The nasal microbiome modulates risk for SARS-CoV-2 infection

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Summary

Background The nasal microbiome may influence host risk for COVID-19 by modulating the expression of key proteins that facilitate SARS-CoV-2 entry, including angiotensin-converting enzyme 2 (ACE2), which binds the virus, and transmembrane serine protease 2 (TMPRSS2), which activates viral entry into nasal epithelial cells. This study examined whether the expression levels of ACE2 and TMPRSS2 in the nasal cavity predict the risk of SARS-CoV-2 infection and whether the host nasal microbiome modulates their expression.

Methods Using 1548 self-collected nasal swabs from a population-based surveillance testing of community-dwelling adults in Washington D.C., we conducted two retrospective case–control studies (cross-sectional: n = 111 cases and 343 controls; longitudinal: n = 97 cases, 286 controls) and a nasal microbiome study (n = 428). Cases, defined as individuals with a positive SARS-CoV-2 test, were matched with controls based on age and test date. Pre-infection samples were analysed. We measured nasal ACE2/TMPRSS2 expression using RT-qPCR and characterized the nasal microbiome using 16S rRNA gene-based qPCR and sequencing. We used machine learning and regression analysis to determine if nasal ACE2/TMPRSS2 expression predicts SARS-CoV-2 infection and whether the nasal microbiome influences their expression.

Findings Elevated nasal ACE2/TMPRSS2 expression was associated with 3.6-fold increased risk of contracting COVID-19 (95% CI = 1.71–7.47) compared to those with no detectable levels of ACE2 or TMPRSS2. Before testing positive for SARS-CoV-2, cases also had significantly higher and more unstable ACE2/TMPRSS2 expression in their nasal cavity than controls. Having high densities of *Staphylococcus aureus*, *Haemophilus influenzae*, or *Moraxella catarrhalis/nonliquefaciens* was linked to increased nasal ACE2/TMPRSS2 expression. In contrast, having high densities of *Dolosigranulum pigrum* was associated with decreased nasal ACE2/TMPRSS2 expression.

Interpretation These results suggest that natural variation in the nasal microbiome significantly impacts ACE2/TMPRSS2 expression in the nasal cavity and the near-term risk of SARS-CoV-2 infection in adults. Modifying the nasal microbiome could potentially reduce COVID-19 risk.

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Introduction

Since 2020, SARS-CoV-2 has caused more than 775 million infections worldwide.[1] SARS-CoV-2 enters cells by binding to the ACE2 receptor in the respiratory epithelium,[2] facilitated by cleavage of the spike protein by another surface protein, the serine protease TMPRSS2.[3] Given the nasal cavity's frequent exposure to viral particles and higher ACE2 receptor expression in nasal

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Research in context

Evidence before this study

Angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) are well-established as key facilitators of SARS-CoV-2 entry into nasal epithelial cells. To evaluate existing evidence regarding nasal ACE2 and TMPRSS2 expression and risk for SARS-CoV-2 infection in adults, we performed an advanced search in PubMed on May 1, 2024, for published research articles using the search terms "nasal", and "ACE2" or "TMPRSS2", and "COVID-19" or "SARS-CoV-2", with no language or date restrictions. One study found that children with low ACE2 and TMPRSS2 expression had lower risk for SARS-CoV-2 infection in households with confirmed SARS-CoV-2 cases. Another study reported that higher ACE2 expression post-SARS-CoV-2 infection was linked to increased secondary transmission in a hospital setting. However, no studies have investigated whether nasal ACE2 or TMPRSS2 expression affects SARS-CoV-2 infection risk in adults.

To further assess existing evidence on the relationship between nasal microbiome and the nasal levels of ACE2 and TMPRSS2 in adults, we performed a second advanced search for (nasal) AND (microbiome) AND ((ace2) OR (tmprss2)). This search found one study reporting that higher proportional abundance of nasopharyngeal *Corynebacterium* spp. was associated with lower rates of SARS-CoV-2 infection among close contacts of two COVID-19 cases. Several *in vitro* studies examined the effects of *Corynebacterium* spp. and *Staphylococcus* spp. bacterial isolates on ACE2/TMPRSS2 expression in respiratory cells and cell lines. However, no studies have investigated how adult nasal microbiome composition impacts the expression levels of ACE2 or TMPRSS2 in the nasal cavity. Further, although studies have linked the nasal microbiome post-SARS-CoV-2 infection with COVID severity, none have evaluated how the nasal microbiome impacts risk for COVID infection.

Added value of this study

This study is the first to evaluate the relationship between nasal ACE2 and TMPRSS2 expression and COVID-19 risk in adults. We found that elevated levels of these proteins in the nasal cavity predict the likelihood of SARS-CoV-2 infection. Moreover, the nasal expression patterns of ACE2 and TMPRSS2 differ significantly in individuals before they become infected, as compared to those who remain uninfected. Our findings also highlight the nasal microbiome as an important driver of nasal ACE2 and TMPRSS2 expression. While some nasal bacteria such as *Staphylococcus aureus*, *Haemophilus influenzae*, and *Moraxella catarrhalis/nonliquefaciens* are associated with increased nasal ACE2 and TMPRSS2 expression, *Dolosigranulum pigrum*, appears to suppress their expression.

Implications of all the available evidence

Natural variations in nasal microbiome and nasal expression of ACE2 and TMPRSS2 in adults could play a crucial role in determining an individual's risk for SARS-CoV-2 infection. Since the nasal microbiome is not fixed by host genetics and is expected to be modifiable, this could represent a novel opportunity to reduce COVID-19 risk by targeting the nasal microbiome.

epithelial cells compared to distal portions of the respiratory tract,[4] it serves as a key site for viral entry. This is especially relevant for recent variants like Omicron, which exhibit increased infectivity and replication in the upper respiratory tract and in nasal cultures.[5,6] This vulnerability emphasizes the need to understand how factors within the nasal cavity influence SARS-CoV-2 infection risk.

