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Original Article

Longitudinal effects of elexacaftor/tezacaftor/ivacaftor on the oropharyngeal metagenome in adolescents with cystic fibrosis

Ruth Steinberg [a,b,c], Alexander Moeller [d], Amanda Gisler [c], Nadja Mostacci [e], Markus Hilty [e,1], Jakob Usemann [c,d,1,*]

[a] Division of Paediatric Respiratory Medicine and Allergology, Department of Paediatrics, Inselspital, Bern University Hospital, University of Bern–Bern Switzerland
[b] Graduate School for Cellular and Biomedical Sciences, University of Bern–Bern Switzerland
[c] University Children's Hospital Basel UKBB Switzerland
[d] Department of Respiratory Medicine, University Children's Hospital Zurich, University of Zurich–Zurich Switzerland
[e] Institute for Infectious Diseases, University of Bern–Bern, Switzerland

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ABSTRACT

Background: Triple modulator therapy elexacaftor/tezacaftor/ivacaftor (ETI) improves lung function and impacts upon the respiratory microbiome in people with Cystic fibrosis (pwCF) with advanced lung disease. However, adolescents with cystic fibrosis (CF) are less colonized with bacterial pathogens than adult pwCF but their microbiota already differs from healthy individuals. The aim of this study was to longitudinally analyze the impact of ETI on the respiratory metagenome in adolescents with predominantly mild CF lung disease.
Methods: In this prospective observational study, we included pwCF aged 12–20 years with at least one F508del mutation, who collected oropharyngeal swabs before and after initiation of ETI therapy twice per week to biweekly over three months. We performed whole metagenome shotgun sequencing, followed by host DNA filtering and taxonomic profiling. We used linear and additive mixed effects models adjusted for known confounders and corrected for multiple testing to study longitudinal development of the microbiome. We analyzed bacterial diversity, abundance, and strain-level phylogeny.
Results: We analyzed the metagenomic data of 297 swabs of 20 pwCF. Microbiome composition changed after initiation of ETI therapy. We observed a slight diversification of the microbiome over time (Inv Simpson, Coef 0.085, 95 %CI 0.003, 0.17, $p = 0.04$). Strain-level analysis and clustering showed that strain retention of the most frequent bacterial species is predominant even during ETI therapy.
Conclusions: During three months of ETI therapy, commensal bacteria increased, which may help to prevent overgrowth of bacterial pathogens.

1. Introduction

People with cystic fibrosis (pwCF) with dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) have reduced airway mucus clearance leading to colonization with opportunistic bacteria including *Pseudomonas aeruginosa, Staphylococcus aureus* and *Haemophilus influenzae* already early in life [1]. Repetitive lower respiratory airway infections with bacterial pathogens are the main drivers for CF lung disease progression [1] and contribute substantially to patient morbidity and mortality [2]. Culture-independent methods show that, even in the absence of typical pathogens, the airway microbiome is disordered in pwCF and different microbiome alterations, including decreased diversity and overall high bacterial load, are associated with pulmonary exacerbations and lung function decline [3].

The triple combination CFTR modulator elexacaftor/tezacaftor/ivacaftor (ETI) restoring CTFR function was approved in Switzerland for pwCF ≥12 years of age and with at least one copy of a F508del mutation in September 2021. ETI therapy led to significant improvement in lung function and markedly reduced pulmonary exacerbations and antibiotic therapy in pwCF [4-7].

Several studies aimed to better understand how ETI affects mucus composition and bacterial colonization in the lung of pwCF [8-12].

* Corresponding author at: University Children's Hospital Zurich, Steinwiesstrasse 75, CH-8032 Zürich, Switzerland.
E-mail address: Jakob.usemann@ukbb.ch (J. Usemann).
[1] Authors contributed equally.

