were extracted into Supplementary Table 2. Data extracted on the expression of Angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) in the olfactory epithelium was extracted into Table 2. Data from imaging studies were extracted into Table 3.

Results

Study selection

1861 records were screened. 215 articles were assessed. In total, 48 studies were included (Figure 1). Characteristics of included studies are shown in Supplement Table 2.

HCoVs

HCoVs infections present as a common cold with nasal obstruction and rhinorrhoea. HCoV-229E was shown to conductively impair olfaction in one study [27]. 78% of 20 participants infected with HCoV-229E developed hyposmia for at least 4 days after infection [27]. The degree of nasal obstruction, determined using nasal peak airflow and acoustic rhinometry, was found to correlate with the severity of olfactory impairment [27].

Viruses in patients with PVOD were investigated in two studies [18,19]. Both only included patients diagnosed with PVOD following an upper respiratory infection and without olfactory cleft obstruction. Of 24 patients with detectable viruses, HCoV-229E and HCoV-OC43 were identified in one patient each [18,19]. The HCoV-229E positive patient reported complete anosmia, nasal obstruction and rhinorrhoea on the first visit [18]. The patient did not report nasal obstruction and rhinorrhoea on later visits, although olfaction remained impaired. Improvement was reported by week 11 and olfaction fully recovered by week 24 [18]. The findings suggest that inflammation of the nasal respiratory epithelium by HCoV-229E can occasionally damage the olfactory epithelium and lead to sensorineural dysfunction [18,19].

MERS-CoV

Olfactory pathology was not described as a feature of MERS. The MERS-CoV entry receptor dipeptidyl peptidase-4 (DPP4) was not detected in the nasal tissue [28,29]. To investigate if the lack of DPP4 or other entry receptors inhibit MERS-CoV, in one study 6 nasal tissue samples were challenged with MERS-CoV's spike protein [29]. Infection was not observed in challenged respiratory or olfactory tissue, suggesting that MERS-CoV lacks nasal tropism and capacity for olfactory pathology [29].

SARS-CoV-1

Olfactory pathology was not an oft-described feature of SARS. One case report described anosmia in a patient 3 weeks after recovering from SARS-CoV-1 infection with chief complaints of fever, cough, headache and diarrhoea [30]. Lack of conductive abnormalities on rhinological examination and MRI suggested a diagnosis of sensorineural PVOD, which persisted for at least 2 years after onset [30]. However, the patient did not report symptoms of the common cold, which usually precede PVOD [30,9].

The replication of SARS-CoV-1 in the nasal turbinates was modelled in one study using immunosuppressed hamsters [31]. Inflammation was not significant, although mild long-lasting damage of the olfactory epithelium was observed [31]. Similar damage is seen in PVOD, suggesting SARS-CoV-1 has some, but restricted, tropism for the olfactory epithelium [14].

SARS-CoV-1's interaction with ACE2 was investigated in two studies [32,33]. SARS-CoV-1 was found to weakly bind ACE2 at neutral pH in the nasal cavity [32,33]. Weak receptor bonds were hypothesized to be vulnerable to nasal airflow, leading to loose SARS-CoV-1 virions being exhaled or drifting towards the lower

Table 2. Co-expression of SARS-CoV-2 entry proteins in the olfactory epithelium.

Cell	Factor	Ueha[40]	Brann[36]	Gupta[38]	Ziegler[41]	Bilinska[35]	Fodoulian[37]	Muus[39]	Chen[42]
OSN	ACE2	+	-	-	-	-	-	NA	-
	TMPRSS2	-	-	-	-	+	NA	NA	NA
SUS	ACE2	+	+	+	+[v]	+	+	+[v]	+
	TMPRSS2	+	+	+	+[v]	+	+	+[v]	NA
BG	ACE2	+	+	+	+[v]	NA	+	+	+
	TMPRSS2	+	+	+	+[v]	+	+	+	NA
MVC	ACE2	+	+	-	+[v]	+	+	+[v]	NA
	TMPRSS2	+	+	-	+[v]	+	+	+[v]	NA
BC	ACE2	+	+	+	+	NA	+	+	NA
	TMPRSS2	+	+	+	+	+	+	+	NA

[v], verified with corresponding authors because non-standard terminology was used to describe cell types in original study. OSN, Olfactory sensory neuron; SUS, Sustentacular cell; BG, Bowman's gland; MVC, Microvillar cell; BC, Basal cell; ACE2, Angiotensin-converting enzyme 2; TMPRSS2, Transmembrane protease, serine 2; NA, Not applicable.

respiratory tract [32,33]. Therefore, SARS-CoV-1 might have limited time to recruit ACE2 before it is dislodged, limiting the capacity for olfactory pathology [32,33].