The nasal cavity is home to diverse nasal bacteria that together form distinct nasal community state types (CSTs), each characterized by a different dominant species: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium* species, *Cutibacterium*, Enterobacteriaceae, *Moraxella*, and *Dolosigranulum*.[7] Recent evidence in adults suggest that nasal bacteria could impact COVID-19 severity,[8,9] including a possible protective effect of pneumococcal vaccination against SARS-CoV-2 infection through decreased carriage,[10] but less is known whether the nasal microbiome can impact host susceptibility to SARS-CoV-2 infection. Answering this question requires data that can establish the connection between nasal microbiome composition and risk for future SARS-CoV-2 infection.

A better understanding of the relationship between nasal microbiome and risk for SARS-CoV-2 infection could inform innovative preventative strategies. Such strategies are still needed as the emergence of new SARS-CoV-2 variants present tremendous challenges to the effectiveness of our existing interventions. While SARS-CoV-2 vaccines have been effective in reducing symptomatic and severe disease, they are limited by waning immunity[11] and new variants adept at evading vaccine-elicited antibody response.[12] Likewise, new variants have diminished the efficacy of monoclonal antibody treatments. However, despite the virus' rapid evolution, all SARS-CoV-2 variants continue to infect hosts via ACE2 receptors in the respiratory tract.[2]

The integral roles of nasal ACE2 receptors and TMPRSS2 in SARS-CoV-2 entry suggest their potential as biomarkers for predicting SARS-CoV-2 infection risk and as therapeutic targets for interventions against emerging SARS-CoV-2 variants. Recent research has found sex- and age-associated difference in nasal ACE2 expression that correlate to variations in SARS-CoV-2 infection rates,[13,14] but a direct link between nasal

ACE2 and TMPRSS2 expression levels and future SARS-CoV-2 infection, particularly in adults, is still lacking. Likewise, the nasal microbiome may influence host susceptibility to SARS-CoV-2 by modulating nasal ACE2 and TMPRSS2 expression, but this is little understood.

Here, we analysed self-collected nasal swab samples from a mandatory SARS-CoV-2 testing program to determine if nasal ACE2 and TMPRSS2 expression levels are associated with subsequent near-term SARS-CoV-2 infection, and if the nasal microbiome could modulate nasal ACE2 and TMPRSS2 expression. Using two case–control studies, we investigated whether nasal ACE2 and TMPRSS2 expression and its temporal patterns could predict risk for near-term SARS-CoV-2 infection by comparing individuals who became infected and those who remained uninfected within the following month. Lastly, we determined if and how the nasal microbiome and specific nasal bacteria influence nasal ACE2 and TMPRSS2 expression.

Methods

Study designs

All students, staff, and faculty with any access to campus at the George Washington University (GWU) in Washington, DC, USA underwent mandatory weekly or biweekly COVID-19 surveillance testing using self-collected anterior nasal swabs from August 2020 to July 2022. We identified cases with a positive SARS-CoV-2 PCR test from February 2021 through December 2021 with remnant samples from at least one available prior negative test. Controls were individuals with negative SARS-CoV-2 tests from the same period, matched by test date and age group (18–24 y, 25–34 y, 35–44 y, 45–54 y, 55–89 y). Additional selection criteria for each nested study were as follows.

Cross-sectional case–control study

Associations between nasal ACE2/TMPRSS2 expression levels with SARS-CoV-2 infection risk were assessed using cases with t_{-1} samples (t_{-1} as the test immediately prior to the positive test) and controls matched 1:3 based on case t_{-1} test date and age group.

Longitudinal case–control study

Association between the dynamics of nasal ACE2/TMPRSS2 expression with infection risk were assessed using cases with available samples from up to five tests prior to the positive test (t_{-5} to t_{-1}). Controls were matched 3:1 based on case test date and age group.

Nasal microbiome study

To determine the association between nasal microbiome and nasal ACE2/TMPRSS2 gene expression levels in a cross-sectional study, we included remnant t_{-2} samples from cases (t_{-2} defined as the test immediately prior to the t_{-1} test) and controls tested on t_{-2} or t_{-1}.

Human subject research

This study was approved by the Institutional Review Board at GWU (NCR213729).

Nucleic acid isolations

Nasal swabs used in all three studies were collected into 1 mL of either DNA/RNA shield (R1100-250, Zymo Research) or Viral Transport Media, stored at 4 °C, and processed within 4 h of collection. From the nasal swab eluent, RNA was extracted using MagMAX™-96 Viral RNA Isolation Kit (AM1836, ThermoFisher) with 50 µL final elution volume as described by the GWU COVID-19 RT-PCR Test (EUA202180). Samples underwent DNA extraction for the nasal microbiome study as previously described.[7]

SARS-CoV-2 testing and ACE2/TMPRSS2 gene expression quantification

Using the extracted RNA, SARS-CoV-2 testing was performed as described by EUA202180 and nasal ACE2 and TMPRSS2 expression levels were quantified by TaqMan assays (Hs01085333_m1 and Hs01122322, ThermoFisher) with QuantiTect One-Step RT-PCR Kit (Qiagen). RPS-18 (F-GTTCCAGCATATTTTGCGAGT; R-GTCAATGTCTGCTTTCCTCAAC; Probe-5HEX/TCTTCGGCC/ZEN/CACACCCTTAATGG//3IABkFQ; IDT) was amplified as an internal control using conditions adapted from Boikos et al.[15]

Nasal ACE2/TMPRSS2 expression levels as predictors for SARS-CoV-2 infection

Nasal samples with RPS-18 Cp >38 were excluded. Having ACE2 or TMPRSS2 Cp <38 was considered detectable.

