

University of Groningen

Determinants of expression of SARS-CoV-2 entry-related genes in upper and lower airways

U-BIOPRED study group; Cambridge Lung Cancer Early Detection Programme; INER-Ciencias Mexican Lung Program; Aliee, Hananeh; Massip, Florian; Qi, Cancan; de Biase, Maria Stella; van Nijnatten, Jos; Kersten, Elin T G; Kermani, Nazanin Z

Published in:
Allergy

DOI:
[10.1111/all.15152](https://doi.org/10.1111/all.15152)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2022

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

U-BIOPRED study group, Cambridge Lung Cancer Early Detection Programme, INER-Ciencias Mexican Lung Program, Aliee, H., Massip, F., Qi, C., de Biase, M. S., van Nijnatten, J., Kersten, E. T. G., Kermani, N. Z., Khuder, B., Vonk, J. M., Vermeulen, R. C. H., Neighbors, M., Tew, G. W., Grimaldeston, M. A., Ten Hacken, N. H. T., Hu, S., Guo, Y., ... Faiz, A. (2022). Determinants of expression of SARS-CoV-2 entry-related genes in upper and lower airways. *Allergy*, 77(2), 690-694. <https://doi.org/10.1111/all.15152>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

DOI: 10.1111/all.15152

Determinants of expression of SARS-CoV-2 entry-related genes in upper and lower airways

To the Editor,

The coronavirus disease 2019 (COVID-19) pandemic is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). To better understand COVID-19 the genetic and environmental factors on susceptibility and severity, detailed knowledge of regulation of genes required for viral entry into respiratory epithelial cells is needed.

We assessed the gene expression of SARS-CoV-2 receptors and activating proteases, and their regulation by smoking, inhaled-corticosteroids (ICS), genetics/epigenetics using nasal and bronchial samples from nine independent cohorts (see extended methods, Table S1).

SARS-CoV-2 cell entry factor (SCEF) genes have higher expression levels in nose than bronchi in matched samples across two cohorts (Figure 1A–D, Table S2), mirroring results from previous smaller studies.^{1,2} Smoking was associated with higher expression of *ACE2*, *TMPRSS2*, *FURIN*, and *BSG* in bronchial brushes, supporting a recent meta-analysis,³ but not in nasal brushes (Figure 1E,F). In contrast, smoking was associated with lower expression of *CTSL* in nasal and bronchial brushings (Table S3). The impact of smoking on the expression of *ACE2* and *BSG* gene expression differs significantly between these tissues (Table S4). None of these genes were associated with sex or age. Cell-type deconvolution of RNA-seq data revealed that all SCEF genes strongly correlated with predicted secretory cell proportions across tissues (Figure 1G–I), in particular *ACE2* (Figure 1J), in line with recent scRNA-seq data.^{3,4} We observed higher proportions of secretory cells (goblet & club cells) in bronchial samples from current smokers compared to ex/never-smokers, which was not observed in nasal brushes (Figure 1K), which may explain the lack of increase of *ACE2* expression in nasal samples. We next performed a cross-sectional analysis for nasal samples in four adult cohorts (NORM/OLIVA ($n = 76$), CRUKPAP ($n = 405$), U-BIOPRED ($n = 89$), and INCI ($n = 79$)); and one pediatric cohort: PIAMA ($n = 291$); and for bronchial samples in five populations: INDURAIN ($n = 184$), U-BIOPRED ($n = 108$), GLUCOLD ($n = 56$), CRUKPAP ($n = 228$) and NORM/TIP ($n = 167$). In upper airways, *CTSL* expression was lower in current smokers compared to non-smokers (Table S5). In lower airways, higher levels of *ACE2* and *TMPRSS2* were identified in current versus never/ex-smokers, whereas smoking was associated with higher *FURIN* and *BSG* levels in brushed cells only (Figure 2A–F, Table

S6). Acute smoke exposure ($n = 63$) and secondhand smoking (infants of parents who smoked $n = 9$ or did not smoke, $n = 13$) were found to associate with higher *ACE2* expression (Figure 2G,H, Table S7 and S8).

