COVID-19 and smoking: is nicotine the hidden link?

To the Editor:

Leung *et al.* [1] have recently published, in the *European Respiratory Journal*, a paper on the expression of angiotensin-converting enzyme II (ACE-2) in the small airway epithelia of smokers and COPD patients, discussing its effects on the risk of severe coronavirus disease 2019 (COVID-19). The authors found an increased expression of the ACE-2 gene in the airways of subjects with COPD and in current smokers. Indeed, a recent systematic review reporting data on the smoking habits of patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), concluded that smoking may be associated with a negative progression of the disease and with the adverse outcome [2]. These conclusions were challenged in a correspondence by Cai [3] on the basis that a reliable mechanism explaining this association was missing. The need for these results to be supported by additional studies is quite clear, but we believe that a robust mechanistic explanation exists. Nicotine has a known influence on the homeostasis of the renin–angiotensin system (RAS) up-regulating the angiotensin-converting enzyme (ACE)/angiotensin (ANG)-II/ANG II type 1 receptor axis, and down-regulating the compensatory ACE-2/ANG-(1–7)/Mas receptor axis, contributing in turn to the development of cardiovascular and pulmonary diseases [4]. Different airway cells, such as bronchial epithelial cells, type II alveolar epithelial cells and interstitial lung fibroblasts, express nicotinic acetylcholine receptors (nAChR), specifically the α7subtype [5]. All these cells express components of the RAS [4]. In addition, nicotine increases the expression and/or activity of ACE in the lung [4], an increase which has been found also in the serum of smokers, and that required at least 20 min to return to control level [4]. ACE-2 serves as a physiologically relevant cellular entry receptor for SARS-CoV, for the human respiratory coronavirus NL63, and probably for SARS-CoV-2 [6]. ACE binds the SARS-CoV-2 S protein, and through its tissutal expression mediates the localisation and the efficiency of the infection [6]. Moreover, nicotine induces the epithelial–mesenchymal transition (EMT) [5, 7], a mechanism sufficient to allow "normal" differentiated cells to acquire the stem cell-like characteristics and properties. We planned experiments on human bronchial epithelial cells (HBEpC), obtained from Cell Applications Inc. (www.cellapplications.com/product number 502K-05a). Cells were maintained as adherent monolayer in complete bronchial/tracheal epithelial cell growth medium (www.cellapplications.com/product) at 37°C in 95% air/5% CO_2, seeded at an initial density of 7.5×10^4 cells·cm^{-2}, and sub-cultured with a 0.25% trypsin–1 mM EDTA solution (Sigma-Aldrich, Milan, Italy) when cultures reached 80% confluence. HBEpC are derived from the surface epithelium of normal human bronchi non-diseased (*i.e.* asthma, COPD or type 2 diabetes). The morphology is consistent with epithelial origin, and is positive for epithelial cell marker cytokeratin 18. Semi-confluent HBEpC at fourth passage (7.5×10^4 cells·cm^{-2}) were treated: 1) for 1 h with zero or 1.0×10^{-7} M nicotine (Sigma-Aldrich, Milan, Italy) dissolved in saline in complete medium; 2) with 1.0×10^{-6} M α-Bungarotoxin (α-BTX; Sigma-Aldrich, Milan, Italy) dissolved in saline, in the continued presence of nicotine at zero or 1.0×10^{-7} M for 1 h; 3) treated continuously with nicotine for additional passages, 1 passage every 48 h for a total of 16 passages. We showed, for the first time, that nicotine at 1×10^{-7} M (the concentration present on the alveolar lining fluids after one cigarette is in the range 6×10^{-6} to 6×10^{-5} M [5]) is able to increase ACE-2 (figure 1a) in HBEpC. Treatment with nicotine induces phospho-S6 ribosomal protein (Ser235/236), Akt1, phospho-Akt (Ser473), phospho-Akt (Thr308) and phospho-p44/42 MAPK (Thr202/Tyr204) (figure 1b). To verify the hypothesis that ACE-2 is induced by nicotine through α7-nAChR, HBEpC, at fourth passage, in the exponential growth phase, plated at a density of 1×10^6 cells·mL^{-1}, were incubated with α7-nAChR siRNA (0.1 µg) diluted in 100 µL of siRNA transfection medium. Transfection was performed as described by Li *et al.* [8], who transfected, successfully, HBE16 human airway epithelial cell line (unaffected cells). A clone of transfected HBEpC that did not express α7-nAChR proteins, also after