SARS-CoV-2

Nine studies used RNA sequencing or immunohistochemistry to find SARS-CoV-2's entry factors ACE2 and TMPRSS2 in olfactory supporting cells, but not in OSNs (Table 2) [34–42]. Sustentacular cells were described to particularly highly co-express ACE2 and TMPRSS2 [35,37]. Infection of supporting cells could impair olfaction without diffuse inflammation, consistent with reports of isolated anosmia in COVID-19 [5].

Co-expression of ACE2 and TMPRSS2 was observed in the nasal respiratory epithelium, but the low prevalence of nasal obstruction and rhinorrhoea in COVID-19 suggest that SARS-CoV-2's tropism for nasal respiratory tissue is restricted [36,37,39–41,8]. In one study, ACE2 was detected in all 13 included olfactory epithelium samples, while ACE2 was only detected in 9 of 19 included nasal respiratory samples [42]. Signal intensities of ACE2 and TMPRSS2 were also higher in the olfactory compared to the respiratory epithelium, suggesting that SARS-CoV-2 can more readily infect tissue lining the olfactory cleft [35,42].

To investigate if SARS-CoV-2 visibly affects the olfactory epithelium, three studies in hamsters were performed [43–45]. They demonstrated extensive damage of sustentacular cells quickly after infection, consistent with their co-expression of ACE2 and TMPRSS2 [43–45]. OSNs were deciliated, reduced in number and infected [43–45]. Olfactory tissue regenerated by day 14, similar to recovery times in COVID-19 patients with anosmia [44,45]. Basal cells were functional but SARS-CoV-2 antigen was detected in the basal layer [45]. This suggests that basal cells were compromised but a sufficient immune response cleared the infection. Basal cell disruption is hypothesized to underlie the longer lasting olfactory dysfunction sometimes reported after SARS-CoV-2 infection [37,36,45].

Several studies investigated these proposed mechanisms in patients infected with SARS-CoV-2. Diffuse inflammation in the nasal cavity was not detected in 12 imaging studies using CT or MRI, confirming that conductive etiologies do not underlie anosmia in COVID-19 (Table 3) [46–57]. However, the olfactory cleft was opacified in 57 of 191 (29.23%) patients suggesting that SARS-CoV-2's tropism for the olfactory epithelium can lead to localized inflammation [47–51,53–55,57].

No extensive cell injury was seen using nasal cytology sampled from COVID-19 patients with anosmia, but positive CD68 staining suggested macrophage presence [58,59,48]. Limited infiltration of helper and cytotoxic T cells was seen in two olfactory tissue biopsies, although signs of mucosal atrophy and axonal neuritis were described [60–62]. Despite limited inflammatory infiltration, patients with mild SARS-CoV-2 infection with olfactory impairment were associated with higher viral burden and longer time to viral clearance compared to patients with normal olfaction [63]. These data suggest that SARS-CoV-2 does not provoke a strong immune response in the nasal cavity.

Proinflammatory cytokines might indirectly affect olfaction [64,65]. Elevated levels of TNF-α and IL-6 were detected in COVID-19 patients with anosmia [65,66]. TNF-α and IL-6 were hypothesized to inhibit olfaction with coagulation cascades which can thicken the olfactory mucosa, activate neuroapoptotic pathways and inhibit basal cells [65,66]. IFN type 1 and 2 upregulated the

Table 3. Olfactory cleft opacification in SARS-CoV-2 infected patients.

Study	Imaging modality	Number of patients	Patients with olfactory cleft opacified
Brelie[46]	MRI	1	0
Galougahi[56]	MRI	1	0
Laurendon[50]	MRI	1	1
Eliezer[57]	CT	1	1
Chung[48]	CT	6	3 (50%)
Tsivgoulis[54]	MRI	8	3 (37%)
Girardeu[49]	MRI	10	2 (20%)
Lechien[51]	CT	16	3 (19%)
Chetrit[47]	MRI	19	9 (47%)
Eliezer[55]	MRI	20	19 (95%)
Naeini[52]	CT	49	0
Spoldi[53]	CT	63	16 (25%)
Total		191	57 (29.23%)

expression of ACE2 and TMPRSS2 in the olfactory epithelium, enhancing the vulnerability of supporting cells [34,36,41]. IFN type 1 also directly reduced odourant binding sites by downregulating odourant receptors [67].