Cross-sectional study

We compared ACE2/TMPRSS2 detection between cases and controls using chi-squared tests and logistic regression. Recognizing the critical roles of ACE2 and TMPRSS2 in facilitating SARS-CoV-2 infection, we aimed to create a combined metric for evaluating infection risk. We utilized decision tree analysis to identify the most informative ACE2 and TMPRSS2 expression attributes for predicting SARS-CoV-2 infection and transformed the identified attributes into three ACE2/TMPRSS2 expression categories. We assessed these categories' association with SARS-CoV-2 infection risk by ordinal logistic regression, adjusting for sex and age. For sensitivity analysis, we excluded case samples within 7 days of a positive test to eliminate cases who potentially had elevated ACE2/TMPRSS2 expression levels due to undetected SARS-CoV-2 infection.

Longitudinal study

To evaluate longitudinal associations between nasal ACE2/TMPRSS2 expression categories with SARS-CoV-2 infection risk, we utilized ACE2/TMPRSS2 expression

data of cases and matched controls from multiple timepoints in a generalized estimating equation model with an independent correlation structure, adjusted for sex. ACE2/TMPRSS2 expression stability was assessed based on Markov transitions across time points and chi-square tests evaluating changes between pairs of time points. Transition patterns between gene expression categories across time were visualized using alluvial plots.

Nasal microbiome characterization

We characterized nasal microbiome by 16S rRNA gene-based sequencing and by a broad-range qPCR (BactQuant)[16] as previously described.[7] Genus-level classification results and amplicon sequence variants (ASVs) for each sample were enumerated to generate a proportional abundance matrix, which was then combined with BactQuant results to generate an absolute abundance matrix. Additional details can be found at https://github.com/araclab/mb_analysis. Additionally, ASVs of indicator genera for known nasal community state types (CSTs) were analysed manually by BLAST and assigned species identification based on published phylogeny.[17] Resultant ASV species assignments and genera comprising >0.025% of total reads were included in the final abundance matrices.

Using the proportional abundance matrix, we assigned nasal CSTs using an iterative approach by hierarchal clustering[7] and identified CST indicators using indicator analysis in the *labdsv* R package (version 1.6–1) with indicator value 0.40 or greater and alpha = 0.10, adjusted for false discovery rate. We visualized the nasal microbiome using heatmaps, boxplots, and stacked barplots.

Nasal microbiome composition and nasal ACE2/TMPRSS2 expression levels

A random forest analysis including all nasal bacteria was performed to identify nasal bacteria attributes associated with ACE2/TMPRSS2 expression categories. After splitting the dataset into training (2/3) and test sets (1/3), we tuned the model using variations of the number of trees, the number of random variables in the tree, and reductions in out-of-bag error rates. Among the 20 most-informative genera or species based on mean-decrease in Gini scores, 13 with a mean proportional abundance ≥1% were selected for further evaluation (which included all but one of the indicator taxa).

To identify informative nasal bacteria attributes for ACE2/TMPRSS2 expression categories, we used absolute abundances of nasal CST indicators in decision tree analysis, including indicator taxa and genera or species identified from the random forest model. Two decision trees were run, using assumptions of expression categories as either categorical or continuous outcomes. Trees were pruned using the complexity parameter of the smallest tree within one standard deviation of the tree with the smallest cross-validation error, and outputs were merged into one set of attributes. A sensitivity analysis was performed using proportional abundance data.

Lastly, we evaluated the association between all indicator taxa and genera and species identified from the random forest analysis and ACE2/TMPRSS2 expression categories using an ordinal logistic regression with backwards selection ($p < 0.6$), adjusted for sex. Thresholds identified from decision tree algorithm were included. If the threshold was not informative based on exclusion using backwards selection in the regression model, we included the absolute abundance of the taxa as a continuous variable unless that was also removed via backwards selection. Model AIC values were utilized to compare model fit. We verified proportional odds assumptions during model validation and confirmed patterns of associations using a multinomial logistic model (results not shown).

All statistical analyses were performed using R (R Core Team, 2018; http://wwwR-project.org/), and SAS (SAS Institute Inc., version 9.4). Graphs were prepared in R. Networks were spatialized in Gephi 0.9.2 using Force Atlas 2.

Role of the funding source

The funders had no role in study design, data collection, data analysis, data interpretation, writing the report, or the decision to submit the report for publication.

Results

Elevated nasal ACE2/TMPRSS2 gene expression predicts risk for SARS-CoV-2 infection

Using remnant samples, participants were included in one or more of three studies: a cross-sectional case–control study (n = 111 cases and 343 controls), a longitudinal case–control study.

(n = 97 cases, 286 controls), and a nasal microbiome study (n = 428) (Table 1) (Fig. 1A).

Among cases, the test immediately before the positive SARS-CoV-2 PCR test (t_{-1}) occurred on average 12 days (SD = 9.3 days) before the positive SARS-CoV-2 test (t_0).

At t_{-1}, we found that cases had higher nasal ACE2 and TMPRSS2 levels than the controls. Specifically, 39.6% of cases had detectable nasal ACE2 expression which was significantly higher as compared to 25.4% of controls (aOR = 1.98, 95% CI = 1.23–3.04) (Fig. 1B). ACE2 density was not significantly higher among cases (Cp mean = 36.4; SD = 1.2) compared with controls (mean = 36.6; SD = 1.1). For TMPRSS2, 91.0% of cases had detectable nasal TMPRSS2 expression as compared to 84.3% of controls (aOR = 1.88, 95% CI = 0.92–3.84). Cases had significantly higher TMPRSS2 density compared with controls (Cp mean = 28.7 vs. 30.3, p = 0.001) (Table S1).