No studies have investigated the longitudinal effects of ICS on SCEF genes in paired biopsies.^{5,6} In steroid-naïve COPD patients, 6 months ICS ± LABA treatment decreased *ACE2* expression ($p = 0.009$, Figure 2I, Table S9) compared to placebo in bronchial biopsies, while *BSG* and *FURIN* increased ($p = 0.012$ and $p = 0.046$, respectively).

No association of genetic variation with expression of SCEF genes was found in a well-powered meta-analysis of nasal: NORM ($n = 93$), CRUKPAP ($n = 339$) and PIAMA ($n = 303$), and bronchial samples: NORM/TIP ($n = 150$) and CRUKPAP ($n = 215$, Table S10). We next investigated whether DNA-methylation is associated with SCEF expression. In pediatric nasal samples (PIAMA; $n = 245$), we identified eQTM for *CTSL*, *BSG*, *NRP1*, *FURIN*, and *TMPRSS2* expression (Table S11). Bronchial eQTMs were analyzed in an adult cohort (INDURAIN; $n = 169$). We identified 143 eQTMs for the different SCEF genes (Figure 2J, Table S12). The nasal eQTMs were influenced by age and sex, but not smoking (Table S13), whereas bronchial eQTMs for *TMPRSS2* were associated with smoking and age (Table S14). *ACE2* expression in bronchial biopsies was associated with 6 CpG sites, two of which were in the promoter region of the adjacent *TMEM27* gene. *ACE2* and *TMEM27* expression was correlated (Figure 2K,L) and both associated with methylation of cg20473453 (Figure 2M), indicating possible co-regulation of *ACE2* and *TMEM27*.

In conclusion, although SCEF genes are more highly expressed in upper airways, first and secondhand smoke exposure only appears to influence the expression of these genes in the lower airways. CpG methylation, but not genetic variation, was associated with expression of several SCEF genes in bronchus and nose, which was associated with age, gender, and smoking. Finally, ICS decreases expression of *ACE2* in bronchial biopsies. Together, these results indicate that the enhanced SCEF expression in the lower airways due to cigarette smoke exposure and the reduced expression in subjects taking ICS may underlie the increased susceptibility to COVID-19 in smokers and the clinical efficacy of ICS.

Shared first author: Hananeh Aliee, Florian Massip, Cancan Qi, Maria Stella de Biase, Jos van Nijnatten, Elin T.G. Kersten, Nazanin Z. Kermani, Basil Khuder.

Shared senior author: Robert C Rintoul, Paul A. Reyfman, Fabian J. Theis, Corry-Anke Brandsma, Ian M. Adcock, Wim Timens, Cheng-Jian Xu, Maarten van den Berge, Roland F. Schwarz, Gerard H. Koppelman, M.C. Nawijn, Alen Faiz.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2021 The Authors. *Allergy* published by European Academy of Allergy and Clinical Immunology and John Wiley & Sons Ltd.

