Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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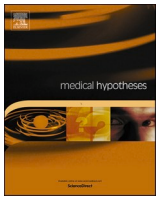

Nicotinic receptors as SARS-CoV-2 spike co-receptors?

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ABSTRACT

Nicotinic acetylcholine receptors (nAChRs) play an important role in homeostasis and respiratory diseases. Controversies regarding the association between COVID-19 hospitalizations and smoking suggest that nAChRs may contribute to SARS-CoV-2 respiratory syndrome. We recently detailed the expression and localization of all nAChR subunits in the human lung. Since virus association with nAChRs has been shown in the past, we hypothesize that nAChR subunits act as SARS-CoV-2 Spike co-receptors. Based on sequence alignment analysis, we report domains of high molecular similarities in nAChRs with the binding domain of hACE2 for SARS-CoV-2 Spike protein. This hypothesis supported by *in silico* pilot data provides a rational for the modelling and the *in vitro* experimental validation of the interaction between SARS-CoV-2 and the nAChRs.

Background

Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) is responsible for the global pandemic of coronavirus disease 2019 (COVID-19) [1,2]. Understanding its structure and molecular interactors are crucial to explain the transmission, viral infection, and replication [3]. Recent controversies regarding the impact of smoking on COVID-19 pointed to the potential role of nicotinic acetylcholine receptors (nAChRs) in human angiotensin converting enzyme-2 (hACE2) interaction with SARS-CoV-2 [4,5]. A similar convergence was established regarding the association of nAChRs with rabies virus where the binding was ultimately experimentally demonstrated a few decades ago [6–10].

Since it has been proposed that nAChRs may play a key role in the SARS-CoV-2-mediated inflammatory syndrome [11,12], additional avenues are currently explored considering that: (i) nAChRs are widely expressed in organs targeted by the virus including lung, nose, brain, gastrointestinal tract, liver, and smooth muscles [13,14]; (ii) nAChRs are also present on endothelial and platelet cells [15] while altered endothelial function induces vascular thrombosis and microangiopathy in COVID-19 [16,17]; (iii) nAChRs are involved in angiogenesis and arterial diseases [18], while significant vascular angiogenesis was found in lungs from patients who died from COVID-19 [19].

The hypothesis

It was recently suggested that smoking may promote cellular uptake mechanisms of SARS-CoV-2 through CHRNA7 signalling [20], and subsequently, CHRNA7 transcripts were correlated with hACE2 levels [21], feeding the possible connections between hACE2 and nAChR localizations in airway epithelial cells. In addition, *in silico* analysis of nAChR ligands suggested an interaction between SARS-CoV-2 and nAChRs [22–24], while nicotine exposure may facilitate SARS-CoV-2 infection *in vitro* [25]. Nicotinic receptors are ligand-gated ion channels consisting of 5 membrane-spanning subunits selected from 16 proteins (CHRNA1-A7, A9-A10, B1-B4, D, E, G) that all harbour at least one extracellular domain of approximately 200 residues. Going one step further, we suggest that nAChRs may bind to SARS-CoV-2-RBD (Receptor Binding Domain) similar to hACE2 and potentially act as co-

Abbreviations: ACE-2, Angiotensin converting enzyme-2; BR, Binding region; COVID-19, Coronavirus disease 2019; ETD, Extracellular topological domain; nAChRs, Nicotinic acetylcholine receptors; PDB, Protein data bank; RBD, Receptor binding domain; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; vdw, Van der Waals.

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receptors for SARS-CoV-2-RBD at least in the airways where we documented the complete atlas of pulmonary nAChR subunits in terms of transcript expression and protein localization [26]. Transcripts of all 16 subunits were detected in airway epithelial cells or whole lung tissues and only subunits α1/α2/α4/β3/γ were not observed via immunofluorescent stainings performed on formalin-fixed paraffin-embedded lung tissues.

Empirical data

The N-terminal domain of hACE2 binding to the spike (S) glycoprotein SARS-CoV-2-RBD and SARS-CoV-1-RBD has been characterized recently [27–30]. Twenty-four hACE2 residues were shown to interact with 21 SARS-CoV-2-RBD residues (region K417 to Y505) and 18 SARS-CoV-1-RBD residues (region R426 to Y491) organized in 4 motifs on hACE2 (Fig. 1A and B).