@ERSpublications
Nicotine *via* alpha7-nicotinic receptor induces ACE-2 overexpression in human bronchial epithelial cells (HBEpC) https://bit.ly/3eJ5b35

Cite this article as: Russo P, Bonassi S, Giacconi R, *et al.* COVID-19 and smoking: is nicotine the hidden link? *Eur Respir J* 2020; 55: 2001116 [https://doi.org/10.1183/13993003.01116-2020].

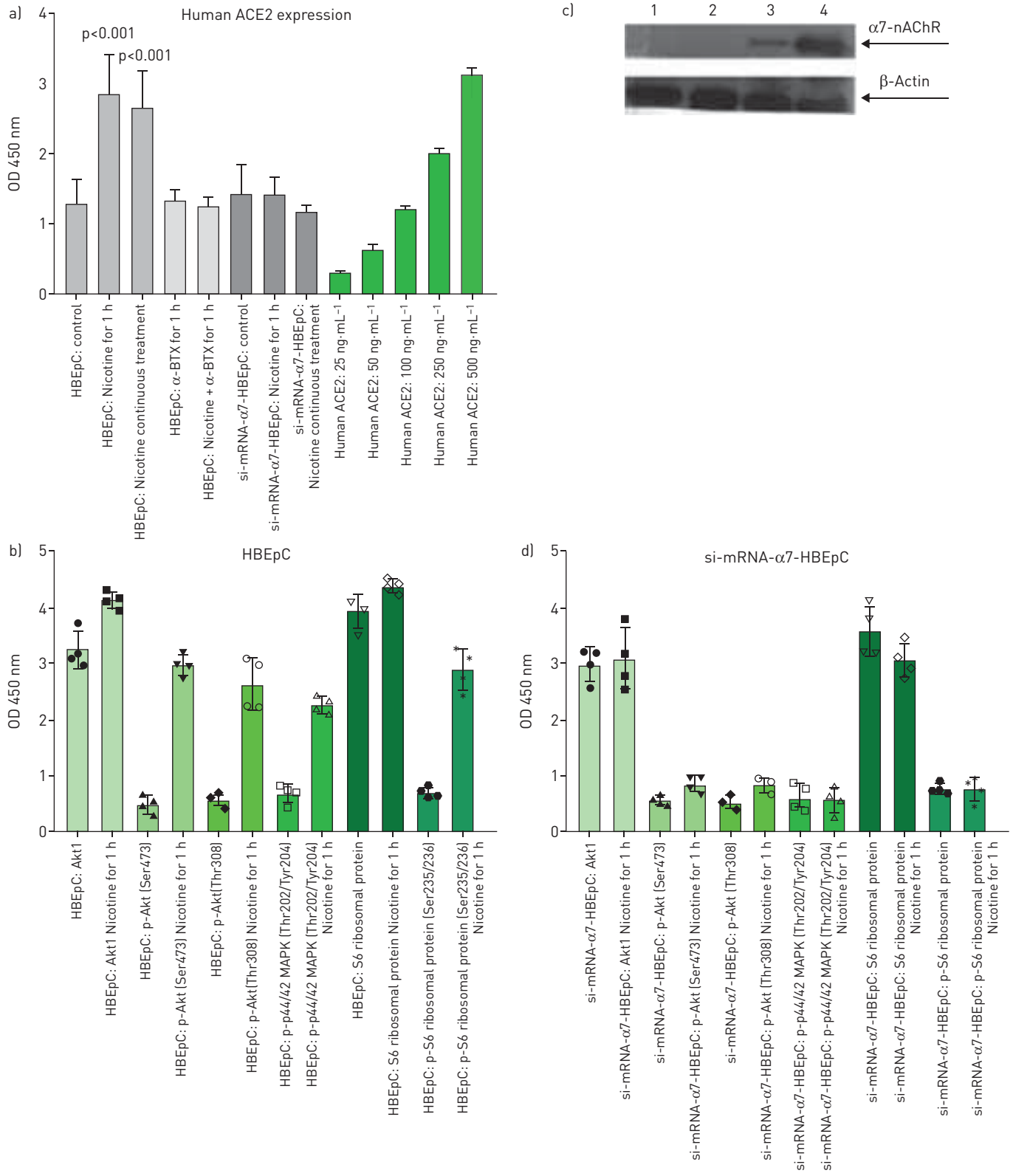

FIGURE 1 Effect of nicotine on human bronchial epithelial cells (HBEpC) or si-mRNA-α7-HBEpC. a) Angiotensin-converting enzyme II (ACE-2) detection. ACE-2 was measured with human ACE-2 ELISA kit ab235649 (www.abcam.com/human-ace2-elisa-kit-ab235649.html) according to the manufacturer's instructions. Data are mean±SEM; p-vaule was evaluated using t-test. The green plots are the human ACE-2 standard. Experiments were performed twice in triplicate. b) Induction of phospho-S6 ribosomal protein (Ser235/236), Akt1, phospho-Akt (Ser473), phospho-Akt (Thr308)

and phospho-p44/42 MAPK (Thr202/Tyr204) in HBEpC. Data were obtained using PathScan® cell growth Multi-target Sandwich ELISA kit n.7239 (Cell Signaling) PathScan® Cell Growth Multi-Target Sandwich ELISA Kit is a solid phase sandwich ELISA that combines the reagents necessary to detect endogenous levels of S6 ribosomal protein, phospho-S6 ribosomal protein (Ser235/236), Akt1, phospho-Akt (Ser473), phospho-Akt (Thr308) and phospho-p44/42 MAPK (Thr202/Tyr204). Data are mean±SEM; p-value was evaluated using t-test. Experiments were performed twice in duplicate. c) α7-nAChR protein detection. Western blotting was performed as described previously [11]. Human α7-nAchR antibody NBP1-49348 was purchased from Novus Biologicals (www.novusbio.com). 