Some studies looked further than ACE2. Neuropilin-1, furin and TMPRSS11D were shown to amplify SARS-CoV-2's olfactory tropism [68,69]. Furin is not required for SARS-CoV-2 infection, as viral replication continued when furin was knocked out [69]. However, furin did enhance direct infection and cell to cell transmission if co-expressed with TMPRSS2 and ACE2 [69]. SARS-CoV-2 could use the enhanced transmission to reach cells of the basal layer. Furin was enriched in microvillar cells and Bowman's gland, but not in nasal respiratory cells [41,36,40]. Neuropilin-1 similarly amplified infection of the olfactory epithelium and was more abundant than ACE2 in olfactory cells facing the nasal cavity [68].

Genotype variations of nasal entry factors might affect ethnic susceptibility for olfactory pathology [70,71]. TMPRSS2 expression was higher in European than in Asian populations which could underlie the higher prevalence of anosmia in Europe [71]. Moreover, nasal ACE2 was less methylated in Black individuals and women of all ethnicities [70]. Hypomethylation can increase ACE2 availability thereby enhancing SARS-CoV-2's nasal tropism and contribute to the higher frequency of anosmia in women [6,70].

SARS-CoV-2's proteins were found to resemble odourant receptors [72]. Therefore, immunoglobulin A (IgA) produced against SARS-CoV-2 may attach to odourant receptors on OSNs and block the transduction of olfactory signals, suggesting an autoimmune component in the pathogenesis of anosmia [72].

In another study, gene expression of odourant receptors was downregulated, which could be a consequence of blocked or impaired OSNs [73].

Discussion

Coronaviruses differently impact olfaction. Transient loss of smell occurs in common colds and can be caused by endemic HCoVs [21]. Evidence on olfactory pathology in HCoV infections was limited by lack of research interest however. In fact, HCoV-NL63 and HCoV-HKU1 were only discovered in the surge of interest after the early 2000s SARS-CoV-1 outbreak [21]. Both viruses had been circulating for decades without great consequence [21]. Loss of smell in common colds is usually not reported and virus identification in PVOD is made difficult by delays in seeking care, as viruses can only be detected in lavage up to 4 weeks after onset [18,19,9]. Olfactory impairment by HCoVs might thus be underreported.

Olfaction is also not a priority in intensive care units. Therefore, detection bias could have masked olfactory pathology in patients infected with SARS-CoV-1 and MERS-CoV, which more frequently had severe outcomes than SARS-CoV-2[74]. SARS-CoV-1 and MERS-CoV also progress faster to severe disease relative to SARS-CoV-2[74,75]. This could reduce the window for detecting changes in olfaction.

However, the absence of nasal receptors plausibly explains the lack of olfactory effects in patients infected with MERS-CoV [29]. Evidence of MERS-CoV's restricted nasal tropism can also be found in the rarity of other sinonasal symptoms and poor infectivity [22,76]. MERS-CoV transmission is usually nosocomial or after contact with camels, where high viral loads can reach DPP4 expressing tissue further down the respiratory tract [22]. Community transmission of MERS-CoV was never documented [22,29]. In contrast, MERS-CoV is endemic in camel herds and the animals develop symptoms resembling the common cold [77]. Camels do express DPP4 nasally, highlighting the importance of nasal receptors in viral tropism and olfactory pathology [29,77].

The only case report of anosmia after SARS-CoV-1 infection is remarkable [30]. Common cold symptoms were not reported by the patient and anosmia developed suddenly, weeks after recovering from SARS [30]. This differs from typical PVOD that begins as conductive dysfunction then gradually becomes sensorineural [9]. The authors hypothesized a relationship with motor neuropathies rarely reported in other SARS-CoV-1 infected patients, however, sensory and motor neuropathies are not interchangeable. The aberrant clinical picture, lack of a validated olfactory test and no comparable reports raise doubts about this case's etiology.

On the contrary, SARS-CoV-1's receptor ACE2 is expressed in the nasal cavity but typical sinonasal symptoms like nasal obstruction and rhinorrhoea were rarely reported during the early 2000s SARS-CoV-1 outbreak [76]. The scarcity of sinonasal symptoms and evidence gathered in this review suggest that SARS-CoV-1's affinity for nasal ACE2 is low. If olfactory pathology reflects transmission efficiency, then it is not surprising that SARS-CoV-1 took 2 years to peak with 8000 cases [78]. Research has indeed