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Treatment with ETI remarkably reduces sputum amount and lowers sputum viscoelasticity in pwCF [10]. Best to our knowledge, the impact of ETI on the lung microbiome has been investigated in 6 studies [10-15]. These studies focused on adults, mainly including pwCF with advanced lung disease [10,11,13,15], and two including adults and adolescents with less severe lung impairment [12,14]. In these studies, during six to twelve months of observation, ETI treatment was associated with increased microbiome diversity and evenness, as well as more bacterial commensals [10,14,15]. In contrast, reduction of overall bacterial load or eradication of opportunistic pathogens such as *Pseudomonas aeruginosa* was only observed in some individuals, but was not found to be a general trend [10-13,15].

A comprehensive longitudinal assessment with frequent sampling is crucial to control for individual variation and the effects of environmental factors [16]. Previous studies assessed the impact of ETI on the lung microbiome using observation times of several months [10-15]. However, early temporal changes of the lung microbiome after ETI therapy have not been studied. It would be of particular interest to understand how ETI treatment modifies the lung microbiome in milder CF lung disease, which has scarcely been studied.

We collected weekly deep throat swabs in pwCF aged 12–20 years between one month before and three months after initiation of ETI treatment to assess in detail the short-term temporal dynamics of the effects of ETI therapy on the airway microbiome. We performed shotgun metagenomic sequencing and applied general and linear mixed models and developments for *in silico* analysis, hypothesizing that even in mild CF diseases, ETI impacts the diversity and composition of the bacterial metagenome.

2. Methods

Additional methods information is available in the supplementary material.

2.1. Study design and participants

In this prospective single center observational study, we included adolescents with CF aged 12–20 years before start of ETI therapy between March 2021 and August 2021. We included pwCF with at least one F508del mutation who were either naïve for CFTR modulator therapy or treated previously with a dual modulator.

2.2. Study visits and collection of oropharyngeal swabs

Parents of study participants were trained to perform standardized oropharyngeal (OP) swabs, which were stored in the refrigerator until they were brought to the study center and until then kept at −80 °C before analysis. For each participant at least two swabs before ETI

