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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.

Keywords: Nicotinic receptors; COVID-19; SARS-CoV-2; hACE2; lung

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-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 $\alpha 1/\alpha 2/\alpha 4/\beta 3/\gamma$ 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 (**Figure 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, **Figure 1 and Figure 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 (**Figure 1**). Interestingly, CHRNA2/A4/A6/A7/A10/B1 presented no overlaps for the 4 motifs (**Figure 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%) (**Figure 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, $\alpha 7$ 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) 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 $\alpha 4\beta 2^*$ containing hetero-, and $(\alpha 7)_5$ homo-pentamers [43]. Being particularly expressed in the cortex, hippocampus, and dopaminergic reward system [44], these receptors are considered to be important drug targets [45]. The $\alpha 6$ and $\alpha 5$ -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 prevent the binding of acetylcholine, or beneficial as it would dampen the virus load that

136 may infect epithelial cells [51]. Structural biology and functional analysis on SARS-CoV-2-RBD
137 in complex with nAChRs subunits alone or as pentamers is required to resolve the possible
138 interactions and unveil additional therapeutic strategies.

139

140

141 **Abbreviations**

142	ACE-2	Angiotensin converting enzyme-2
143	BR	Binding region
144	COVID-19	Coronavirus disease 2019
145	ETD	Extracellular topological domain
146	nAChRs	Nicotinic acetylcholine receptors
147	PDB	Protein data bank
148	RBD	Receptor binding domain
149	SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
150	vdw	Van der Waals
151		

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169 **References**

- 170 [1] Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of
171 2019 novel coronavirus: implications for virus origins and receptor binding. *The Lancet*
172 2020;395:565–74. [https://doi.org/10.1016/S0140-6736\(20\)30251-8](https://doi.org/10.1016/S0140-6736(20)30251-8).
- 173 [2] Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with
174 Pneumonia in China, 2019. *N Engl J Med* 2020;382:727–33.
175 <https://doi.org/10.1056/NEJMoa2001017>.
- 176 [3] Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19
177 associated with acute respiratory distress syndrome. *The Lancet Respiratory Medicine*
178 2020;8:420–2. [https://doi.org/10.1016/S2213-2600\(20\)30076-X](https://doi.org/10.1016/S2213-2600(20)30076-X).
- 179 [4] Rossato M, Russo L, Mazzocut S, Di Vincenzo A, Fioretto P, Vettor R. Current smoking is not
180 associated with COVID-19. *Eur Respir J* 2020;55:2001290.
181 <https://doi.org/10.1183/13993003.01290-2020>.
- 182 [5] Leung JM, Sin DD. Smoking, ACE-2 and COVID-19: ongoing controversies. *Eur Respir J*
183 2020;56:2001759. <https://doi.org/10.1183/13993003.01759-2020>.