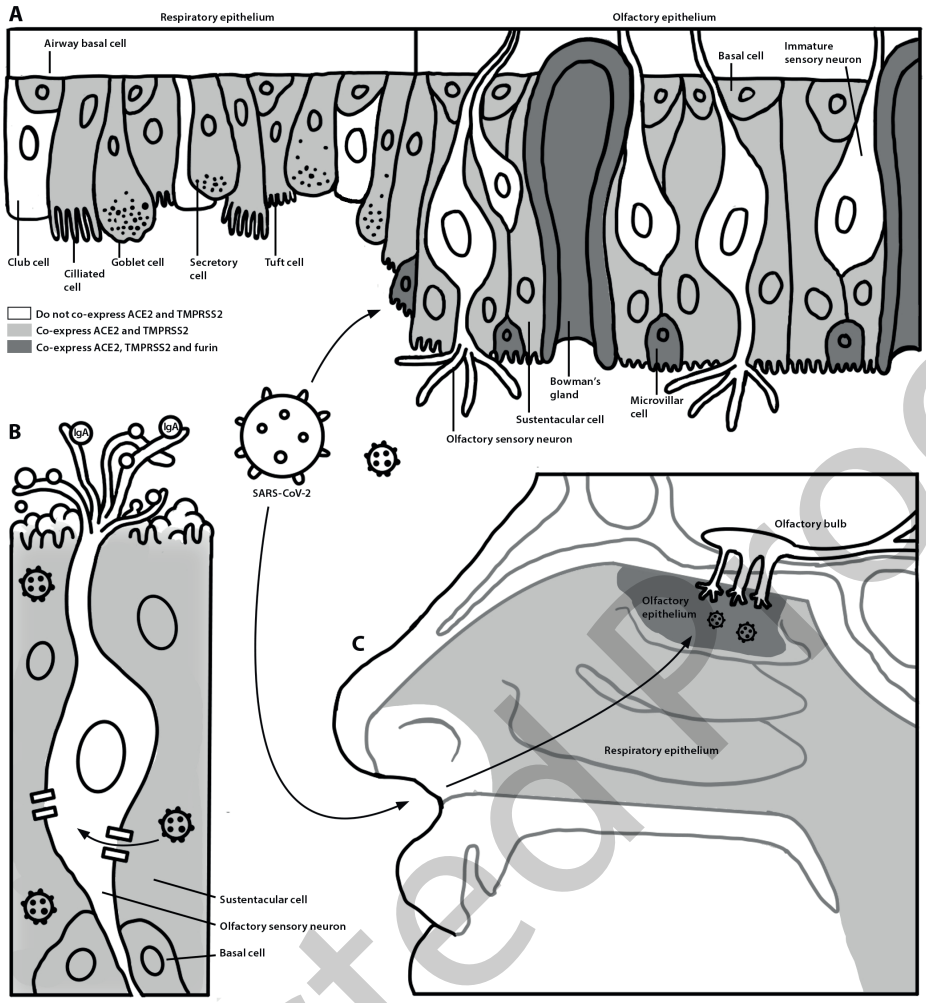

Figure 2. Possible mechanisms of anosmia in SARS-CoV-2 infection (A) Expression of SARS-CoV-2 entry factors in the nasal respiratory and olfactory epithelia. ACE2 and TMPRSS2 co-expressing cells are coloured light grey. Cells additionally expressing furin are coloured dark grey. Respiratory club cells and OSNs were not found to co-express ACE2 and TMPRSS2. Bowman's glands and microvillar cells' apical location and expression of furin could make them especially vulnerable to SARS-CoV-2 infection. Furin significantly enhances direct infection and cell to cell transmission of SARS-CoV-2 when co-expressed with ACE2 and TMPRSS2. (B) SARS-CoV-2's proteins resemble odourant receptors. IgA made against SARS-CoV-2 can then block odourant receptors. Therefore, anosmia might be a temporary sacrifice for a robust immune response. In addition, sustentacular cells and olfactory are connected with tight junctions, potentially providing an alternative route for olfactory neuroinfection. (C) SARS-CoV-2 appears to preferentially target the olfactory epithelium while bypassing the nasal respiratory epithelium.

indicated that SARS-CoV-1's recognition of ACE2 is impaired compared to SARS-CoV-2 [79]. This is due to structural differences in the receptor binding domain of SARS-CoV-1's spike protein, which also leads to less robust bonds with ACE2 [79].

Geographic spread could further explain the lack of olfactory impairment by SARS-CoV-1. Anosmia in patients infected with the closely related SARS-CoV-2 is reported more in Europe than in Asia [1]. Ethnic variations of entry factors have been proposed to explain this discrepancy [1,8]. SARS-CoV-1 was successfully contained in the early 2000s in Asia, suggesting that SARS-CoV-1 was limited to populations resistant to olfactory impairment [78].