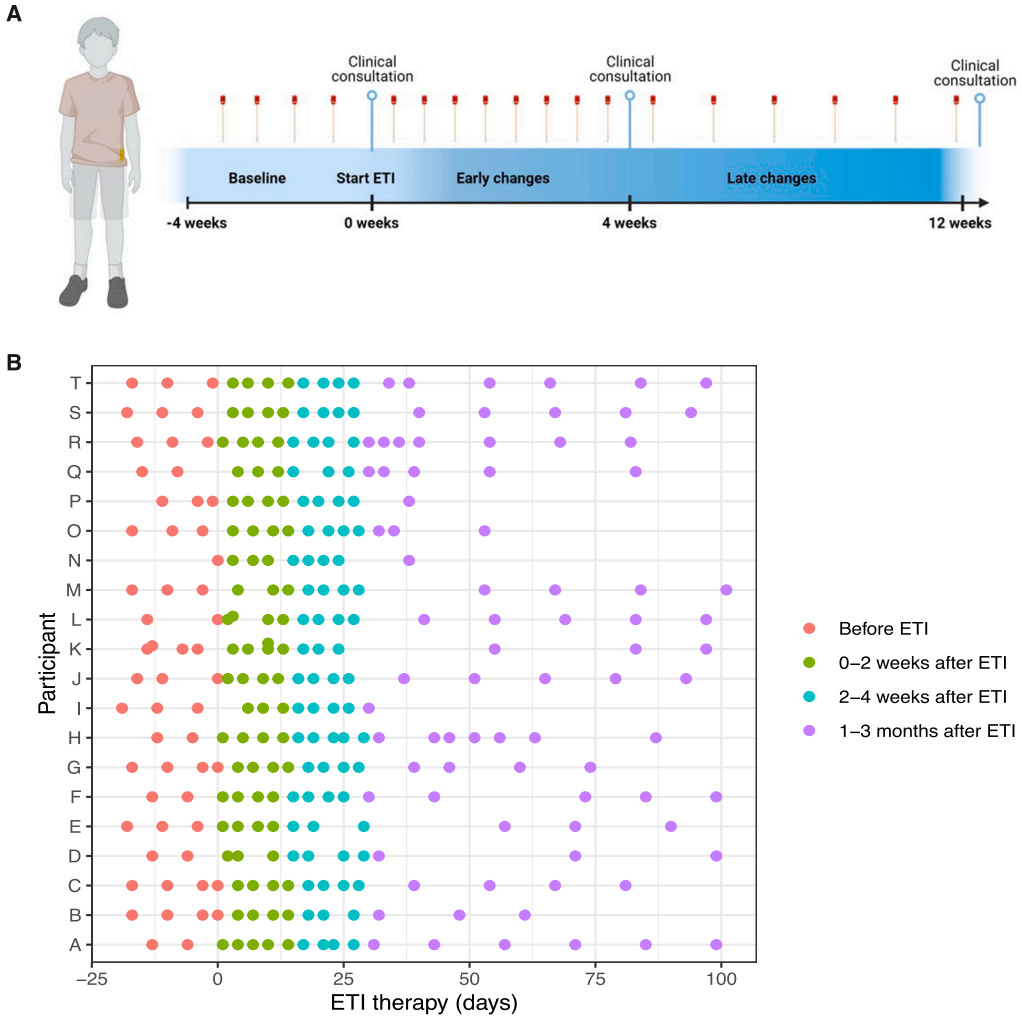

Fig. 1. Study timeline and collected swabs. (A) We collected at least two swabs before ETI therapy start. In the first four weeks of ETI therapy, two swabs per week were taken, and from four to twelve weeks the frequency was reduced to biweekly. Participants visited the outpatient clinic for consultations at start, 4 weeks, and 12 weeks after ETI therapy. (B) shows an overview of the swabs collected (points). The different phases of the study are shown with colors. The duration of ETI therapy is displayed on the x-axis, and each row of the y-axis shows one study participant. ETI: Triple modulator therapy elexacaftor/tezacaftor/ivacaftor.

therapy start were collected. In the first four weeks of ETI therapy, two swabs per week were taken, and from four to twelve weeks the frequency was reduced to biweekly (Fig. 1). Study participants attended the outpatient clinics at the start of ETI therapy, four and twelve weeks apart, where spirometry according to ERS/ATS standards [17] and a clinical examination were performed. A sweat test and a traditional bacterial culture of OP swabs were done before ETI therapy and after three months.

2.3. DNA extraction and processing of metagenomes

DNA of OP swabs was extracted with the QIAmp® DNA Mini Kit. Shotgun-metagenomic sequencing of the OP microbiome was done with a NovaSeq 6000 PE150. Human reads were removed from the raw reads in two steps with BBmap as described before [18,19] (Supplemental table 1).

2.4. Taxonomic classification and normalization

We profiled the bacterial taxonomies with MetaPhlAn4 with default parameters and newest database [20]. We included samples with at least 10,000 reads mapped to bacterial marker genes with MetaPhlAn4 (n = 297) (Supplemental table 1). We normalized microbial reads by total sum scaling. We assessed strain level resolution with StrainPhlAn4 and used parameters adapted for low-biomass samples as described previously for StrainPhlAn3 [19,20]. We displayed phylogenetic strain trees for the six most abundant bacterial taxa observed on strain level with high resolution (R packages *ggtree, ggplot2*).

2.5. Microbiome outcome measures

We assessed α-diversity of MetaPhlAn4 mapped reads with Shannon-diversity index, Inverse-Simpson index, observed species, and evenness without additional transformation. To analyze β-diversity, we created a distance matrix using Bray–Curtis dissimilarity. We analyzed the intra-individual microbiota variability by comparing consecutive swabs (maximum two weeks of time difference) of the same participant. We investigated the relative number of polymorphisms on strain level by StrainPhlAn4 and performed PAM-Clustering (*cluster*) on a dissimilarity matrix for bacterial species. The number of clusters was chosen based on best average silhouette width.