1–2 si-mRNA-α7-HBEpC treated with zero (lane 1) or 1.0×10^{-7} M nicotine (lane 2) for 1 h. 3–4 HBEpC treated with zero (lane 3) or 1.0×10^{-7} M nicotine (lane 4) for 1 h. Experiments were performed twice. d) as in panel b, but treated cells are si-mRNA-α7-HBEpC.

treatment with nicotine (figure 1c), and is not able to induce phospho-S6 ribosomal protein (Ser235/236), Akt1, phospho-Akt (Ser473), phospho-Akt (Thr308) and phospho-p44/42 MAPK (Thr202/Tyr204) after nicotine treatment (figure 1d), was selected for further experiments. Nicotine did not induce ACE-2 in this clone (si-mRNA-α7-HBEpC) (figure 1a). This observation supports the hypothesis that ACE-2 increase is specifically mediated by α7-nAChR. Moreover, when HBEpC were incubated simultaneously with nicotine and α-BTX, an α7 nicotine antagonist [9], no induction of ACE-2 was observed (figure 1d). Importantly, treatment with nicotine, α-BTX or with the combination is not cytotoxic (data not shown). On these bases, we suggest that smoking may promote cellular uptake mechanisms of SARS-CoV-2 through α7-nAChR signalling. A possible α7-nAChR down-stream mechanism may be the induction of phospho-Akt and phospho-p44/42 MAPK. This mechanism was hypothesised, partially, by OLDS and KABBANI [10] on their schematic model explaining how nicotine exposure increases the risk of SARS-CoV-2 entry into lung cells. α7-nAChR is present both in neuronal and non-neuronal cells (*i.e.* lung, endothelial, lymphocyte); consequently, smoking may impact COVID-19 pathophysiology and clinical outcome in several organ systems, including the brain.