To understand loss of smell in SARS-CoV-2 infection, scientists used RNA sequencing and immunohistochemistry to define the nasal distribution of ACE2 and TMPRSS2 (Figure 2A). ACE2 and TMPRSS2 were found in the olfactory supporting cells but not in OSNs (Table 2). Sustentacular cells and Bowman's glands provide structural support as they stretch the length from the lamina propria to the apical surface [11]. Damage to these structures could disorganize the olfactory epithelium and put cilia of OSNs in positions suboptimal for odourant binding [11]. Various critical functions would cease such as electrolyte balance, odourant transport, the mucous layer where odourants dissolve and OSN regeneration [8]. However, the rapid recovery of olfaction usually seen in COVID-19 cases indicates that basal cells remain functio-

nal, as confirmed in one animal model [45].

OSNs seem spared by not co-expressing ACE2 and TMPRSS2, consistent with reports of anosmia as an early but not immediate feature of COVID-19 [80]. However, indirection infection of OSNs might still be possible through tight junctions with sustentacular cells (Figure 2B) [11]. Infected OSNs were observed in animal models but it is unclear if the same occurs in humans and if SARS-CoV-2 can propagate further along the olfactory nerve [44,45].

Low prevalence of nasal obstruction and rhinorrhoea suggest the nasal respiratory epithelium is avoided by SARS-CoV-2. Instead, olfactory cleft inflammation occasionally seen with imaging is consistent with the proposed vulnerability of the olfactory epithelium (Figure 2C). This respiratory avoidance is confusing because ACE2 and TMPRSS2 were described across the nasal cavity. In fact, the respiratory lining of the nasal cavity expresses more ACE2 than the lower respiratory tract, the site of severe COVID-19 [81]. SARS-COV-2 should encounter ample opportunity for infection before ever reaching the olfactory cleft.

Does SARS-CoV-2 bypass the respiratory epithelium?

The olfactory epithelium occupies a small area of the nasal cavity (Figure 2C). This could explain the high signal intensities of ACE2 and TMPRSS2 seen in biopsies of olfactory tissue [35,42]. Furthermore, only 8% of nasal airflow passes along the olfactory cleft, while the rest passes along the respiratory epithelium [82]. Turbulence in nasal respiratory zones might lead to challenging conditions for SARS-CoV-2, as suggested by studies on the dynamics of ACE2 binding [82,32,33]. Although SARS-CoV-2 binds nasal ACE2 more strongly than SARS-CoV-1, nasal airflow could break these bonds. Under those conditions, SARS-CoV-2 could find refuge in the olfactory cleft and replicate undisturbed using ACE2, TMPRSS2, neuropilin-1 and furin.

Besides protective airflow, nasal breathing was suggested to protect against SARS-CoV-2 via nitric oxide produced in the paranasal sinuses [83]. Patients with inefficient nasal respiration might thus be at higher risk of severe disease because the nasal respiratory epithelium leads directly to the lungs. Anosmia could therefore indicate better infection control, a theory consistent with the symptom's predominance in outpatient COVID-19 cases and an association with higher viral loads in mild cases (84,85, 63). Indeed, IgA made against SARS-CoV-2 may block OSNs because SARS-CoV-2's proteins resemble odourant receptors (Figure 2B) [72]. Downregulation of odourant receptor genes was observed in SARS-CoV-2 clinical specimens, which could be a consequence of IgA occupied odourant receptors [73]. Lower mortality and less severe lower respiratory disease were also observed in hospitalized SARS-CoV-2 patients with anosmia compared to those without olfactory impairment [85]. Different viral loads could underlie the varied clinical presentations but the data is conflicting, as both the upper and lower respiratory tract have been reported as areas of highest viral replication [1, 42, 2]. Whether patients reporting anosmia better resist an initial viral challenge, or are equally vulnerable to infection but better control SARS-CoV-2, remains an important question. While anosmia is the best predictor of a SARS-CoV-2 diagnosis, it would be interesting to see if nasal obstruction and rhinorrhoea are prognostic for severe disease [86]. Importantly, bypass of the nasal respiratory epithelium raises doubts if ACE2 is sufficient to explain anosmia.

Furin and neuropilin-1 might explain this gap [69]. Furin enhances direct cell infection and cell to cell transmission when co-expressed with ACE2 and TMPRSS2. Co-expression of Furin, ACE2 and TMPRSS2 was observed in apical supporting cells (Figure 2A). Similar observations were made on neuropilin-1 [68]. Interestingly, SARS-CoV-1 was only capable of using furin to enhance cell to cell transmission, additionally restricting SARS-CoV-1's olfactory tropism compared to SARS-CoV-2 [87].