2.6. Statistical analysis

Statistical analyses were performed with the statistical software R (version 4.1.2) [21]. Demographic and clinical data at study visits were compared with Kruskal–Wallis rank sum tests. Microbiota changes were assessed longitudinally and in four categories: −4–0 weeks before start ETI; 0–2 weeks after ETI; 2–4 weeks after ETI; 4–12 weeks after ETI. All statistical tests were corrected for sex and temporal correlation within subject. We calculated linear mixed-effect models (*nlme*) for α-diversity and additionally corrected for library size. Consecutive dissimilarities (within and between) were calculated with generalized additive mixed-effect models (*mgcv*). β-diversity was tested in categories with permutational multivariate analysis of variance (PERMANOVA) (*vegan*). Differential abundance analysis of bacterial genus was calculated with MaAsLin2 and obtained p-values were corrected for multiple testing [22,23]. For MaAsLin2 analysis, we considered adjusted p-value <0.25 significant, as suggested by the authors [22,23].

3. Results

3.1. Study population and clinical outcomes

We collected and sequenced 323 swabs and analyzed 297 swabs of 20 pwCF with a median age of 14 years (Table 1). Specific reasons for sample exclusion are detailed in the Methods section and Supplemental table 1. All participants showed a significant clinical response to ETI therapy with improved lung function and sweat chloride tests (Table 1, Supplemental figure 1). During three months of ETI treatment, the percentage predicted forced expiratory volume in one second increased (mean difference baseline compared to 4–12 weeks after ETI = 10.4 %; 95 % CI 6.12 %, 14.68 %; $p < 0.001$) and sweat chloride decreased (mean difference baseline compared to three months after ETI = −68.05 mmol/l; 95 % CI 59.78 mmol/l, 76.33 mmol/l; $p < 0.001$).

Table 1
Characteristics of adolescents included in the study.

Characteristic	Before ETI, N = 20*	4 weeks ETI, N = 20*	12 weeks ETI, N = 20*	p-value†
Age (years)	14.00 (14.00, 15.00) 12, 21			
Sex (female) ‡	4 (20 %)			
BMI	19.28 (16.99, 20.79) 16.53, 22.96			
BMI (z-Score)	−0.26 (−0.54, 0.24) −1.65, 1.24			
Modulator before ETI (yes) ‡	6 (30 %)			
Number of swabs per child	16.00 (14.00, 19.00) 10.00, 21.00			
Library size (mean reads)	298,690 (181,876, 813,054) 4156, 9892245			
F508del‡				
Homozygous	14 (70 %)			
Heterozygous	6 (30 %)			
FEV1 (%)	84 (80, 103) 53, 114	100 (88, 110) 71, 116	100 (92, 109) 59, 119	0.037
FEV1 (z-Score)	−1.31 (−1.73, 0.24) −3.79, 1.21	−0.05 (−0.99, 0.93) −2.35, 1.37	0.01 (−0.74, 0.81) −3.30, 1.62	0.037
FEV1/FVC (z-Score)	−0.80 (−1.79, −0.32) −2.81, 1.86	0.28 (−0.91, 0.74) −2.13, 2.16	−0.18 (−1.14, 0.59) −2.65, 1.62	0.073
Sweat Chloride (mmol/l)	112 (98, 115) 79, 135	NA	39 (32, 50) 16, 84	<0.001
Pseudomonas aeruginosa ‡,§	2 (10 %)		1 (5 %)	>0.9
Staphylococcus aureus ‡,§	17 (85 %)		13 (65 %)	0.3
Haemophilus influenzae ‡,§	2 (10 %)		0 (0 %)	0.5

* Median (IQR), Range.
† Kruskal–Wallis rank sum test, Fisher's exact test.
‡ n (%).
§ Bacterial cultures obtained from different oropharyngeal swab
ETI: Triple modulator therapy elexacaftor/tezacaftor/ivacaftor, FEV1: forced expiratory volume in 1 second, FVC: forced vital capacity, IQR: interquartile range, BMI: body mass index.