Postulating that nAChRs may contain extracellular hACE2-like regions mimicking their interactions with SARS-CoV-2, and since structural data of the 16 nAChR subunits are incomplete, we performed bioinformatics analysis using the Clustal Omega program (Uniprot) to align each of the 16 nAChR subunits' extracellular topological domain protein sequences with the 4 motifs containing the binding residues of hACE2 with SARS-CoV-1 and 2. We identified identical residues in the 4 regions of interests (ROI) as well as conservations between groups of similar properties with a particular focus on residues located within van der Waals (vdw) contact distance between the viral ligand and the receptor (24 red residues, Figs. 1 and 2).

Key residues to form a solid network between hACE2 and SARS-CoV-2-RBD are localized in the motif-1 (S19-Q42) and the motif-4 (K355-R357). The last residue (R393) was not included in the analysis since it was not in the proximity of other key residues involved in complex formation (Fig. 1). Interestingly, CHRNA2/A4/A6/A7/A10/B1 presented no overlaps for the 4 motifs (Fig. 2 and Table 1).

We established a score based on the percentage of matching residues either in the entire proposed binding region or solely considering the residues involved in vdw contacts (Table 1). Each of the nAChR subunits presented on average 68.5% ([59.1–81.8]) of resemblance with hACE2 binding regions to SARS-CoV-2-RBD and 64.4% ([47.8–73.9]) solely taking into account vdw contacts. The best scores for the 2 parameters were obtained by CHRNA6 (61.4%/73.9%), and CHRNB1 (81.8%/69.5%) (Fig. 2 and Table 1). Most of nAChRs possessed mutations in the possible binding domains where a loss or a strong inhibition of the interaction with SARS-CoV-1 and 2 was reported (Table 1, [31]). Mutagenesis might favour a beneficial role of nAChRs if they were found to bind SARS-CoV-2 but their levels of expression and specific localizations in various tissues weigh up the pros and cons in a complex and not fully elucidated biological scale.

Consequences of the hypothesis and discussion

Although these findings do not demonstrate that nAChRs can function as co-receptors for the ligand SARS-CoV-2-RBD, here we report a significant molecular similarity between hACE2 binding sites and extracellular domains of nAChRs. Structural biology to match protein structures in 3D would ideally complement this bioinformatics analysis but crystallized structures of all human nAChR subunits are currently not fully resolved. Notably, the protein folding of nAChRs subunit should reveal the critical residues to allow the interaction with SARS-CoV-2-RBD. In addition, nAChRs function as pentamers, the complexity of the composition of the channel may also contribute to the potential interaction between SARS-CoV-2 and one of the subunits where binding would be possible. Interestingly, CHRNA6 and CHRNB1 transcripts were highly expressed in lung tissues and small airway epithelial cells, and the proteins were strongly detected in bronchi strengthening the possibility of the direct interaction between nAChR subunits and SARS-CoV-2 [26].

Although we focus here on similarities of a potential binding domain with hACE2, we do not exclude the possibility that SARS-CoV-2 may interact with alternative domains on nAChRs. The hypothesis of SARS-CoV-2 binding to nAChRs was originally based on the identification of similar known nAChR antagonist/agonist motif sequences on SARS-CoV-2 spike protein. This possible interaction has been sparsely discussed *in silico* [22,23,32]. Finally, although several pentamers are functionally characterized in the nervous system or muscles, the association of subunits in the airways is only partially elucidated and requires further investigations in the context of COVID-19 [26]. Conversely, nAChRs may play a role in COVID-19 progression beyond the respiratory system including systemic inflammation and the nervous system.

The modulation of nAChRs in the lung may increase pro-inflammatory cytokine production, a process potentially leading to the "cytokine storm" during infection [33,34]. Moreover, α7 nAChR has been involved in the control of inflammation associated with influenza virus infection [35]. nAChR can also control COVID-19 physiopathology by modulating the Renin-Angiotensin System (RAS) [36]. This nicotine-induced imbalance of the two arms of the RAS is likely responsible for cardio-vascular dysfunction, and acute/chronic lung diseases associated with severe forms of COVID-19.