An important note regarding this review is that the diverse literature complicates addressing pathophysiology. This is compounded by the inclusion of 14 preprints. Preprints gained relevance with COVID-19 but lack of peer review makes their inclusion risky. Our conclusion on the vulnerability of supporting cells is largely drawn from 24 studies, 5 of which are preprints, with results in line with the peer reviewed literature [34, 39, 40, 43, 49]. However, the dynamics of furin mediated viral entry, pH dependant ACE2 bond stability, the contribution of nasal airflow, odourant receptor downregulation and ethnic variation of entry receptors were only investigated in preprinted studies [32, 33, 40, 67, 69-71, 73]. These findings need to be validated, but their inclusion highlights gaps in research for future study.

An alternative design can be found in Widadgo and Raj's 2016 paper where olfactory tissue was challenged with MERS-CoV's spike protein fused to a mouse antibody [29]. Biosafety level 3 labs are required to investigate SARS-CoV-2, but their study design can address olfactory pathophysiology even in smaller laboratories [88]. Physiological studies are needed because our conclusions are drawn from evidence using small sample sizes, datasets and animals.

We also excluded neurological studies, although changes in the olfactory bulb were described in several papers [36,49,50,54,56,66]. Future studies should explore if this is because of infection in the olfactory bulb or neuroplasticity due to reduced signalling from the olfactory epithelium.

Conclusion

In this review we highlighted SARS-CoV-2's tropism for olfactory supporting cells. Their vulnerability stems from co-expression of ACE2 and TMPRSS2. However, despite ACE2 and TMPRSS2 co-expression in the nasal respiratory epithelium, sinonasal inflammation is not prevalent in SARS-CoV-2 infection. This raises doubts about the predominant focus on ACE2 in research. Furin and neuropilin-1 could be important in the pathogenesis

of anosmia in SARS-CoV-2 infection, while mechanisms such as odourant receptor mimicry warrant further attention. Fortunately, anosmia after SARS-CoV-2 appears transient in most cases [89]. The complex response to SARS-CoV-2 in the nasal cavity deserves continued research however. For example, prophylactic antagonism of toll-like receptors and neuropilin-1 in the nasal epithelium were shown to inhibit SARS-CoV-2 [68,90]. With concerns about new mutations and vaccines' long-term efficacy, the distinct effects of SARS-CoV-2 on olfaction could reveal novel targets for intervention.

Authorship contribution

MZ: design, search, analysis, manuscript. NvD: design, analysis, revision. KG: expert opinion, revision, WF: design, analysis, expert opinion, revision.

Conflict of interest

No conflict of interest.

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SUPPLEMENTARY DATA

Supplement Table 1. Search queries.

Database	Virus	(AND) Anosmia	(AND) Mechanism
PubMed EMBASE Web of Science	(covid-19 OR covid* OR novel coronavirus OR nCoV OR SARS-CoV-2 OR coronavirus OR coronavirus* OR coronavirus infections OR (severe acute respiratory syndrome OR SARS-COV OR SARS coronavirus OR SARS-related coronavirus) OR (middle east respiratory syndrome OR middle east respiratory syndrome coronavirus OR MERS-CoV OR MERS OR MERS coronavirus OR MERS-related coronavirus) OR (betacoronavirus OR alphacoronavirus OR human coronavirus OR human coronavirus* OR HCOV OR coronavirus NL63 OR coronavirus 229E OR coronavirus HKU1 OR coronavirus OC43))	(olfact* OR smell OR anosmia OR hyposmia OR microsmia OR hyperosmia OR dysosmia OR parosmia OR PVOD OR ((postviral OR post-viral OR post viral OR viral OR post-infectious OR post infectious OR postinfectious) AND (olfactory OR olfaction OR smell) AND (disorder OR dysfunction OR impairment)) OR ((smell OR olfaction OR olfactory OR nasal OR sino-nasal OR sinonasal OR chemosensory OR sensory) AND (symptoms OR sequelae OR blindness OR loss OR impairment OR damage OR dysfunction OR neuropathy OR pathology OR pathophysiology)))	(histology OR immunopathology OR pathology OR pathophysiology OR physiopathology OR pathogenesis OR mechanism OR etiology OR aetiology OR otorhinolaryngology OR otolaryngology OR rhinology OR immunology OR (nasal epithelium OR olfactory epithelium OR nasal neuroepithelium OR respiratory epithelium OR epithelial cells) OR ((olfaction OR olfactory OR nasal) AND (mucosa OR epithelium OR tissue OR system OR nerves OR anatomy)) OR olfactory perception OR (olfactory receptor OR olfactory receptor neurons OR OSN OR ORN) OR olfactory bulb OR olfactory cleft OR olfactory pathway OR nasal cavity)
DOAJ	(SARS-CoV-2 OR COVID-19 OR coronavirus*)	(anosmia OR olfactory+dysfunction)	NA
bioRxiv MedRxiv	covid-19+sars-cov-2+corona*	smell+nasal+anosmia+ "olfactory dysfunction"+ hyposmia+parosmia+olfact*	NA

NA, not applicable.