Patrizia Russo[1,2], Stefano Bonassi[1,2], Robertina Giacconi[3], Marco Malavolta[3], Carlo Tomino[4] and Fabrizio Maggi[5,6]

[1]Clinical and Molecular Epidemiology, IRCSS San Raffaele Pisana, Rome, Italy. [2]Dept of Human Sciences and Quality of Life Promotion, San Raffaele University, Rome, Italy. [3]Advanced Technology Center for Aging Research, Scientific Technological Area, Italian National Institute of Health and Science on Aging (INRCA), Ancona, Italy. [4]Scientific Direction, IRCSS San Raffaele Pisana, Rome, Italy. [5]Dept of Translational Research, University of Pisa, Pisa, Italy. [6]Virology Division, Pisa University Hospital, Pisa, Italy.

Correspondence: Patrizia Russo, Clinical and Molecular Epidemiology, IRCSS San Raffaele Pisana, Via di Val Cannuta, 247, I-00166 Rome, Italy. E-mail: patrizia_russo@hotmail.it

Received: 10 April 2020 | Accepted after revision: 16 April 2020

Conflict of interest: None declared.

References

1 Leung JM, Yang CX, Tam A, *et al.* ACE-2 expression in the small airway epithelia of smokers and COPD patients: implications for COVID-19. *Eur Respir J* 2020; 55: 2000688.
2 Vardavas CI, Nikitara K. COVID-19 and smoking: a systematic review of the evidence. *Tob Induc Dis* 2020; 18: 20.
3 Cai H. Sex difference and smoking predisposition in patients with COVID-19. *Lancet Respir Med* 2020; 8: e20.
4 Oakes JM, Fuchs RM, Gardner JD, *et al.* Nicotine and the renin-angiotensin system. *Am J Physiol Regul Integr Comp Physiol* 2020; 315: R895–R906.
5 Cardinale A, Nastrucci C, Cesario A, *et al.* Nicotine: specific role in angiogenesis, proliferation and apoptosis. *Crit Rev Toxicol* 2012; 42: 68–89.
6 Zhou P, Yang XL, Wang XG, *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; 579: 270–273.
7 Santoro A, Tomino C, Prinzi G, *et al.* Tobacco smoking: risk to develop addiction, chronic obstructive pulmonary disease, and lung cancer. *Recent Pat Anticancer Drug Discov* 2019; 14: 39–52.
8 Li Q, Zhou X, Kolosov VP, *et al.* The expression and pharmacological characterization of nicotinic acetylcholine receptor subunits in HBE16 airway epithelial cells. *Cell Biochem Biophys* 2012; 62: 421–431.
9 Wang YY, Liu Y, Ni XY, *et al.* Nicotine promotes cell proliferation and induces resistance to cisplatin by α7 nicotinic acetylcholine receptor-mediated activation in Raw264.7 and El4 cells. *Oncol Rep* 2014; 31: 1480–1488.
10 Olds JL, Kabbani N. Is nicotine exposure linked to cardiopulmonary vulnerability to COVID-19 in the general population? *FEBS J* 2020; in press [https://doi.org/10.1111/febs.15303].
11 Trombino S, Cesaro A, Margaritora S, *et al.* Alpha7-nicotinic acetylcholine receptors affect growth regulation of human mesothelioma cells: role of mitogen-activated protein kinase pathway. *Cancer Res* 2004; 64: 135–145.