- 184 [6] Gastka M, Horvath J, Lentz TL. Rabies virus binding to the nicotinic acetylcholine receptor alpha
185 subunit demonstrated by virus overlay protein binding assay. *J Gen Virol* 1996;77 (Pt 10):2437–
186 40. <https://doi.org/10.1099/0022-1317-77-10-2437>.
- 187 [7] Lentz TL. Rabies virus binding to an acetylcholine receptor alpha-subunit peptide. *J Mol*
188 *Recognit* 1990;3:82–8. <https://doi.org/10.1002/jmr.300030205>.
- 189 [8] Donnelly-Roberts DL, Lentz TL. Synthetic peptides of neurotoxins and rabies virus glycoprotein
190 behave as antagonists in a functional assay for the acetylcholine receptor. *Pept Res*
191 1989;2:221–6.
- 192 [9] Lentz TL, Hawrot E, Wilson PT. Synthetic peptides corresponding to sequences of snake venom
193 neurotoxins and rabies virus glycoprotein bind to the nicotinic acetylcholine receptor. *Proteins*
194 1987;2:298–307. <https://doi.org/10.1002/prot.340020406>.
- 195 [10] Lentz TL, Burrage TG, Smith AL, Crick J, Tignor GH. Is the acetylcholine receptor a rabies virus
196 receptor? *Science* 1982;215:182–4. <https://doi.org/10.1126/science.7053569>.
- 197 [11] Gonzalez-Rubio J, Navarro-Lopez C, Lopez-Najera E, Lopez-Najera A, Jimenez-Diaz L, Navarro-
198 Lopez JD, et al. Cytokine Release Syndrome (CRS) and Nicotine in COVID-19 Patients: Trying to
199 Calm the Storm. *Front Immunol* 2020;11:1359. <https://doi.org/10.3389/fimmu.2020.01359>.
- 200 [12] Lutchman D. Could the smoking gun in the fight against COVID-19 be the (rh)ACE-2? *Eur Respir J*
201 2020;56:2001560. <https://doi.org/10.1183/13993003.01560-2020>.
- 202 [13] Zoli M, Pucci S, Vilella A, Gotti C. Neuronal and Extraneuronal Nicotinic Acetylcholine Receptors.
203 *Curr Neuropharmacol* 2018;16:338–49.
204 <https://doi.org/10.2174/1570159X15666170912110450>.
- 205 [14] Keiger CJH, Jones KR, Case LD, Drake AF, Kendal-Reed M, Walker JC. Nicotinic Cholinergic
206 Receptor Expression in the Human Nasal Mucosa. *Ann Otol Rhinol Laryngol* 2003;112:77–84.
207 <https://doi.org/10.1177/000348940311200115>.
- 208 [15] Schedel A, Thornton S, Schloss P, Klüter H, Bugert P. Human Platelets Express Functional $\alpha 7$ -
209 Nicotinic Acetylcholine Receptors. *Arterioscler Thromb Vasc Biol* 2011;31:928–34.
210 <https://doi.org/10.1161/ATVBAHA.110.218297>.
- 211 [16] Sardu C, Gambardella J, Morelli MB, Wang X, Marfella R, Santulli G. Hypertension, Thrombosis,
212 Kidney Failure, and Diabetes: Is COVID-19 an Endothelial Disease? A Comprehensive Evaluation
213 of Clinical and Basic Evidence. *JCM* 2020;9:1417. <https://doi.org/10.3390/jcm9051417>.
- 214 [17] Levi M, Thachil J, Iba T, Levy JH. Coagulation abnormalities and thrombosis in patients with
215 COVID-19. *The Lancet Haematology* 2020;7:e438–40. [https://doi.org/10.1016/S2352-3026\(20\)30145-9](https://doi.org/10.1016/S2352-3026(20)30145-9).
- 216 [18] Santanam N, Thornhill BA, Lau JK, Crabtree CM, Cook CR, Brown KC, et al. Nicotinic
217 acetylcholine receptor signaling in atherogenesis. *Atherosclerosis* 2012;225:264–73.
218 <https://doi.org/10.1016/j.atherosclerosis.2012.07.041>.
- 219