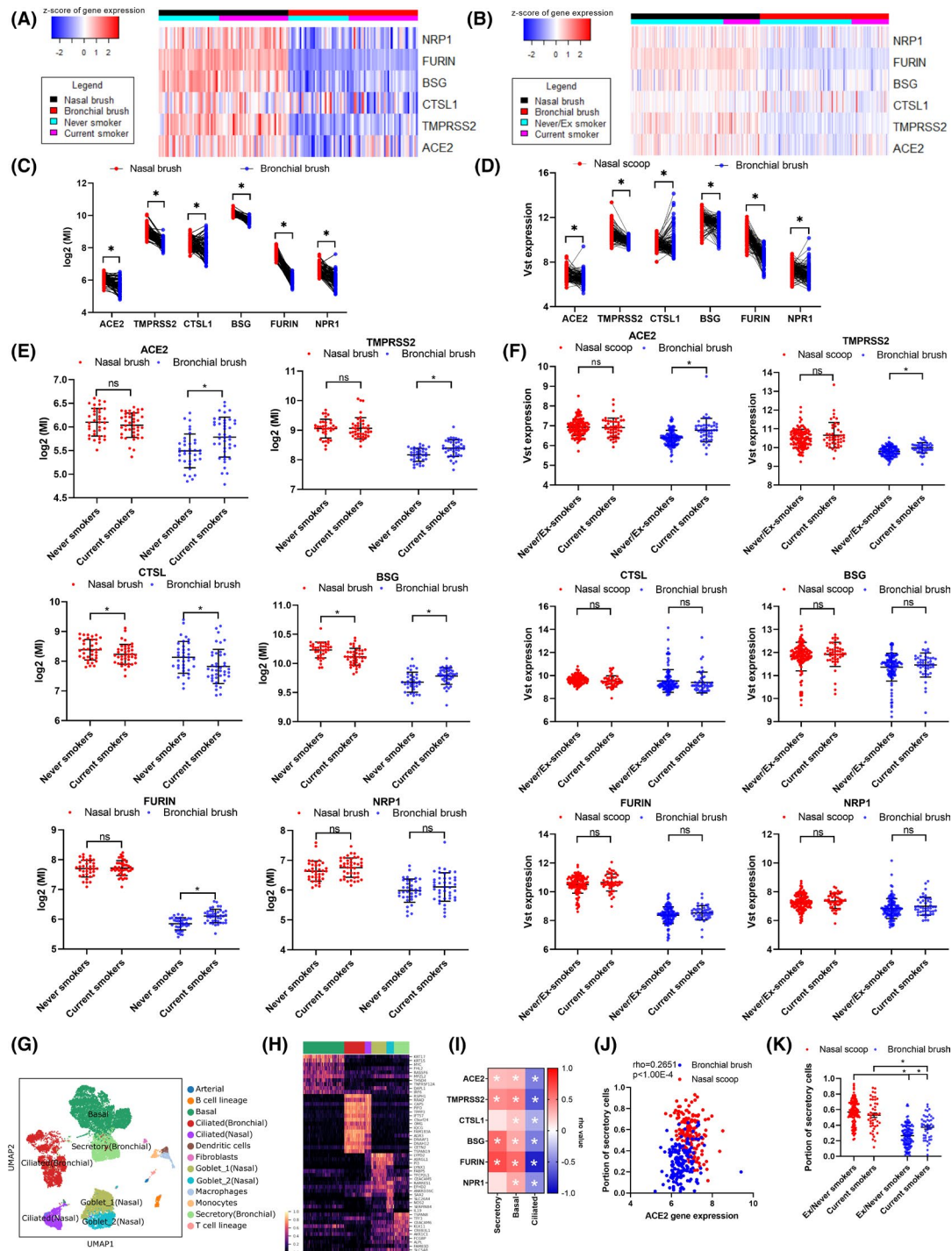


FIGURE 1 Expression genes required for SARS-CoV-2 entry into cells in nasal and bronchial brushes and relationship with goblets cells. Heatmaps and plots of SARS-CoV-2 cell entry related in matched nasal and bronchial brushes from the NORM ($n = 77$) (A & C) and CRUKPAP ($n = 162$) cohorts (B & D). Plots comparing ACE2, TMPRSS2, CTSL, BSG, FURIN, and NPR1 expression in current and ex/never-smokers in nasal and bronchial brushes, (E) NORM and (F) CRUKPAP. Plots comparing ACE2 expression in ex-smokers and duration of smoke cessation in nasal and bronchial brushes. (G) UMAP of merged bronchial biopsy and nasal brush single cell datasets. (H) Heatmap of selected genes associated with each epithelial cell type. (I) Correlation heatmap of cellular deconvolution cell proportions compared to SARS-CoV-2 cell entry related (Spearman's rho) correlation was conducted. (J) Association of cellular deconvolution of Goblet cells with ACE2 expression. (K) Goblet/secretory cell fraction separated based on tissue type and smoking status. Cellular deconvolution was performed using AutoGeneS. Statistics for deconvolution results were conducted using Mann-Whitney test, while the correlation heatmap was analyzed using Spearman correlation. * $p < 0.05$, *** p value < 0.001 Abbreviations: MI, microarray intensity; VST, variance-stabilizing transformation. Statistics for deconvolution results were conducted using Mann-Whitney test for unpaired and Wilcoxon for paired