3.2. Metagenome biodiversity increases after initiation of ETI therapy

The sum of bacterial reads had a large range, and we thus corrected the statistical model for sample library size. Based on the MetaPhlAn4 outputs, we observed a slight increase in α-diversity during the three months of ETI treatment (Fig. 2 (A-E)) for inversed Simpson diversity (Coef 0.09, 95 %CI 0.00, 0.17, p = 0.04). Shannon diversity and the number of observed species showed a trend towards diversification (Shannon: Coef 0.01, 95 %CI 0.0, 0.02, p = 0.08; observed species: Coef 0.24, 95 %CI 0.00, 0.49, p = 0.07), whereas evenness did not change

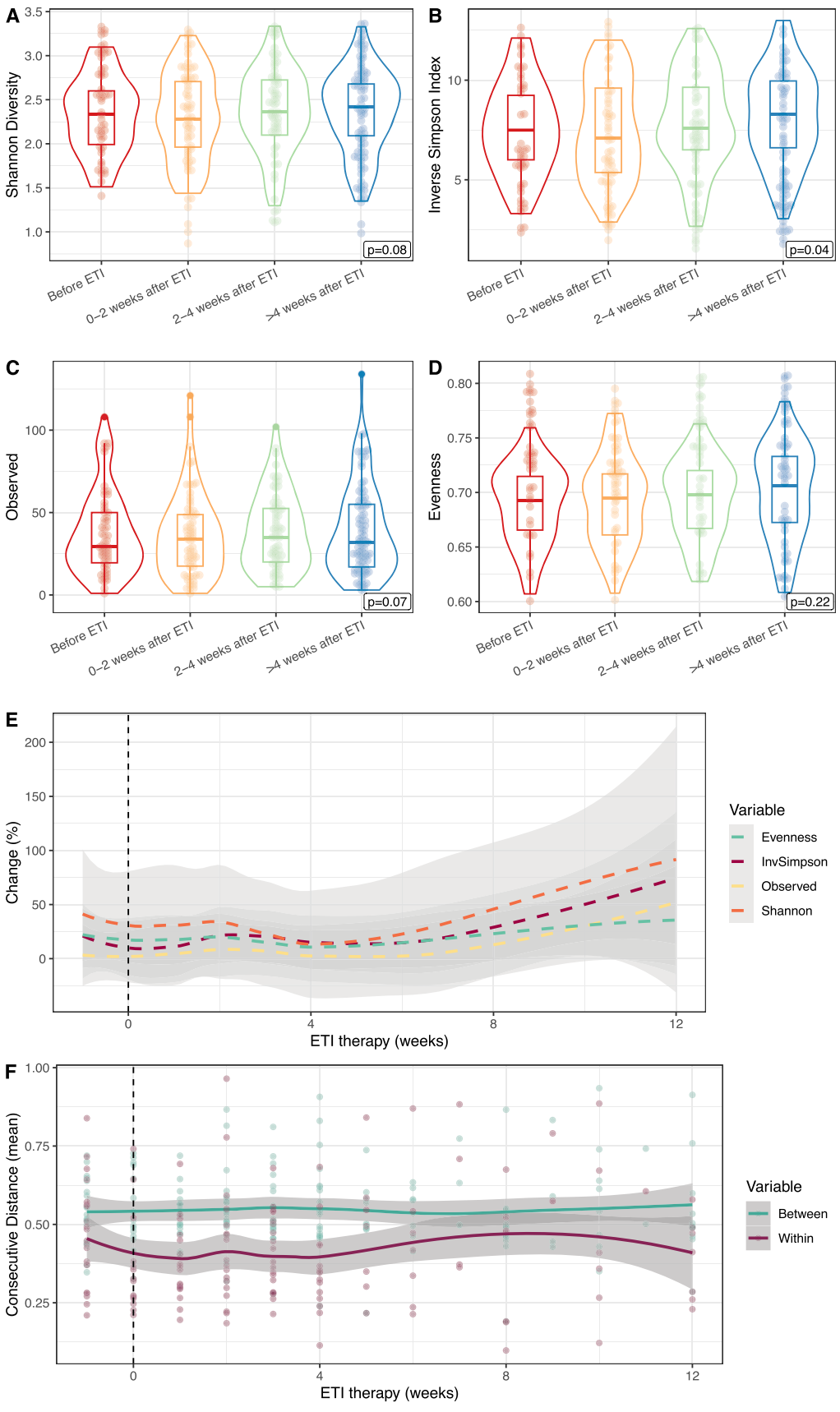

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Fig. 2. Changes in α-diversity and β-diversity related to start of ETI therapy. Displayed are (A) the model fitted values of Shannon Diversity Index, (B) the model fitted values of Inverse Simpson Index, (C) number of observed species, and (D) Evenness before and after ETI therapy. Violin plots represent the density of observed values (lower and upper boundaries of violins represent minimum and maximum value, respectively). Boxplots represent the 25th and 75th percentiles (lower and upper boundaries of boxes, respectively), the median (middle horizontal line) and measurements that fall within 1.5 times the interquartile range (IQR; distance between 25th and 75th percentiles; whiskers). Statistical significance was assessed using mixed-effect linear models with library size and sex as fixed effects and subject as random effect. (E) displays the % increase of different α-diversity measures in a longitudinal plot (smoothed by loess function). (F) displays the mean of Bray–Curtis dissimilarities between consecutive taken swabs (y-axis) between the individual participants ("Between") in petrol and of the same study participant ("Within") in dark red per week of ETI therapy (x-axis). Two swabs were considered consecutive if they were taken within a time range of maximum 14 days. ETI: Triple modulator therapy elexacaftor/tezacaftor/ivacaftor.

(Coef 0.00, 95 %CI 0.0, 0.01, $p = 0.22$). For sensitivity analysis, we performed the same analysis after transformation, as well as with samples with a library size >30.000 to control for confounding and received a similar result (Supplemental figure 2).