Characteristic	Cross-sectional case–control study (T_{-1})			Longitudinal case–control study ($T_{-1 \text{ to } -5}$)			Microbiome study[a]
	Cases N = 111	Controls N = 343	p-value	Cases N = 97	Controls N = 286	p-value	Overall N = 428
Total samples	111	343		265	829		428
Samples/person, mean (Q1–Q3)	NA	NA		3.0 (2.0–4.0)	3.2 (2.0–4.0)		NA
Sex, n (col %)			0.58			0.65	
Female	67 (60.4)	217 (63.3)		63 (64.9)	193 (67.5)		275 (64.3)
Male	44 (39.6)	126 (36.7)		34 (35.1)	93 (32.5)		153 (35.8)
Mean age (SE)	24.0 (0.8)	24.2 (0.5)	0.70	23.7 (0.8)	24.1 (0.6)	0.44	24.0 (0.4)
Test date of samples, n (col %)			0.70			0.12	
Spring	0	0		39 (11.1)	80 (7.7)		0
Summer	23 (20.7)	72 (21.0)		93 (26.6)	315 (30.3)		76 (17.8)
Fall	87 (78.4)	270 (78.7)		202 (57.7)	611 (58.8)		350 (81.8)
Winter	1 (0.9)	1 (0.3)		16 (4.6)	34 (3.3)		2 (0.5)

NA, Not applicable; SE, Standard error. [a]Controls with T-1 or T-2 samples and cases with T-2 samples with gene expression and microbiome data.

Table 1: **Participant demographics and sample characteristics from our three studies.**

We identified three nasal ACE2/TMPRSS2 gene expression thresholds—low (ACE2 and TMPRSS2 not detected), medium (ACE2 not detected and TMPRSS2 Cp >32), and high (ACE2 detected or TMPRSS2 Cp ≤ 32)—that best predicted SARS-CoV-2 infection risk (Fig. 1C, Table 2). Among cases, the medium category was the most common (48.7%), followed by high category (41.4%), while the low category was uncommon (9.9%). In contrast, while the medium category was also the most common among controls (52.2%), there were relatively fewer controls with high category (26.5%) and more with the low expression category (21.3%) (Table 2). Overall, females were more likely to be in the medium or high gene expression categories compared with males (85.9% vs. 74.1%, p = 0.003); however, among cases there were no differences in gene expression category by sex (91.0% vs. 88.6%, p = 0.44).

Having the high expression category at t_{-1} was associated with increased risk for subsequent SARS-CoV-2 infection (aOR = 3.58, 95% CI = 1.71–7.47) (Table 2). Males tended to have a greater risk increase with high expression (aOR = 4.67, 95% CI = 1.51–14.46) compared with females (aOR = 2.70, 95% CI = 1.02–7.12), although interaction by sex was not statistically significant (p = 0.34). The medium expression category at t_{-1} was also associated with increased risk for SARS-CoV-2 infection in the overall population (aOR = 2.10, 95% CI = 1.03–4.26) (Table 2). Expression categories were not significantly different by age (p = 0.33) (Table S3).

To test potential confounding between undetected SARS-CoV-2 infection and elevated nasal ACE2 expression, we conducted a sensitivity analysis excluding cases who were ≤7 days from their positive COVID-19 test; findings were consistent with the primary analysis (Table S2A).

Longitudinal nasal ACE2/TMPRSS2 expression patterns also predict risk for SARS-CoV-2 infection

In a longitudinal study of 97 cases and 286 matched controls at up to five timepoints (t_{-5} to t_{-1}) (Table 1), cases also had distinct longitudinal nasal ACE2/TMPRSS2 gene expression patterns prior to SARS-CoV-2 infection. Cases had less stable ACE2/TMPRSS2 expression and were more likely to switch between expression categories across any pair of sequential time points when compared with controls (54.4% vs. 45.8%, p = 0.029). When evaluating the specific transitions, cases had higher Markov transition frequencies into the high gene expression category from low (0.28 vs. 0.17) and medium (0.31 vs. 0.25), and were less stable within the low category (0.26 vs. 0.34) (Fig. S1). At t_{-2} to t_{-1}, cases were more likely to shift toward higher nasal ACE2/TMPRSS2 expression category—from low to medium or high, or from medium to high—than controls (42.6% vs. 22.0%, p = 0.002) (Fig. 1D). Cases were more likely to have persistently high expression categories across timepoints (t_{-5} to t_{-1}) leading up to the infection (aOR = 1.62, p = 0.016) (Fig. 1D, Table S2B).

Absolute abundances of four nasal taxa correlate with nasal ACE2/TMPRSS2 expression in a threshold-dependent manner

Next, we characterized the nasal microbiome and nasal ACE2/TMPRSS2 expression of 428 individuals to determine if nasal microbiome can influence host SARS-CoV-2 infection susceptibility through their modulations (Table 1). Among participants, 22.0% belonged to the low expression category, 51.0% belonged to medium, and 27.0% belonged to high, similar to the case–control studies (Fig. 2B). We detected six of the seven previously identified nasal CSTs. While Enterobacteriaceae-dominated CST (CST2) was absent, we detected a new nasal CST dominated by