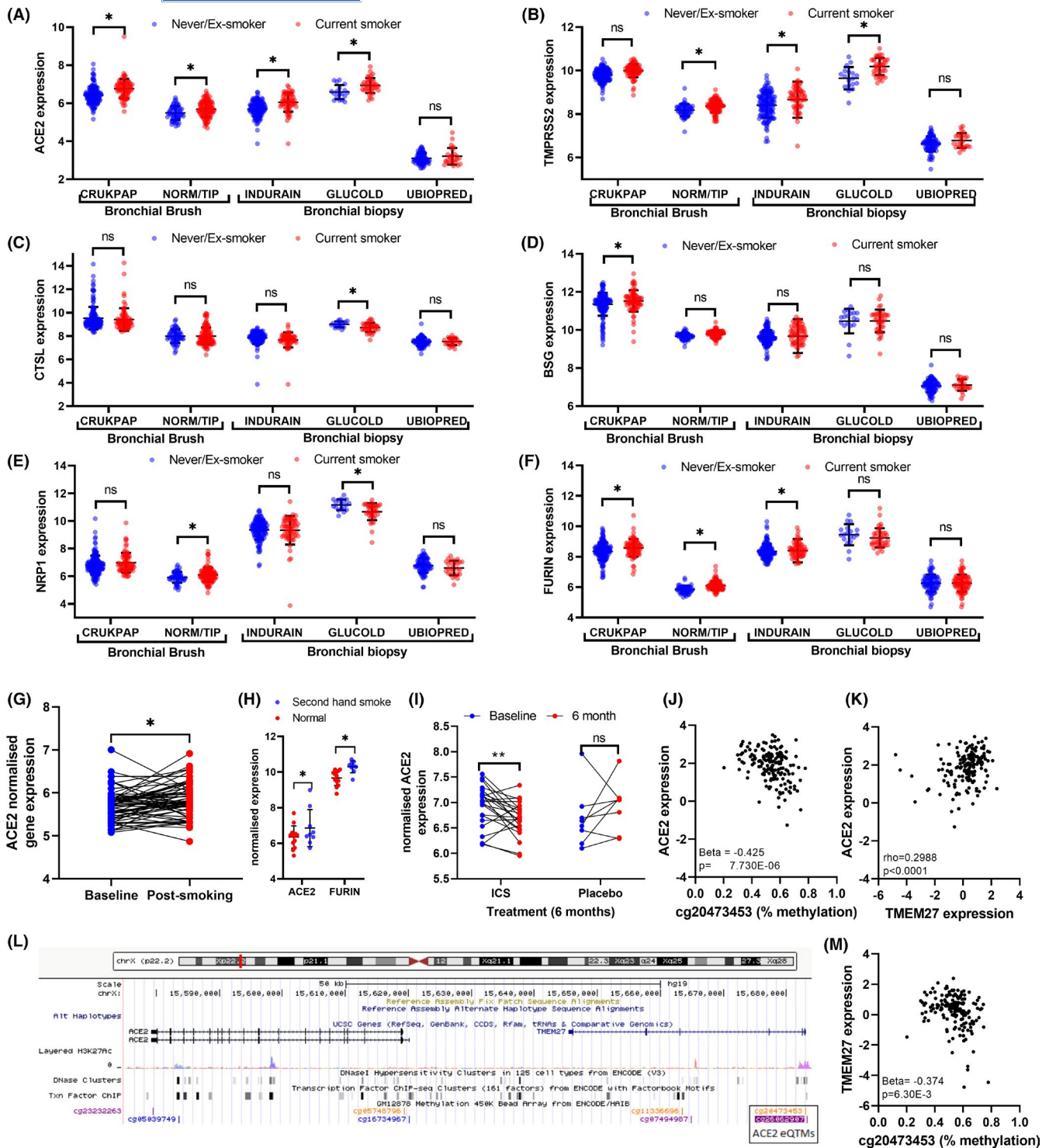





FIGURE 2 Transcriptional response of SARS-CoV-2 cell entry related genes to clinical characteristics, methylation, and environmental stimuli. Expression of (A) ACE2, (B) TMPRSS2, (C) CTSL, (D) BSG, (E) NRP1, and (F) FURIN in bronchial biopsies; INDURAIN ($n = 207$), U-BIOPRED ($n = 108$) and GLUCOLD ($n = 56$) and bronchial brushes CRUKPAP dataset ($n = 228$) and NORM/TIP ($n = 167$), separated based on smoking status. The effect of acute smoke exposure on (G) ACE2 in bronchial brushings 24 h after smoking and not smoking 3 cigarettes. (H) The influence of secondhand smoke in children of SARS-CoV-2 cell entry related in bronchial biopsies. The influence of 6 month ICS and Placebo compared to baseline from bronchial biopsies of COPD patients, (I) ACE2. Top eQTM for (J) ACE2. (K) Correlation of ACE2 and TMEM27 (Spearman's rho correlation was conducted). (L) Diagram of the top CpG site associated with ACE2 expression. (M) EQTM for TMEM27 and the top CpG site associated with ACE2 expression. Statistics was done using an unpaired t-test. * $p < 0.05$ Abbreviations: MI, microarray intensity VST, variance-stabilizing transformation

FUNDING INFORMATION

Chan Zuckerberg Initiative; European Union's H2020 Research and Innovation Program, Grant/Award Number: 874656; Innovative Medicines Initiative Joint, Grant/Award Number: 115010; European Union's Seventh Framework Programme, Grant/Award Number: FP7/2007-2013; Longfonds Junior Fellowship; Helmholtz Association, Grant/Award Number: NIH K08HL146943; Parker B. Francis Fellowship; ATS Foundation/Boehringer Ingelheim Pharmaceuticals Inc. Research