The potential neurotropism of SARS-CoV-2 has been discussed extensively. SARS-CoV-2 has been identified in the brain of patients [37]. There is by now a substantial amount of literature, and cases, that link neurological and psychiatric sequelae of COVID-19 to an implication of the human brain. The current state of the discussion has been reviewed [38]. Of specific interest is a recent description of a case of Parkinson's Disease directly linked to the infection [39]. The working hypothesis is that SARS-CoV-2 infection can be a trigger precipitating the onset of neuropathology. Nicotinic acetylcholine receptors (nAChRs)

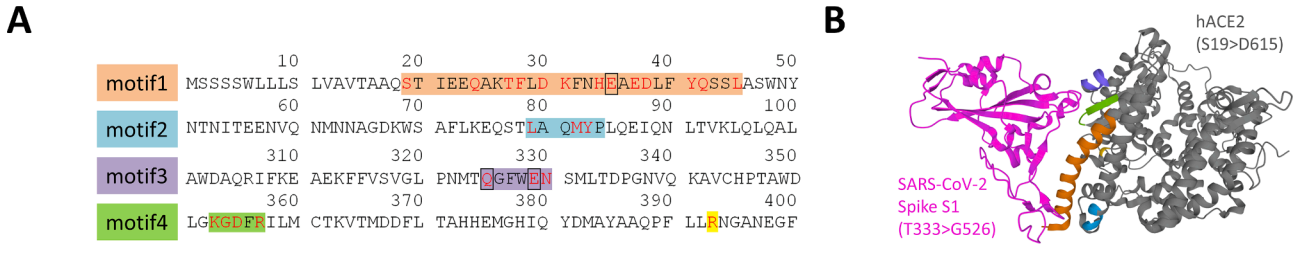

Fig. 1. hACE2 binding motifs to SARS-CoV-1 and 2 sequences. (A) Localizations of motifs 1 to 4 on hACE2 sequence (Q9BYF1) are highlighted in orange, blue, purple, and green respectively. Red residues are residues involved in van der Waals contact distance between hACE2 and SARS-CoV-1 and 2. Framed residues correspond to residues that are either interacting with SARS-CoV-2-RBD only (E35) or SARS-CoV-1-RBD only (Q325, E329). The residue highlighted in yellow corresponds to the 24th residue involved in van der Waals contact distance but not in the immediate proximity of other residues involved in the binding, therefore it was not included in the analysis. (B) The crystal structure (Protein Data Bank: 6M0J) shows the 4 motifs highlighted in (A) with similar colour codes. The crystal structure of SARS-CoV-2 spike receptor-binding domain bound with hACE2 (PDB ID: 6M0J [52]) was highlighted and exported from the viewer of the Research Collaboratory for Structural Bioinformatics (RCSB; www.rcsb.org).