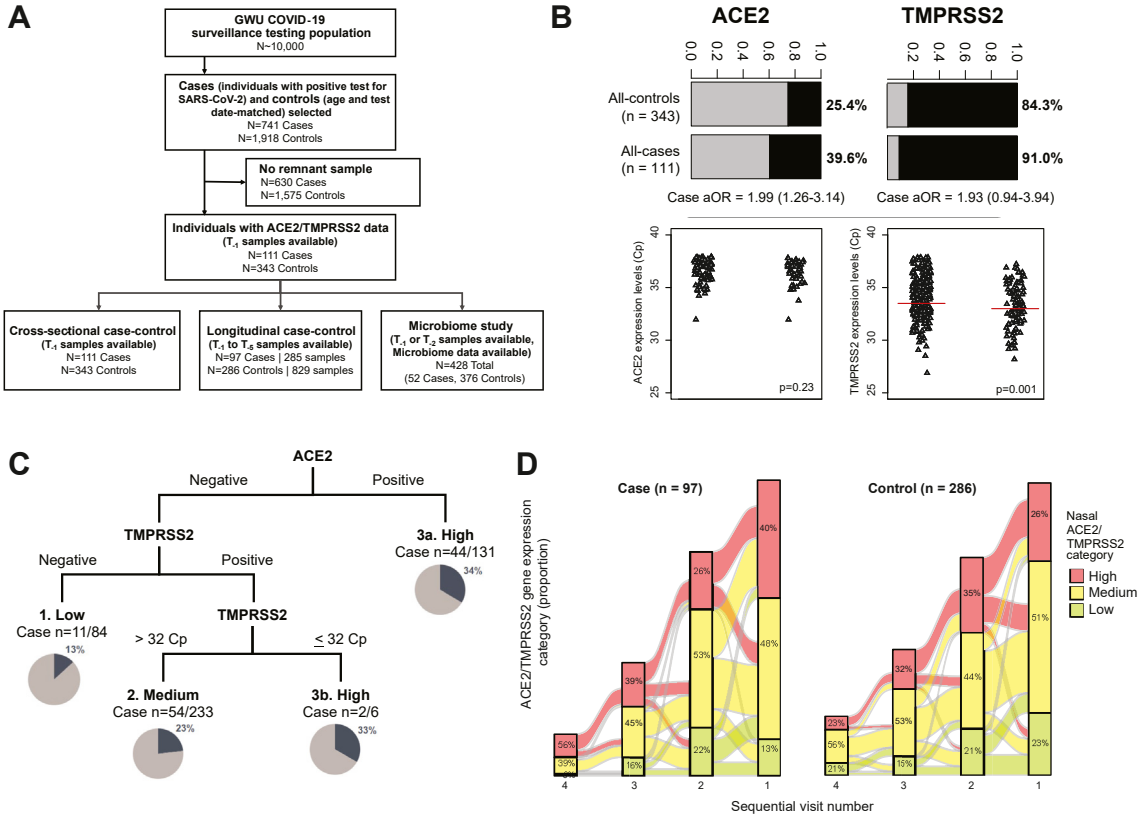


***Fig. 1:* A–D. Summary of samples analysed in our three studies and nasal ACE2 and TMPRSS2 gene expression in cases and controls**. A: Flowchart illustrating sample selection in our three studies. B. Nasal ACE2 and TMPRSS2 gene expression in the cross-sectional case–control study, showing ACE2 detection rates (top) and TMPRSS2 expression levels (Cp-values) (bottom). Cp-values are inversely correlated with expression levels, where higher Cp-values correlate with lower expression levels and vice versa. C. Informative nasal ACE2/TMPRSS2 expression categories for COVID-19 risk. Decision tree establishing the informative thresholds for ACE2 and TMPRSS2 expression levels for distinguishing COVID-19 cases from controls. The decision tree shows that individuals with detectable ACE2 are more likely to become cases, with 34% (n = 44/131) becoming COVID-19 positive in the next surveillance test. In contrast, individuals with no detectable ACE2 or TMPRSS2 have the lowest risk of becoming COVID-19 cases, with only 13% becoming COVID-19 positive in the next surveillance test. Among individuals negative for ACE2 but TMPRSS2-positive, higher TMPRSS2 expression correlated with higher risk of becoming COVID-19 cases (33%), compared to lower TMPRSS2 expression (23%). D. Dynamics of nasal ACE2/TMPRSS2 gene expression categories across sequential visits by case/control status. Alluvial diagram illustrating transitions between ACE2 and TMPRSS2 gene expression categories across sequential time points (up to 5 sequential visits prior to positive COVID-19 test of cases or age- and date-matched controls). Time points with missing gene expression data were excluded for visualization, and available longitudinal visits are plotted as sequential visits. Cases spent more time in medium and high ACE2/TMPRSS2 categories over time (generalized estimating equation model p = 0.016, p < 0.001, respectively) and were less stable over time, with 54% of cases shifting between categories over time, compared with 46% of controls (p = 0.029).

Haemophilus influenzae (CST8) (Fig. 2A, Table S4). *Corynebacterium*-dominated CST (CST5) was the most prevalent (36.9%), followed by *Cutibacterium*-dominated CST (CST4) (18.2%), *Staphylococcus epidermidis*-dominated CST (CST3) (15.9%), *Dolosigranulum*-dominated CST (CST7) (12.1%), and *S. aureus*-dominated CST (CST1) (11.4%). *H. influenza* -dominated CST (CST8) (4.2%) and *Moraxella catarrhalis/nonliquefaciens*-dominated CST (CST6) (1.2%) were the least common CSTs (Fig. 2C).

All nasal CSTs were present in each of the three nasal ACE2/TMPRSS2 categories, except for CST6, which was not detected in the low expression category (Fig. 2C). However, the CSTs did not differ significantly across nasal expression categories (χ^2 p = 0.58).

As nasal CSTs represent the overall microbiome composition, we investigated whether other nasal microbiome attributes, such as absolute and proportional abundance of nasal bacteria, influence host susceptibility to SARS-CoV-2 infection. Using random forest analysis, we identified 13 taxa that predicted nasal ACE2/TMPRSS2 expression, including seven of the eight nasal CST indicators (Table S5). Further investigation showed that four species—*D. pigrum*,

Nasal ACE2/TMPRSS2 expression categories[b]	Overall (n = 454)	Cases (n = 111)	Control (n = 343)	Logistic regression	
	N (%)	n (%)	n (%)	aOR (95% CI)[a]	p-value
All					**0.002**
High	137 (30.2)	46 (41.4)	91 (26.5)	3.58 (1.71–7.47)	
Medium	233 (51.3)	54 (48.7)	179 (52.2)	2.10 (1.03–4.26)	
Low	84 (18.5)	11 (9.9)	73 (21.3)	ref	
Female					**0.045**
High	97 (34.2)	31 (46.3)	66 (30.4)	2.70 (1.02–7.12)	
Medium	147 (51.8)	30 (44.8)	117 (53.9)	1.47 (0.56–3.82)	
Low	40 (14.1)	6 (9.0)	34 (15.7)	ref	
Male					**0.027**
High	40 (23.5)	15 (34.1)	25 (19.8)	4.67 (1.51–14.46)	
Medium	86 (50.6)	24 (54.6)	62 (49.2)	3.11 (1.09–8.86)	
Low	44 (25.9)	5 (11.4)	39 (31.0)	ref	

aOR, adjusted odds ratio; CI, confidence intervals; ACE2, angiotensin-converting enzyme 2; TMPRSS2, transmembrane serine protease 2. Bold values indicate statistical significance at the $p < 0.05$ level. [a]Adjusted for age. [b]High, TMPRSS2 ≤32 and/or ACE2 detected; Medium, TMPRSS2 Cp 32–37 & ACE2 not detected; Low, TMPRSS2 & ACE2 not detected.