3.3. Change in microbial communities increases with longer duration of ETI therapy

To compare the development of the microbial community before and during the three months of ETI treatment, we performed PERMANOVA tests between the baseline microbial community using the a priori defined time-points after initiation of therapy. Therapy time-points were associated with the microbial community (F-model = 1.46, $R^2 = 0.01$, $p = 0.014$), but they explained only one percent of variance between swabs, while study participant explained almost 40 % of variance between swabs (Table 2). Compared to baseline, microbial community changed already within two weeks after initiation of ETI treatment and variance increased with longer duration of ETI (Table 2). We accounted for confounding by time difference between the swabs by including the time difference in days between the first swab and the following collection date as fixed effect in the model.

3.4. Intra-individual microbiota changes are associated with ETI therapy

We analyzed intra-individual microbiota stability (dissimilarity between consecutive swabs of the same child). After 5–6 weeks of ETI therapy, the microbiota community changed on an intra-individual level, reflected by an increase in within-subject dissimilarities (Fig. 2 (F)). However, the intra-dissimilarity was consistently lower compared to the inter-dissimilarity (Fig. 2(F)). The time difference related to the start of ETI therapy showed a non-linear association with the within-subject dissimilarity (gamm: $F = 2.91$, $p = 0.03$).

3.5. Higher abundance of commensal bacteria already after 2–4 weeks of ETI therapy

Differential abundance analysis showed a subtle increase of commensal bacteria after 2–4 weeks of ETI therapy, which was clearly visible after more than 4 weeks of ETI therapy (Fig. 3, Supplemental figure 3). For example, we observed a relative decrease of *Haemophilus* (Coef −1.22, adjusted $p < 0.25$) and an increase of *Actinomyces* (Coef 0.75, adjusted $p < 0.25$) or *Alloprevotella* (Coef 0.64, adjusted $p < 0.25$) after 2–4 weeks. In addition, *Peptostreptococcus* increased (Coef 1.27, adjusted $p < 0.05$) and *Gemella* increased (Coef 0.99, adjusted $p < 0.25$) after more than 4 weeks of ETI therapy.