- [19] Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. *N Engl J Med* 2020;383:120–8. <https://doi.org/10.1056/NEJMoa2015432>.
- [20] Russo P, Bonassi S, Giacconi R, Malavolta M, Tomino C, Maggi F. COVID-19 and smoking: is nicotine the hidden link? *Eur Respir J* 2020;55:2001116. <https://doi.org/10.1183/13993003.01116-2020>.
- [21] Leung JM, Yang CX, Sin DD. COVID-19 and nicotine as a mediator of ACE-2. *Eur Respir J* 2020;55:2001261. <https://doi.org/10.1183/13993003.01261-2020>.
- [22] Alexandris N, Lagoumintzis G, Chasapis CT, Leonidas DD, Papadopoulos GE, Tzartos SJ, et al. Nicotinic cholinergic system and COVID-19: In silico evaluation of nicotinic acetylcholine receptor agonists as potential therapeutic interventions. *Toxicol Rep* 2021;8:73–83. <https://doi.org/10.1016/j.toxrep.2020.12.013>.
- [23] Farsalinos K, Eliopoulos E, Leonidas DD, Papadopoulos GE, Tzartos S, Poulas K. Nicotinic Cholinergic System and COVID-19: In Silico Identification of an Interaction between SARS-CoV-2 and Nicotinic Receptors with Potential Therapeutic Targeting Implications. *Int J Mol Sci* 2020;21. <https://doi.org/10.3390/ijms21165807>.
- [24] Stefano ML, Kream RM, Stefano GB. A Novel Vaccine Employing Non-Replicating Rabies Virus Expressing Chimeric SARS-CoV-2 Spike Protein Domains: Functional Inhibition of Viral/Nicotinic Acetylcholine Receptor Complexes. *Med Sci Monit* 2020;26:e926016. <https://doi.org/10.12659/MSM.926016>.
- [25] Lupacchini L, Maggi F, Tomino C, De Dominicis C, Mollinari C, Fini M, et al. Nicotine Changes Airway Epithelial Phenotype and May Increase the SARS-COV-2 Infection Severity. *Molecules* 2020;26:101. <https://doi.org/10.3390/molecules26010101>.
- [26] Diabasana Z, Perotin J-M, Belgacemi R, Ancel J, Mulet P, Delepine G, et al. Nicotinic Receptor Subunits Atlas in the Adult Human Lung. *IJMS* 2020;21:7446. <https://doi.org/10.3390/ijms21207446>.
- [27] Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z, et al. Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. *Cell* 2020;181:894-904.e9. <https://doi.org/10.1016/j.cell.2020.03.045>.
- [28] Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H, et al. Structural basis of receptor recognition by SARS-CoV-2. *Nature* 2020;581:221–4. <https://doi.org/10.1038/s41586-020-2179-y>.
- [29] Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh C-L, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 2020;367:1260–3. <https://doi.org/10.1126/science.abb2507>.
- [30] Turoňová B, Sikora M, Schürmann C, Hagen WJH, Welsch S, Blanc FEC, et al. In situ structural analysis of SARS-CoV-2 spike reveals flexibility mediated by three hinges. *Science* 2020;370:203–8. <https://doi.org/10.1126/science.abd5223>.
- [31] Li W, Zhang C, Sui J, Kuhn JH, Moore MJ, Luo S, et al. Receptor and viral determinants of SARS-coronavirus adaptation to human ACE2. *EMBO J* 2005;24:1634–43. <https://doi.org/10.1038/sj.emboj.7600640>.
- [32] Oliveira ASF, Ibarra AA, Bermudez I, Casalino L, Gaieb Z, Shoemark DK, et al. A potential interaction between the SARS-CoV-2 spike protein and nicotinic acetylcholine receptors. *Biophysical Journal* 2021;120:983–93. <https://doi.org/10.1016/j.bpj.2021.01.037>.
- [33] Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 2000;405:458–62. <https://doi.org/10.1038/35013070>.
- [34] Pavlov VA, Tracey KJ. Neural regulation of immunity: molecular mechanisms and clinical translation. *Nat Neurosci* 2017;20:156–66. <https://doi.org/10.1038/nn.4477>.
- [35] Gao Z, Li L, Huang Y, Zhao C, Xue S, Chen J, et al. Vagal- $\alpha 7$ nAChR signaling is required for lung anti-inflammatory responses and arginase 1 expression during an influenza infection. *Acta Pharmacol Sin* 2021;42:1642–52. <https://doi.org/10.1038/s41401-020-00579-z>.