Table 2: **Association between COVID-19 case status and nasal TMPRSS2/ACE2 expression categories at T_{-1}, adjusted for age.**

M. catarrhalis/nonliquefaciens, *H. influenzae*, and *S. aureus*—significantly affected nasal ACE2/TMPRSS2 gene expression when their colonizing abundances reached specific thresholds (Tables S6 and S7).

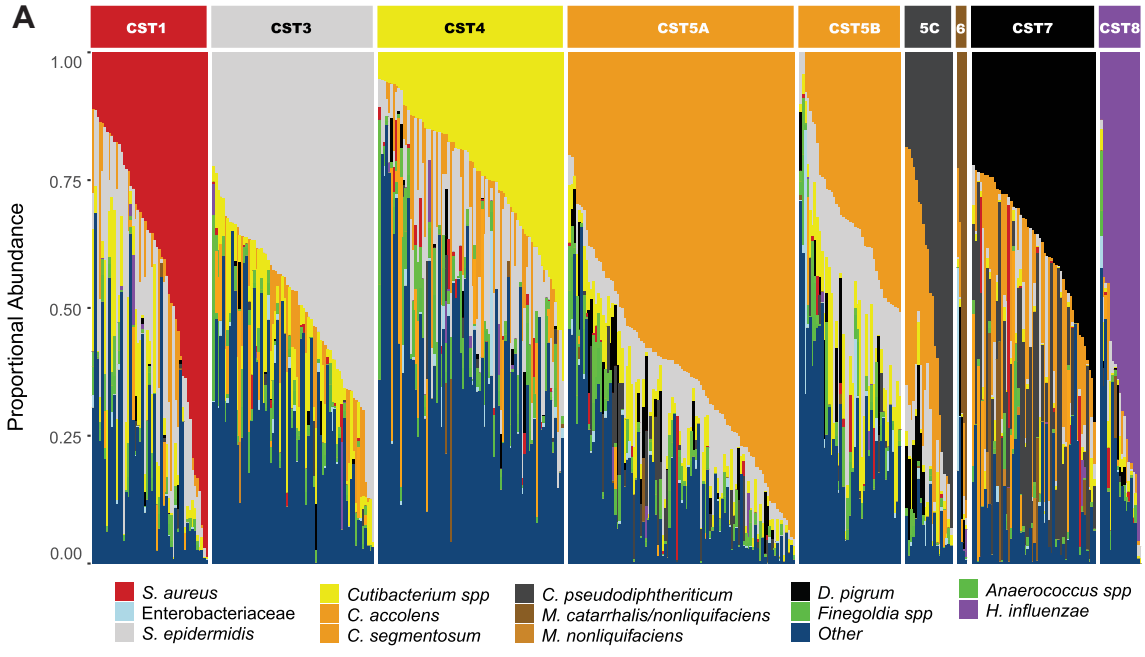
D. pigrum was the only nasal bacteria species for which high absolute abundance was linked to reduced nasal ACE2/TMPRSS2 expression (aOR = 0.57; 95% CI = 0.30–1.11; p = 0.10) (Fig. 3A). In contrast, having *S. aureus*, *M. catarrhalis/nonliquefaciens*, and *H. influenzae* above specific absolute abundance thresholds was associated with elevated nasal ACE2/TMPRSS2 expression and thus increased risk for SARS-CoV-2 infection. Specifically, if *M. catarrhalis/nonliquefaciens* absolute abundance reached 6.3×10^4 16S rRNA gene copies per swab, there were 7.4-fold higher odds of elevated nasal ACE2/TMPRSS2 expression ($p < 0.001$), while if *H. influenzae* absolute abundance reached 5.0×10^5, there were 4.7-fold higher odds of elevated ACE2/TMPRSS2 expression (p = 0.01) (Fig. 3A). In contrast, *S. aureus* showed two distinct patterns; when compared to individuals with low or no *S. aureus*, those with *S. aureus* absolute abundance ranging from 3.2×10^3 to 7.9×10^5 16S rRNA gene copies per swab had nearly double the odds of having elevated nasal ACE2/TMPRSS2 expression (aOR = 1.9; 95% CI = 1.2–3.0; p = 0.011). However, although not statistically significant, having 7.9×10^5 or more *S. aureus* 16S rRNA gene copies per swab was associated with reduced nasal ACE2/TMPRSS2 expression (Table S6).

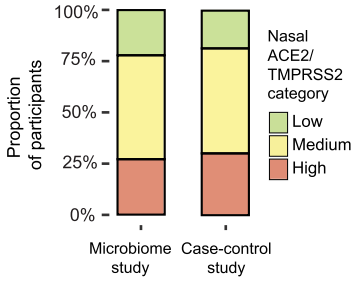
Further analysis indicated that a high absolute abundance of *D. pigrum* may uniquely reduce nasal ACE2/TMPRSS2 expression (aOR = 0.6; 95% CI = 0.3–1.1; p = 0.10), whereas a high proportional abundance of *D. pigrum* had limited effect (aOR = 0.9; 95% CI = 0.7–1.3; p = 0.60). In contrast, having *S. aureus*, *M. catarrhalis/nonliquefaciens*, or *H. influenzae* above specific proportional abundance thresholds also impacted nasal ACE2/TMPRSS2 expression (Table S7). These findings suggest that the impact of *D. pigrum* on host susceptibility to SARS-CoV-2 infection is likely driven by its absolute abundance rather than its proportional abundance relative to other bacteria. The overall patterns for these four taxa were generally consistent when evaluating any ACE2 detection and TMPRSS2 gene expression as independent outcomes (Tables S8 and S9).