Table 2
Changes in β-diversity after ETI therapy.

Baseline compared to	F model	R^2	Pr (>F)*
0–2 weeks after ETI	0.88	0.01	0.17
2–4 weeks after ETI	1.1	0.01	0.03
1–3 months after ETI	1.88	0.01	<0.01
Variable	**F model**	**R^2**	**Pr (>F)†**
ETI time-point	1.46	0.01	0.014
Age (years)	1.14	0.002	0.27
Sex	0.0	NaN	
Study participant	39.48	0.38	<0.001

* PERMANOVA: Terms were added sequentially, model corrected for age, sex, days between time-points as fixed effects and study participant as random effect.
† PERMANOVA: All terms were added marginally and as fixed effects.
Definitions of abbreviations: ETI= Triple modulator therapy elexacaftor/tezacaftor/ivacaftor.

3.6. Strain-level analysis reveals strain retention of microbes in the oropharyngeal niche

Strain-level analysis and phylogenetic clustering showed that although individual strains may evolve within a person, there is a general strain retention over time (Fig. 4, Supplemental figure 4). We focused on the six most prevalent bacterial species that were sequenced with strain-level resolution (*Prevotella melaninogenica, Veillonella dispar, Veillonella atypica, Prevotella histicola, Neisseria subflava, Porphyromonas porpheri*). We plotted phylogenetic trees to obtain information about genetic similarity of the assessed strains (Supplemental figure 4) and clustered strains longitudinally per participant (Fig. 4). For both analyses, we observed an apparent strain retention. We did not observe an increased changing of clusters within an individual after start of ETI therapy. Strain replacement was observed on average three times (25 % of swabs with detection on strain-level) per study participant (range: 0 (0 %) to 10 (57 %)) during the study period. We observed strain replacement most commonly for *Veillonella atypica* (replacement in 11 study participants) and *Veillonella dispar* (replacement in 10 participants). To asses a strain's response towards habitat changes mediated by ETI therapy, we analyzed mutation rates based on a dissimilarity matrix and percentage of polymorphic sites in all of the six strains (Supplemental figure 5). The relative number of polymorphisms or the mutation rates did not change after ETI therapy start.

4. Discussion

Here, we investigate the longitudinal effects of ETI therapy on the metagenome of adolescent pwCF with predominantly mild lung disease. We focused on frequent airway sampling before and after ETI therapy, allowing us to assess early changes of the microbiome. We observed changes in microbiome composition after two weeks of therapy, which became more pronounced over the three months observation time. On the one hand, this shows how quickly ETI alters host airway biology, and on the other hand, it may indicate that changes persist in young pwCF after three months of ETI therapy. In addition, we observed a trend towards diversification of microbiota explained by a relative increase of commensal bacterial species.

Our findings are similar to previous studies mainly conducted in adult pwCF showing an increase in microbiome diversity and evenness, as well as a relative increase in commensal species after ETI treatment during observation times up to one year [10,12-15]. In most previous studies, CF lung disease was more advanced and bacterial pathogens such as *Pseudomonas aeruginosa* were more frequently present as in our study [10-13,15].

Nichols et al. studied 200 adolescents over a period of 6 months with similarly mild CF disease as in our study. In that study, *Pseudomonas aeruginosa* was more frequent before ETI start compared to our population (44% vs 10 %) [12]. In contrast to our findings, the abundance of