COVID-19 and nicotine as a mediator of ACE-2

From the authors:

We recently reported that current smokers and those with COPD had higher airway epithelial cell expression of the angiotensin-converting enzyme II (ACE-2) viral entry receptor [1]. We thus read with great interest the work of P. Russo and co-workers, which proposes a mechanism for this finding, namely that this upregulation is mediated by nicotine exposure specifically through the α7 subtype of nicotine acetylcholine receptors (α7-nAChR). While exposure to increasing concentrations of nicotine caused epithelial cells to increase ACE-2 levels, subsequent gene silencing of α7-nAChR appeared to significantly dampen this response. A secondary transcriptome sequencing analysis of our cohort (consisting of 42 subjects who underwent bronchoscopy for epithelial cell brushings [1]) reveals evidence in support of this hypothesis. We found that airway epithelial cell expression of *CHRNA7*, encoding α7-nAChR, was significantly correlated with the expression of *ACE2* (Pearson r=0.54, $p=2.31\times10^{-8}$) (figure 1). There was significantly higher *CHRNA7* expression in those with COPD (2.75±0.73 *versus* 2.14±0.43 in those without COPD; $p=1.47\times10^{-4}$), with a trend towards higher expression in current smokers compared to former and never smokers (2.86±0.92 in current smokers, 2.35±0.57 in former smokers, and 2.27±0.45 in never smokers; $p=6.16\times10^{-2}$). *CHRNA7* was also negatively correlated with forced expiratory volume in 1 s percent predicted (Pearson r=−0.37, $p=2.83\times10^{-4}$). Interestingly, *CHRNA7* was positively if weakly correlated with body mass index (Pearson r=0.14, $p=6.31\times10^{-3}$), raising the intriguing possibility that nicotine receptor mediation of ACE-2 may also be related to why obese individuals have made up a considerable proportion of coronavirus disease 2019 (COVID-19) cases [2].

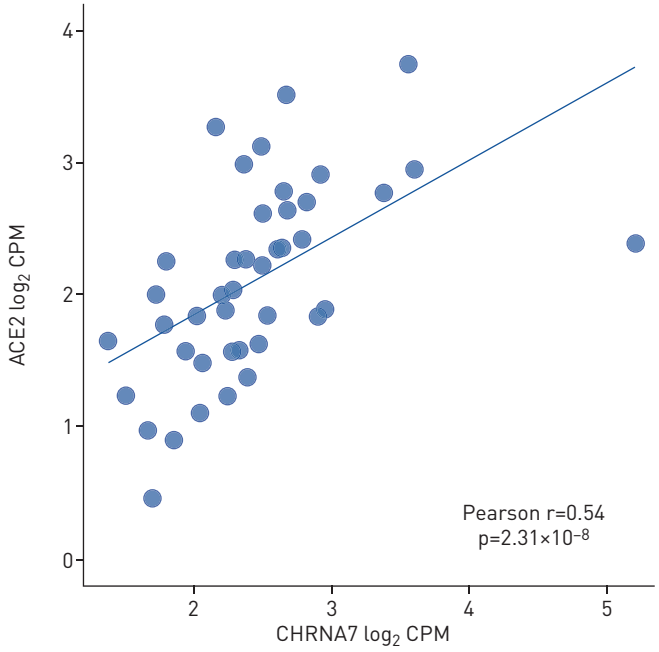

FIGURE 1 Transcriptome profiles generated through RNA-Seq of airway epithelial cells demonstrated a significant positive correlation between *ACE2* and *CHRNA7* expression.