Supplement Table 2. Characteristics and key outcomes of included studies.

Journal	Year	First author	Virus	Design	Outcomes
Laryngoscope	2007	Suzuki[18]	HCoVs	Prospective	HCoV-229E in one PVOD patient
Laryngoscope	2020	Tian[19]	HCoVs	Prospective	HCoV-OC43 in one PVOD patient
Acta Otolaryngol	1995	Akerlund[27]	HCoVs	Observational	hyposmia correlated with nasal obstruction after HCoV-229E
J Virol	2016	Widagdo[29]	MERS-CoV	immunhistochemistry	MERS-CoV did not infect challenged respiratory and olfactory tissue, suggesting no DPP4 or alternative entry receptors
Am J Pathol	2016	Meyerholz[28]	MERS-CoV	immunhistochemistry	no DPP4 in nasal mucosa
Acta Neurol Taiwan	2006	Hwang[30]	SARS-CoV-1	Case report	Case report of anosmia after SARS-CoV-1 infection with no preceding sinonasal inflammation
Virology	2008	Schaecher[31]	SARS-CoV-1	Animal model	mild long-lasting olfactory epithelium damage
preprint	2020	Paris[32]	SARS-CoV-1, SARS-CoV-2	Simulation	SARS-CoV-2 lacks pH switch, leading to tighter ACE2 bods SARS-CoV-1 has a pH switch leading to looser ACE2 bonds
preprint	2020	Paris[33]	SARS-CoV-1, SARS-CoV-2	Simulation	SARS-CoV-2 lacks pH switch, leading to tighter ACE2 bonds SARS-CoV-1 has a pH switch leading to looser ACE2 bonds
JAMA Otolaryngol Head Neck Surg	2020	Eliezer[57]	SARS-CoV-2	Case report, CT	Olfactory cleft opacification 1/1
Open Forum Infect Dis	2020	Chung[48]	SARS-CoV-2	Case cohort, CT	Olfactory cleft opacification 3/6
Am J Otolaryngol	2020	Naeini[52]	SARS-CoV-2	Case cohort, CT	Olfactory cleft opacification 0/49
preprint	2020	Baxter[34]	SARS-CoV-2	RNAseq	IFN type I and II induced by response to SARS-CoV-2 upregulates nasal ACE2 expression