Fellowship; Cancer Research UK Cambridge Centre; Cambridge NIHR Biomedical Research Centre; Cambridge Bioresource; The Netherlands Organization for Health Research and Development; The Netherlands Organization for Scientific Research; The Netherlands Lung Foundation, Grant/Award Number: AF 4.1.14.001; The Netherlands Ministry of Spatial Planning, Housing, and the Environment; The Netherlands Ministry of Health, Welfare, and Sport; China Scholarship Council

CONFLICT OF INTEREST

The authors declare that there are no competing interest in relation to this work.

Hananeh Aliee¹
 Florian Massip²
 Cancan Qi^{3,4} 
 Maria Stella de Biase²
 Jos van Nijnatten^{3,5,6}
 Elin T. G. Kersten^{3,4}
 Nazanin Z. Kermani⁷
 Basil Khuder⁸
 Judith M. Vonk^{3,9}
 Roel C. H. Vermeulen^{10,11}
 U-BIOPRED study group
 Cambridge Lung Cancer Early Detection Programme
 INER-Ciencias Mexican Lung Program
 Margaret Neighbors¹²
 Gaik W. Tew¹³
 Michele A. Grimbaldeston¹²
 Nick H. T. ten Hacken⁵
 Sile Hu¹⁴
 Yike Guo⁷
 Xiaoyu Zhang⁷ 
 Kai Sun⁷
 Pieter S. Hiemstra¹⁵
 Bruce A. Ponder^{16,17}
 Mika J. Mäkelä¹⁸
 Kristiina Malmström¹⁸
 Robert C Rintoul^{17,19}
 Paul A. Reyfman⁸ 
 Fabian J. Theis^{1,20}
 Corry-Anke Brandsma^{3,21}
 Ian M. Adcock²²
 Wim Timens^{3,21}
 Cheng-Jian Xu^{23,24,25}
 Maarten van den Berge^{3,5}

Roland F. Schwarz²

Gerard H. Koppelman^{3,4}

M.C. Nawijn^{3,21}

Alen Faiz^{3,5,6}

¹*Institute of Computational Biology, Helmholtz Centre, Munich, Germany*

²*Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany*

³*University of Groningen, University Medical Center Groningen, Groningen Research Institute for Asthma and COPD, Groningen, the Netherlands*