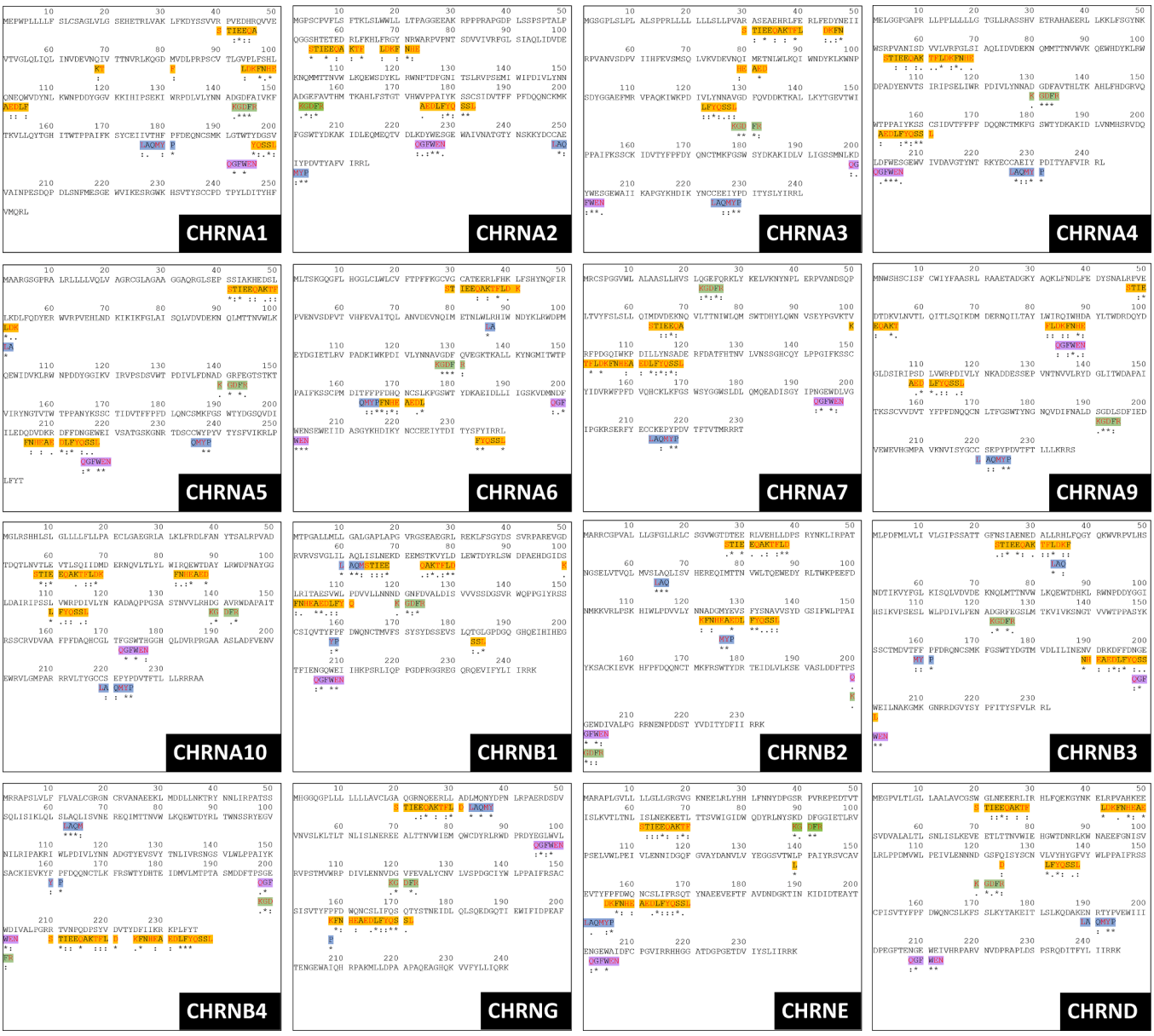

Fig. 2. Clustal alignment of nAChRs with hACE2 binding motifs. Data from the UniProt Knowledgebase (UniProtKB, [53]) were used to perform a sequence alignment study of matching residues between hACE2/SARS-CoV-2 binding sites and nAChR protein sequences. The UniProtKB/Swiss-Prot accession numbers of the sequences used for analyzing the similarities are the following: ACE2 (Q9BYF1), CHRNA1 (P02708), CHRNA2 (Q15822), CHRNA3 (P32297), CHRNA4 (P43681), CHRNA5 (P30532), CHRNA6 (Q15825), CHRNA7 (P36544), CHRNA9 (Q9UGM1), CHRNA10 (Q9GZZ6), CHRNB1 (P11230), CHRNB2 (P17787), CHRNB3 (Q05901), CHRNB4 (P30926), CHRNG (P07510), CHRNE (Q04844), CHRND (Q07001). Motifs 1 to 4 were aligned with the 16 nAChR sequences to identify molecular similarities. The nAChR extracellular topological domains' subunit sequences are represented with the partial alignment obtained with Clustal for the 4 motifs. *, position with a single fully conserved residue; :, position with a residue showing conservation between groups of strongly similar properties (scoring > 0.5 in the Gonnet PAM 250 matrix); ., position with a residue showing conservation between groups of weakly similar properties (scoring =<0.5 in the Gonnet PAM 250 matrix).

are important transmembrane neurotransmitter receptors for acetylcholine in the mammalian brain [40]. They modulate key neuronal functions such as excitability, synaptic transmission, and plasticity. They can change neuronal network states and consequently whole-brain responses to internal and external inputs and have been linked to disease [41]. They are also exogenously activated by nicotine and are responsible for the events leading to nicotine addiction [42]. The predominant receptors in the brain are α4β2* containing hetero-, and (α7)5 homopentamers [43]. Being particularly expressed in the cortex, hippocampus, and dopaminergic reward system [44], these receptors are considered to be important drug targets [45]. The α6 and α5-containing receptors are more restricted, but still very present in sub-cortical modulatory areas. The presence of SARS-CoV-2 in the brain parenchyma can therefore potentially interfere with higher cognitive function. nAChRs are potentially implicated in the penetration of SARS-CoV-2 into the central nervous system, as they are expressed in many nerve endings [46]. The olfactory route has been established in previous types of coronaviruses able to enter nerve endings and use retrograde axonal transport [47]. A possibility is also the vagus nerve. It is well established that it contains most nAChR subunits [48].