A substantial portion of individuals with nasal CSTs defined by *M. catarrhalis/nonliquefaciens* (CST6), *H. influenzae* (CST8), and *S. aureus* (CST1) have the density attributes associated with elevated SARS-CoV-2 infection risk. Specifically, all individuals with CST6 (n = 5/5), 61.1% of individuals with CST8 (n = 11/18), and 75.5% of individuals with CST1 (n = 37/49) have these density attributes. Similarly, the majority (82.7%) of individuals with CST7 (n = 43/52) have the high-density *D. pigrum* attribute that may reduce SARS-CoV-2 infection risk (Fig. 3B).

Individuals who do not have CST6, CST8, or CST1 may also have density attributes associated with elevated risk for SARS-CoV-2 infection, but this is less common. Specifically, 2.8% of those without CST6 (n = 12/423), 0.2% without CST8 (n = 1/410), and 13.5% without CST1 (n = 51/379) have the high-risk density attributes associated with *M. catarrhalis/nonliquefaciens*, *H. influenzae*, and *S. aureus*, respectively, and 4% (n = 15/361) of individuals without CST7 have the high-density *D. pigrum* attribute that may reduce SARS-CoV-2 infection risk.

While *S. aureus* was associated with relatively lower odds of elevated nasal ACE2/TMPRSS2 expression (1.9-fold higher) than *M. catarrhalis/nonliquefaciens* (7.4-fold





C

Nasal CST	N	Nasal Gene Expression Category N (%)		
		Low	Medium	High
CST1	49	11 (22.4)	20 (40.8)	18 (36.7)
CST3	68	12 (17.6)	30 (55.9)	18 (26.5)
CST4	78	20 (25.6)	36 (46.2)	22 (28.2)
CST5A	95	22 (23.2)	49 (51.6)	24 (25.3)
CST5B	43	14 (32.6)	21 (48.8)	8 (18.6)
CST5C	20	2 (10.0)	12 (60.0)	6 (30.0)
CST6	5	0 (0.0)	4 (80.0)	1 (20.0)
CST7	52	11 (21.2)	29 (55.8)	12 (23.0)
CST8	18	2 (11.1)	9 (50.0)	7 (38.9)

***Fig. 2:* A–C. Nasal microbiome composition and nasal ACE2/TMPRSS2 expression**. A. Nasal microbiome composition across detected nasal community state types (CSTs). Waterfall plot showing the proportional abundance of nasal taxa that comprise nasal microbiome of 428 participants. Each nasal taxon is represented by a colour specified in the colour legend. Seven CSTs were detected in our study, including a new *H. influenzae*-dominated CST (CST8). However, a previously identified CST—Enterobacteriaceae-dominated CST2—was not detected in our study. B. Prevalence of nasal ACE2/TMPRSS2 gene expression categories in each study. C. Nasal CSTs prevalence across nasal ACE2/TMPRSS2 gene expression categories.

higher) or *H. influenzae* (4.7-fold higher), the high-risk *S. aureus* nasal microbiome attributes are much more common. High-risk *S. aureus* absolute abundance was detected in 20.6% of our study population (n = 88), as compared to high-risk *M. catarrhalis/nonliquefaciens* (n = 17, 3.2%) or high-risk *H. influenzae* (n = 12, 2.8%) absolute abundance.

Discussion

In this study, we demonstrated that the combined expression of nasal ACE2 and TMPRSS2 was a significant and a strong predictor for future SARS-CoV-2 infection in adults. We also establish the first connection between the nasal microbiome and susceptibility to SARS-CoV-2 infection in adults. To our knowledge, our study is the first to report the predictive value of nasal ACE2/TMPRSS2 expression for future SARS-CoV-2 infection.

Our data showed that higher absolute abundance of *D. pigrum* reduces nasal ACE2/TMPRSS2 expression, likely providing protection against SARS-CoV-2 infection. Earlier research has also linked nasal microbiome dominated by *Corynebacterium* and *Dolosigranulum* to

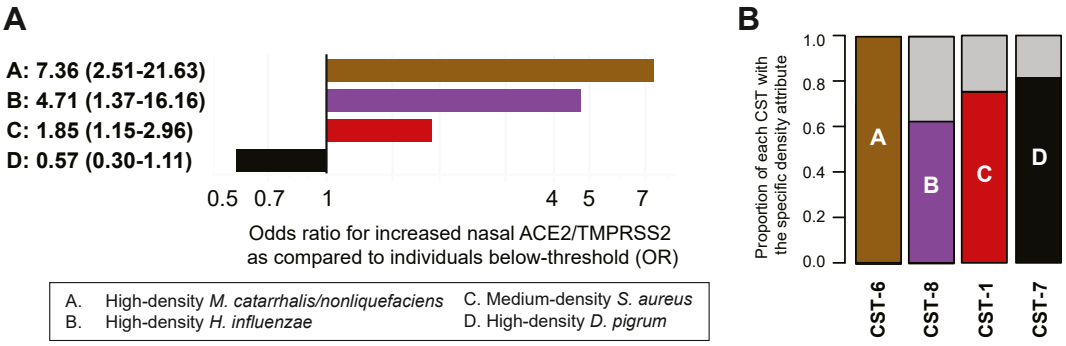


***Fig. 3:* A and B. Associations between nasal microbiome composition and nasal ACE2/TMPRSS2 expression**. A. Odds ratio (OR) for increased nasal ACE2/TMPRSS2 expression with each density attribute, as compared to individuals below threshold. Odds ratio (OR) for increased nasal ACE2/TMPRSS2 expression with high-density absolute abundance of *Moraxella catarrhalis/nonliquenfaciens*, *Haemophilus influenzae*, *S. aureus*, and *D. pigrum*, as compared to individuals below threshold. aOR calculated using an ordinal logistic regression, adjusted for sex and age. B. Proportion of individuals with each nasal community state type (CST) exhibiting specific density attributes. Proportion of individuals with CST6, CST8, CST1, and CST7 exhibiting specific density attributes for each CST (CST6: High-density *M. catarrhalis/nonliquenfaciens*; CST8: High-density *H. influenzae*; CST1: Medium-density *S. aureus*; CST7: High-density *D. pigrum*).