Together, these data further help to characterise the connections between airway epithelial ACE-2, and α7-nAChR, and the unique vulnerability of patients with COPD to severe COVID-19. α7-nAChR's

@ERSpublications
α7-nAChR may upregulate ACE-2 https://bit.ly/2xS0cfT

Cite this article as: 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].

widespread abundance in the human body, from neuronal tissue to immune cells to the lung and digestive tract, and its various roles in diseases such as schizophrenia [3], Alzheimer's disease [4] and Parkinson's disease [5] has meant that considerable work has already been done to target α7-nAChR as a therapeutic modality. As an example, α7-nAChR antagonists for the purpose of smoking cessation have long been proposed [6] and the idea of potentially repurposing these compounds for a pandemic with few therapeutic options currently available is certainly appealing. Whether α7-nAChR-selective antagonists, such as methyllycaconitine [7] and α-conotoxin [8], can meaningfully alter ACE-2 expression to prevent severe acute respiratory syndrome coronavirus 2 entry into the airway epithelium seems the next logical investigation in our furious pursuit for better therapeutics.

Janice M. Leung[1,2], Chen Xi Yang[1] and Don D. Sin[1,2]
[1]Centre for Heart Lung Innovation, University of British Columbia, Vancouver, BC, Canada. [2]Division of Respiratory Medicine, Dept of Medicine, St. Paul's Hospital, Vancouver, BC, Canada.

Correspondence: Don D. Sin, Division of Respiratory Medicine, Dept of Medicine, St. Paul's Hospital, 1081 Burrard Street, Vancouver, BC V6Z1Y6, Canada. E-mail: Don.Sin@hli.ubc.ca

Received: 18 April 2020 | Accepted after revision: 20 April 2020

Conflict of interest: J.M. Leung has nothing to disclose. C.X. Yang has nothing to disclose. D.D. Sin reports grants from Merck, personal fees for advisory board work from Sanofi-Aventis and Regeneron, grants and personal fees for lectures from Boehringer Ingelheim and AstraZeneca, personal fees for lectures and advisory board worm from Novartis, outside the submitted work.

References

1 Leung JM, Yang CX, Tam A, *et al.* ACE-2 expression in the small airway epithelia of smokers and COPD patients: implications for COVID-19. *Eur Respir J* 2020; 55: 2000688.
2 Garg S, Kim L, Whitaker M, *et al.* Hospitalization rates and characteristics of patients hospitalized with laboratory-confirmed coronavirus disease 2019—COVID-NET, 14 States, March 1–30, 2020. *MMWR Morb Mortal Wkly Rep* 2020; 69: 458–464.
3 Freedman R, Leonard S, Gault JM, *et al.* Linkage disequilibrium for schizophrenia at the chromosome 15q13-14 locus of the alpha7-nicotinic acetylcholine receptor subunit gene (CHRNA7). *Am J Med Genet* 2001; 105: 20–22.
4 Guan ZZ, Zhang X, Ravid R, *et al.* Decreased protein levels of nicotinic receptor subunits in the hippocampus and temporal cortex of patients with Alzheimer's disease. *J Neurochem* 2000; 74: 237–243.
5 Banerjee C, Nyengaard JR, Wevers A, *et al.* Cellular expression of alpha7 nicotinic acetylcholine receptor protein in the temporal cortex in Alzheimer's and Parkinson's disease—a stereological approach. *Neurobiol Dis* 2000; 7: 666–672.
6 Ramachandran Nair L, Liu X. Targeting the alpha4beta2- and alpha7-subtypes of nicotinic acetylcholine receptors for smoking cessation medication development. *J Addict Res Ther* 2019; 10: 381.
7 Panagis G, Kastellakis A, Spyraki C, *et al.* Effects of methyllycaconitine (MLA), an alpha 7 nicotinic receptor antagonist, on nicotine- and cocaine-induced potentiation of brain stimulation reward. *Psychopharmacology (Berl)* 2000; 149: 388–396.
8 Liang J, Tae HS, Xu X, *et al.* Dimerization of alpha-conotoxins as a strategy to enhance the inhibition of the human alpha7 and alpha9alpha10 nicotinic acetylcholine receptors. *J Med Chem* 2020; 63: 2974–2985.