- [36] Alifano M, Alifano P, Forgez P, Iannelli A. Renin-angiotensin system at the heart of COVID-19 pandemic. *Biochimie* 2020;174:30–3. <https://doi.org/10.1016/j.biochi.2020.04.008>.
- [37] Song E, Zhang C, Israelow B, Lu-Culligan A, Prado AV, Skriabine S, et al. Neuroinvasion of SARS-CoV-2 in human and mouse brain. *Journal of Experimental Medicine* 2021;218:e20202135. <https://doi.org/10.1084/jem.20202135>.
- [38] Iadecola C, Anrather J, Kamel H. Effects of COVID-19 on the Nervous System. *Cell* 2020;183:16-27.e1. <https://doi.org/10.1016/j.cell.2020.08.028>.
- [39] Cohen ME, Eichel R, Steiner-Birmanns B, Janah A, Ioshpa M, Bar-Shalom R, et al. A case of probable Parkinson's disease after SARS-CoV-2 infection. *The Lancet Neurology* 2020;19:804–5. [https://doi.org/10.1016/S1474-4422\(20\)30305-7](https://doi.org/10.1016/S1474-4422(20)30305-7).
- [40] Picciotto MR, Higley MJ, Mineur YS. Acetylcholine as a Neuromodulator: Cholinergic Signaling Shapes Nervous System Function and Behavior. *Neuron* 2012;76:116–29. <https://doi.org/10.1016/j.neuron.2012.08.036>.
- [41] Higley MJ, Picciotto MR. Neuromodulation by acetylcholine: examples from schizophrenia and depression. *Current Opinion in Neurobiology* 2014;29:88–95. <https://doi.org/10.1016/j.conb.2014.06.004>.
- [42] Changeux J-P. Nicotine addiction and nicotinic receptors: lessons from genetically modified mice. *Nat Rev Neurosci* 2010;11:389–401. <https://doi.org/10.1038/nrn2849>.
- [43] Corringer P-J, Novère NL, Changeux J-P. Nicotinic Receptors at the Amino Acid Level. *Annu Rev Pharmacol Toxicol* 2000;40:431–58. <https://doi.org/10.1146/annurev.pharmtox.40.1.431>.
- [44] Besson M, Granon S, Mameli-Engvall M, Cloez-Tayarani I, Maubourguet N, Cormier A, et al. Long-term effects of chronic nicotine exposure on brain nicotinic receptors. *Proceedings of the National Academy of Sciences* 2007;104:8155–60. <https://doi.org/10.1073/pnas.0702698104>.
- [45] Taly A, Corringer P-J, Guedin D, Lestage P, Changeux J-P. Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system. *Nat Rev Drug Discov* 2009;8:733–50. <https://doi.org/10.1038/nrd2927>.
- [46] Marchi M, Grilli M, Pittaluga AM. Nicotinic modulation of glutamate receptor function at nerve terminal level: a fine-tuning of synaptic signals. *Front Pharmacol* 2015;6. <https://doi.org/10.3389/fphar.2015.00089>.
- [47] Dubé M, Le Coupanec A, Wong AHM, Rini JM, Desforges M, Talbot PJ. Axonal Transport Enables Neuron-to-Neuron Propagation of Human Coronavirus OC43. *J Virol* 2018;92. <https://doi.org/10.1128/JVI.00404-18>.
- [48] Cooper E. Nicotinic Acetylcholine Receptors on Vagal Afferent Neurons. *Annals of the New York Academy of Sciences* 2006;940:110–8. <https://doi.org/10.1111/j.1749-6632.2001.tb03670.x>.
- [49] Cai G, Bossé Y, Xiao F, Kheradmand F, Amos CI. Tobacco Smoking Increases the Lung Gene Expression of ACE2, the Receptor of SARS-CoV-2. *Am J Respir Crit Care Med* 2020;rccm.202003-0693LE. <https://doi.org/10.1164/rccm.202003-0693LE>.
- [50] Zhang H, Rostami MR, Leopold PL, Mezey JG, O'Beirne SL, Strulovici-Barel Y, et al. Expression of the SARS-CoV-2 ACE2 Receptor in the Human Airway Epithelium. *Am J Respir Crit Care Med* 2020;rccm.202003-0541OC. <https://doi.org/10.1164/rccm.202003-0541OC>.
- [51] Farsalinos K, Niaura R, Le Houezec J, Barbouni A, Tsatsakis A, Kouretas D, et al. Editorial: Nicotine and SARS-CoV-2: COVID-19 may be a disease of the nicotinic cholinergic system. *Toxicology Reports* 2020;7:658–63. <https://doi.org/10.1016/j.toxrep.2020.04.012>.
- [52] Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* 2020;581:215–20. <https://doi.org/10.1038/s41586-020-2180-5>.
- [53] The UniProt Consortium. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Research* 2019;47:D506–15. <https://doi.org/10.1093/nar/gky1049>.

Figure Legends

Figure 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).

Figure 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).

351 **Table 1. Table presenting the sequence similarities with SARS-CoV-hACE2 binding motifs**
352 **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

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

360

A

	10	20	30	40	50
motif1	MSSSSWLLLS	LVAVTAAQ ST	IEE QAKTFLD	KFNH E AEDLF	YQSSL ASWNY
	60	70	80	90	100
motif2	NTNITEENVQ	NMNNAGDKWS	AFLKEQST LA	QMY PLQEIQN	LTVKLQLQAL
	310	320	330	340	350
motif3	AWDAQRI FKE	AEKFFVSVGL	PNMT Q GFW EN	SMLTDPGNVQ	KAVCHPTAWD
	360	370	380	390	400
motif4	LG KGDFR ILM	CTKVTMDDFL	TAHHEMGHIQ	YDMAYAAQPF	LL R NGANEGF

B