milder COVID-19 symptoms in adults.[8,9] In contrast to the potential protective effect of *D. pigrum*, we found that higher abundances of *S. aureus*, *M. catarrhalis/nonliquefaciens*, and *H. influenzae* were associated with elevated nasal ACE2 and TMPRSS2 expression, indicating an increased risk for SARS-CoV-2 infection. These findings are consistent with earlier studies that linked *Staphylococcus*-dominated nasal microbiome profiles to more severe COVID-19 symptoms in adults,[8] while *Haemophilus* and *Moraxella* are established risk factors for acute respiratory viral infections in children.[18]

Potential explanations for the links between nasal microbiome and SARS-CoV-2 infection risk observed in this study include bacterial competition and microbiome's effects on the nasal immune environment. Several studies have shown a strong inverse relationship between *D. pigrum* and *S. aureus*,[7] supported by *in vitro* studies demonstrating *D. pigrum's* ability to suppress *S. aureus* growth. This suggests that the ability of *D. pigrum* to reduce nasal ACE2 and TMPRSS2 expression could involve inhibiting *S. aureus*, and potentially *M. catarrhalis/nonliquefaciens* and *H. influenzae*. Furthermore, certain nasal bacteria species have been shown to impact nasal innate immune responses, including interferon production.[19,20] Since ACE2 is an interferon-stimulated gene in the respiratory epithelium, the nasal microbiome could feasibly modulate nasal ACE2 expression, potentially increasing or decreasing its levels.[21]

Our study identified notable sex differences in nasal ACE2/TMPRSS2 expression and associated risks for future SARS-CoV-2 infection. While women generally have higher levels of nasal ACE2 and TMPRSS2 and constituted the majority (60.4%) of the cases in the study, men with elevated nasal ACE2/TMPRSS2 expression tended to have higher odds of infection. This suggests that nasal ACE2/TMPRSS2 expression may present a greater risk for COVID-19 in men. ACE2 expression could be expected to be higher in women due to its escape of X-chromosome inactivation; however, oestrogen has been shown to down-regulate ACE2 expression.[22,23] Further, previous studies have reported conflicting findings on sex-based differences in ACE2/TMPRSS2 expression and their association with SARS-CoV-2 infection risk or disease severity.[24–26] While mortality and disease severity are unequivocally higher in men than women, epidemiological data have been more heterogeneous by setting.[27]

There are several study limitations. Although we curated a unique longitudinal sample collection from a university surveillance testing program, there were limited demographic, clinical, and environmental exposure data available. Therefore, we could not adjust for confounders such as smoking status, which may impact nasal microbiome profiles, ACE2 and TMPRSS2 expression, and SARS-CoV-2 susceptibility.[28,29] Further, the study sampled from the GWU population, which may limit generalizability. Sample sizes were limited by availability of remnant samples, which may preclude findings of statistical significance for some associations. Also, our findings from the nasal cavity may not generalize to other body sites such as the lung. Future studies, including mechanistic studies and larger, more diverse cohorts with extensive metadata are needed. SARS-CoV-2 variants may vary in their tropism for utilizing ACE2 and the TMPRSS2 pathway.[30,31] For example, the Omicron variant appears to have reduced reliance on the TMPRSS2 pathway.[30] However, Omicron displays enhanced infectivity and replication in the upper respiratory tract and in nasal epithelial cells, potentially attributable to increased ACE2 binding affinity and the higher density of ACE2 receptors in the

upper respiratory tract.[5,6,31] Study findings remained consistent when assessing ACE2 independently of TMPRSS2, supporting generalizability of our findings. Furthermore, our study period included a transitional period between multiple variants (e.g. Beta, Gamma, Delta, and Omicron), suggesting that the findings may apply across several variants.

In conclusion, while post-infection ACE2 and TMPRSS2 expression in the upper respiratory tract has been linked to increased disease severity among adults,[32] and while higher post-infection ACE2 expression has been linked to increased secondary transmission among adults,[33] and risk of infection in children,[13,14] our study demonstrates that adults with higher nasal ACE2 and TMPRSS2 expression had markedly higher risk of contracting COVID-19 in the near-term. Moreover, our findings revealed specific attributes of the nasal microbiome that may modulate nasal ACE2 and TMPRSS2 expression and consequently impact an individual's risk for SARS-CoV-2 infection. Understanding how the nasal microbiome impacts viral entry receptors and co-factors levels and SARS-CoV-2 infection could inform new microbiome-driven strategies to prevent or treat SARS-CoV-2 infections.

Contributors

Conceptualization, CML; Methodology, DEP, BAH, CML; Investigation, JES, TP, SGN, and JV; Writing — Original Draft, DEP and CML.; Writing — Review & Editing, DEP, BAH, CML, LBP, MA, JES, TP, SGN, JV, and NOW; Funding Acquisition, CML and LBP; Resources, CML, JV; Formal Analysis, DEP and CML; Data Curation, DEP and MA; Data Visualization, DEP, CML, LBP, and NOW; Supervision, CML and BAH; Data verification, DEP and CML. All authors had full access to all the data in the study and accept responsibility for the decision to submit for publication.

Data sharing statement

Requests for access to study protocols, deidentified participant data, including individual clinical or laboratory-generated data that underlie the results reported in this article, are available from the corresponding authors on reasonable request. Our microbiome analysis pipeline is available at https://github.com/araclab/mb_analysis. All other data and code are accessible upon request by emailing the corresponding authors.

Declaration of interests

CML is a shareholder and a scientific advisor of Trench Therapeutics. LBP has received research funding from Trench Therapeutics. DEP, MA, NOW, TP, LBP, and CML have a patent application related to the subject matter of the contribution.

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During the preparation of this work the authors used ChatGPT 3.5 to edit the text for clarity. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2025.105660.

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