Journal	Year	First author	Virus	Design	Outcomes
ACS Chem Neurosci	2020	Bilinska[35]	SARS-CoV-2	Immunohistochemistry	ACE2 and TMPRSS2 co-expression in SUS TMPRSS2 expressed in all support cells, BG, BC expression not determined ACE2 express in RE of nasal cavity, but with lower intensity
Dtsch Arztebl Int	2020	Brelie[46]	SARS-CoV-2	Case report, MRI	Olfactory cleft opacification 0/1
preprint	2020	Bryche[43]	SARS-CoV-2	Animal model	Sustentacular cells rapidly infected at day 4, OSN significantly reduced,
preprint	2020	Butler[73]	SARS-CoV-2	RNAseq	Odourant receptors pathways down regulated as a result of infection
Science	2020	Cantuti-Castelvetri[68]	SARS-CoV-2	Immunohistochemistry	Neuropilin-1 is highly expressed in olfactory epithelial cells and can enhance infection of the olfactory epithelium
preprint	2020	Cardenas[70]	SARS-CoV-2	Biopsy	Nasal ACE2 hypomethylation in women and Black invididuals
ACS Chem Neurosci	2020	Cazzolla[66]	SARS-CoV-2	Case cohort	Il-6 is elevated in patients with anosmia
J Infect	2020	Chetrit[47]	SARS-CoV-2	Case cohort, MRI	Olfactory cleft opacification 9/19
Neurology	2020	Eliezer[55]	SARS-CoV-2	Case cohort, MRI	Olfactory cleft opacification 19/20
iScience	2020	Fodoulian[37]	SARS-CoV-2	RNAseq	ACE2 and TMPRSS2 in support cells, No ACE2 in OSN
Acad Radiol	2020	Galougahi[56]	SARS-CoV-2	Case report, MRI	Olfactory cleft opacification 0/1
Acta Cytol	2020	Gelardi[58]	SARS-CoV-2	Cytology	No cytological signs of extensive cell injury
Am J Otolaryngol	2020	Gelardi[59]	SARS-CoV-2	Cytology	No cytological signs of extensive cell injury
preprint	2020	Girardeau[49]	SARS-CoV-2	Case cohort, MRI	Olfactory cleft opacification 2/10
Brief Bioinform	2020	Gupta[38]	SARS-CoV-2	RNAseq	ACE2 and TMPRSS2 in support cells, No ACE2 and TMPRSS2 in OSN
Lancet	2020	Kirschenbaum[60]	SARS-CoV-2	Immunohistochemistry	Mucosal atrophy and neuritis in biopsy of olfactory tissue
Neurology	2020	Laurendon[50]	SARS-CoV-2	Case report, MRI	Olfactory cleft opacification 1/1
Laryngoscope	2020	Lechien[51]	SARS-CoV-2	Case cohort, CT	Olfactory cleft opacification 3/16
preprint	2020	Melo[64]	SARS-CoV-2	RNAseq	Increase of pro inflammatory cytokines may damage the olfactory epithelium
JAMA Otolaryngol Head Neck Surg	2020	Morbini[61]	SARS-CoV-2	Immunohistochemistry	Macrophage activation in olfactory epithelium
preprint	2020	Muus[39]	SARS-CoV-2	RNAseq	ACE2 and TMPRSS2 in all support cells ACE2 and CTSL in olfactory epithelium
Int J Infect Dis	2020	Nakagawara[63]	SARS-CoV-2	Case cohort	Patients with anosmia have higher viral loads and longer time to viral clearance
preprint	2020	Papa[69]	SARS-CoV-2	Immunohistochemistry, CRISPR	Furin enhances infection and cell to transmission but not essential for infection
preprint	2020	Rodriguez[67]	SARS-CoV-2	Immunhistochemistry	IFN1 secretion from sustentacular cells stimulates ACE2 expression in olfactory epithelium IFN1 and other cytokines activate OSN immune cascades, resulting in downregulation of olfactory receptors
preprint	2020	Santos[71]	SARS-CoV-2	Observational cohort	Less TMPRSS2 in European than in Asian individuals
Nature	2020	Sia[44]	SARS-CoV-2	Animal model	moderate inflammatory cell infiltrate in nasal turbinate, viral antigen detectable in nasal mucosa and ORN reduced number of OSN at day 2, nasal epithelial attenuation on day 7, tissue repair at day 14
Eur Arch Otorhinolaryngol	2020	Spoldi[53]	SARS-CoV-2	Case cohort, CT	Olfactory cleft opacification 16/63
ACS Chem Neurosci	2020	Torabi[65]	SARS-CoV-2	Case cohort, biopsy	increased TNFa, c an lead to olfactory submucosa expansion and inhibit basal cells

Journal	Year	First author	Virus	Design	Outcomes
Eur J Neurol	2020	Tsivgoulis[54]	SARS-CoV-2	Case cohort, MRI	Olfactory cleft opacification 3/8
preprint	2020	Ueha[40]	SARS-CoV-2	Immunhistochemistry	ACE and TMPRSS2 co-expression in all tissue, including OSN Furin in SUS and BG's
Clin Infect Dis	2020	Zhang[45]	SARS-CoV-2	Animal model	Rapid disorganisation of olfactory epithelium after challenge, including OSNs
Cell	2020	Ziegler[41]	SARS-CoV-2	RNAseq	ACE2 and TMPRSS2 in support cells, No ACE2 in OSN
preprint	2020	Meinhardt[62]	SARS-CoV-2	Case cohort, biopsy	Virus present in olfactory mucosa and signs of axon damage
Sci Adv	2020	Brann[36]	SARS-CoV-2	RNAseq	ACE2 and TMPRSS2 in support cells No ACE2 and TMPRSS2 in OSN ACE2 and TMPRSS2 upregulation after tissue damage
Eur Respir J	2020	Chen[42]	SARS-CoV-2	Immunohistochemistry	ACE2 and TMPRSS2 in SUS and BG No ACE2 and TMPRSS2 in OSN
Rhinology Online	2020	Root-Bernstein[72]	SARS-CoV-2	Proteonomic similarity searching (BLAST)	SARS-CoV-2 mimics human odourant receptors, which could block by OSNs by IgA made against the virus