⁴*Department of Pediatric Pulmonology and Pediatric Allergy, University of Groningen, University Medical Center Groningen, Beatrix Children's Hospital, Groningen, the Netherlands*

⁵*Department of Pulmonary Diseases, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands*

⁶*Respiratory Bioinformatics and Molecular Biology (RBMB), School of Life Sciences, University of Technology Sydney, Sydney, New South Wales, Australia*

⁷*Department of computing, Data Science Institute, Imperial College London, London, UK*

⁸*Division of Pulmonary and Critical Care Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA*

⁹*Department of Epidemiology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands*

¹⁰*Julius Global Health, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands*

¹¹*Division of Environmental Epidemiology (EEPI), Institute for Risk Assessment Science (IRAS), Utrecht University, Utrecht, The Netherlands*

¹²*OMNI Biomarker Development, Genentech Inc., South San Francisco, California, USA*

¹³*Product Development Immunology, Infectious Disease & Ophthalmology, Genentech Inc., South San Francisco, California, USA*

¹⁴*Department of statistics, University of Oxford, Oxford, UK*

¹⁵*Department of Pulmonology, Leiden University Medical Center, Leiden, The Netherlands*

¹⁶*Cancer Research UK Cambridge Institute, University of Cambridge, Cambridge, UK*

¹⁷*Department of Oncology, University of Cambridge, Hutchison/MRC Research Centre, Cambridge, UK*

¹⁸*Department of Allergy, University of Helsinki and Helsinki University Hospital, Helsinki, Finland*

¹⁹*Royal Papworth Hospital, Cambridge, UK*

²⁰*Department of Mathematics, Technical University of Munich, Munich, Germany*

²¹Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

²²National Heart and Lung Institute, London, UK

²³Research group Bioinformatics and Computational Genomics, Centre for Individualised Infection Medicine, CiiM, Hannover Medical School, Helmholtz Centre for Infection Research, Hannover, Germany

²⁴Department of Gastroenterology, Hepatology and Endocrinology, TWINCORE, Centre for Experimental and Clinical Infection Research, Hannover Medical School, Helmholtz Centre for Infection Research, Hannover, Germany

²⁵Department of Internal Medicine, Radboud University Medical Center, Nijmegen, The Netherlands

Correspondence

Alen Faiz, School of Life Sciences, Building 4, Room 04.07.418, University of Technology Sydney, Thomas St, Ultimo NSW 2007, Australia.
Email: alen.faiz@uts.edu.au

ORCID

Cancan Qi  <https://orcid.org/0000-0003-3825-5802>

Xiaoyu Zhang  <https://orcid.org/0000-0002-6033-0525>

Paul A. Reyfman  <https://orcid.org/0000-0002-6435-6001>

REFERENCES

1. Hou YJ, Okuda K, Edwards CE, et al. SARS-CoV-2 reverse genetics reveals a variable infection gradient in the respiratory tract. *Cell*. 2020;182(2):429-446.e14.
2. Chen M, Shen W, Rowan NR, et al. Elevated ACE-2 expression in the olfactory neuroepithelium: implications for anosmia and upper respiratory SARS-CoV-2 entry and replication. *Eur Respir J*. 2020;56(3):2001948.
3. Cai G, Bossé Y, Xiao F, et al. Tobacco smoking increases the lung gene expression of ACE2, the receptor of SARS-CoV-2. *Am J Respir Crit Care Med*. 2020;201(12):1557-1559.
4. Muus C, Luecken MD, Eraslan G, et al. Single-cell meta-analysis of SARS-CoV-2 entry genes across tissues and demographics. *Nat Med*. 2021;27(3):546-559.
5. Finney LJ, Glanville N, Farne H, et al. Inhaled corticosteroids down-regulate the SARS-CoV-2 receptor ACE2 in COPD through suppression of type I interferon. *Allergy Clin Immunol*. 2021;147(2):510-519.
6. Peters MC, Sajuthi S, Deford P, et al. COVID-19 related genes in sputum cells in asthma: relationship to demographic features and corticosteroids. *Am J Respir Crit Care Med*. 2020;202(1):83-90.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.