According to each individual clinical context including but not limited to respiratory diseases and smoking history [49,50], nAChR binding to SARS-CoV-2-RBD may facilitate virus attachment to cell surface before virus entry, or serve as a decoy either harmful as it would

Table 1
Table presenting the sequence similarities with SARS-CoV-hACE2 binding motifs for all nAChRs.

CHRN	ETDL	BRO	BR in ETD (44 R)			vdw contacts in ETD (23 R)			Mutagenesis
			Id (R, (%))	Sim (R, (%))	Tot (R, (%))	Id (R, (%))	Sim (R, (%))	Tot (R, (%))	
A1	21–255	Y	11 (25)	17 (38.6)	28 (63.6)	4 (17.4)	9 (39.1)	13 (56.5)	353 K > D 357 R > A
A2	27–264	N	14 (31.8)	16 (36.4)	30 (68.2)	5 (21.7)	8 (34.8)	13 (56.5)	353 K > D 357 R > A
A3	32–240	Y	12 (27.3)	20 (45.4)	32 (72.7)	6 (26.1)	10 (43.4)	16 (69.5)	31 K > D
A4	29–242	N	14 (31.8)	17 (38.6)	31 (70.4)	5 (21.7)	8 (34.8)	13 (56.5)	353 K > D 357 R > A
A5	23–254	Y	13 (29.6)	18 (40.9)	31 (70.5)	6 (26.1)	11 (47.8)	17 (73.9)	31 K > D 353 K > D
A6	26–239	N	16 (36.4)	11 (25)	27 (61.4)	9 (39.1)	8 (34.8)	17 (73.9)	
A7	23–230	N	12 (27.3)	14 (31.8)	26 (59.1)	5 (21.7)	9 (39.1)	14 (60.8)	
A9	26–237	Y	10 (22.7)	22 (50)	32 (72.7)	5 (21.7)	12 (52.2)	17 (73.9)	
A10	25–237	N	12 (27.3)	17 (38.6)	29 (65.9)	6 (26.1)	7 (30.4)	13 (56.5)	353 K > D 355 D > A
B1	24–244	N	16 (36.4)	20 (45.4)	36 (81.8)	6 (26.1)	10 (43.4)	16 (69.5)	353 K > D
B2	26–233	Y	15 (34.1)	16 (36.4)	31 (70.5)	7 (30.4)	9 (39.1)	16 (69.5)	
B3	25–232	Y	13 (29.6)	20 (45.4)	33 (75)	4 (17.4)	13 (56.5)	17 (73.9)	353 K > D
B4	22–236	Y	14 (31.8)	15 (34.1)	29 (65.9)	5 (21.7)	11 (47.8)	16 (69.5)	31 K > D
G	23–240	Y	14 (31.8)	14 (31.8)	28 (63.6)	5 (21.7)	7 (30.4)	12 (52.1)	353 K > D
E	21–239	Y	12 (27.3)	17 (38.6)	29 (65.9)	2 (8.7)	9 (39.1)	11 (47.8)	
D	22–245	Y	13 (29.6)	17 (38.6)	30 (68.2)	6 (26.1)	10 (43.4)	16 (69.5)	353 K > D

BRO present overlap in binding regions (BR) in Extracellular topological domains (ETD). The binding regions correspond to the 4 motifs containing a total of 44 residues (R). The van der Waals (vdw) contacts include 23 residues. ETDL, ETD position on protein sequence; BRO, BR overlaps; Id, fully conserved residues (Number, % of total); Sim, residues with similar properties (Number, % of total); Tot, sums of the fully conserved and similar residues (Number, % of total). Mutagenesis indicates the positions of the residues that were shown to be involved in a loss of SARS-CoV interaction and that are also present on nAChR sequences.

prevent the binding of acetylcholine, or beneficial as it would dampen the virus load that may infect epithelial cells [51]. Structural biology and functional analysis on SARS-CoV-2-RBD in complex with nAChRs subunits alone or as pentamers is required to resolve the possible interactions and unveil additional therapeutic strategies.

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CRediT authorship contribution statement

Valérian Dormoy: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition. **Jeanne-Marie Perotin:** Formal analysis, Writing – review & editing. **Philippe Gosset:** Formal analysis, Writing – review & editing, Funding acquisition. **Uwe Maskos:** Formal analysis, Writing – review & editing, Funding acquisition. **Myriam Polette:** Formal analysis, Writing – review & editing. **Gaëtan Deslée:** Formal analysis, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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