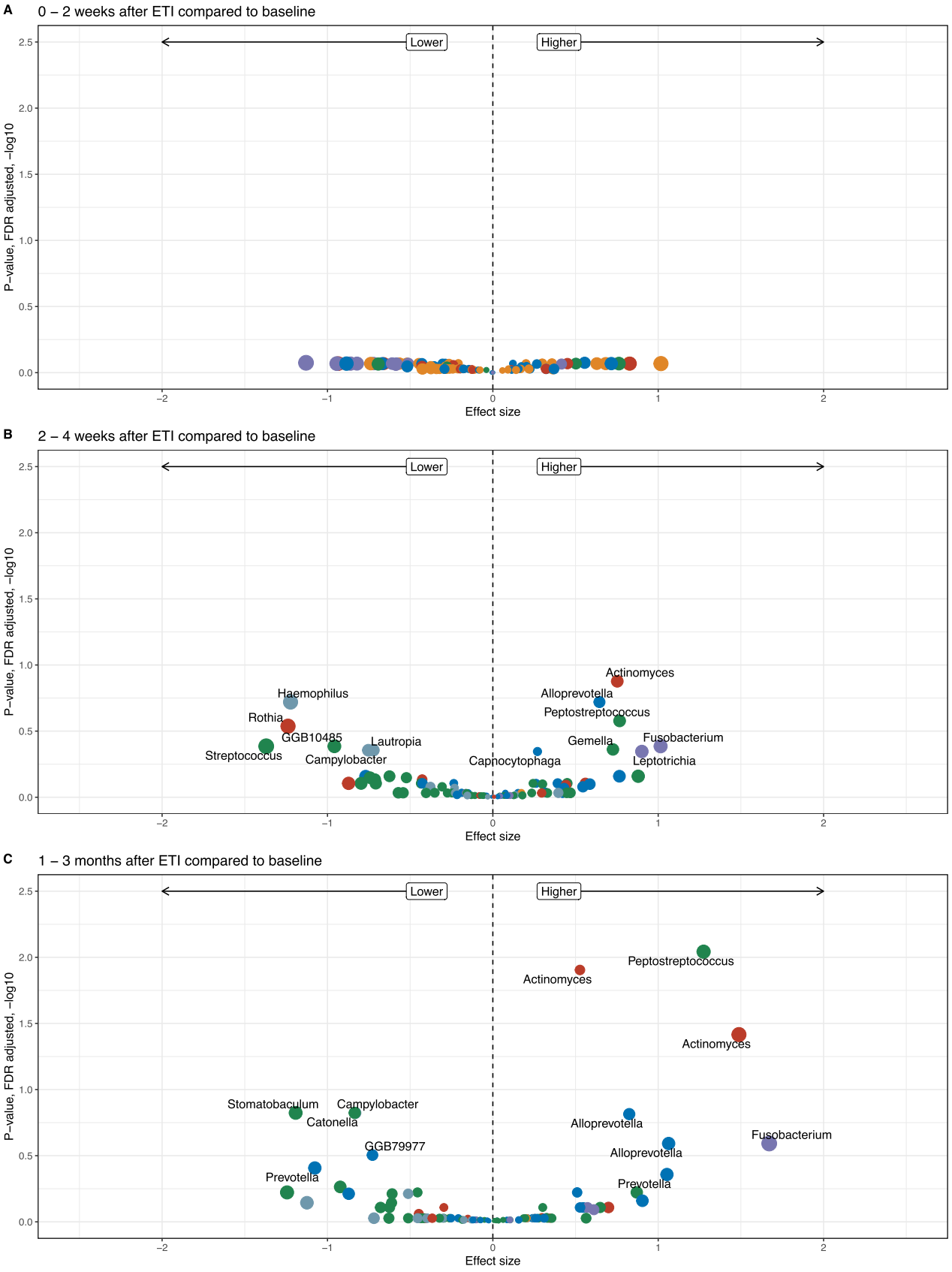

Fig. 3. Differential abundance analysis (MaAsLin2). Differential abundance analysis (MaAsLin2) is plotted with effect size on the x-axes and –log10 FDR adjusted p-values (q-values) on the y-axes. (A) 0–2 weeks after start of ETI, (B) 2–4 weeks after ETI therapy, and (C) 1–3 months after ETI therapy. ETI: Triple modulator therapy elexacaftor/tezacaftor/ivacaftor.

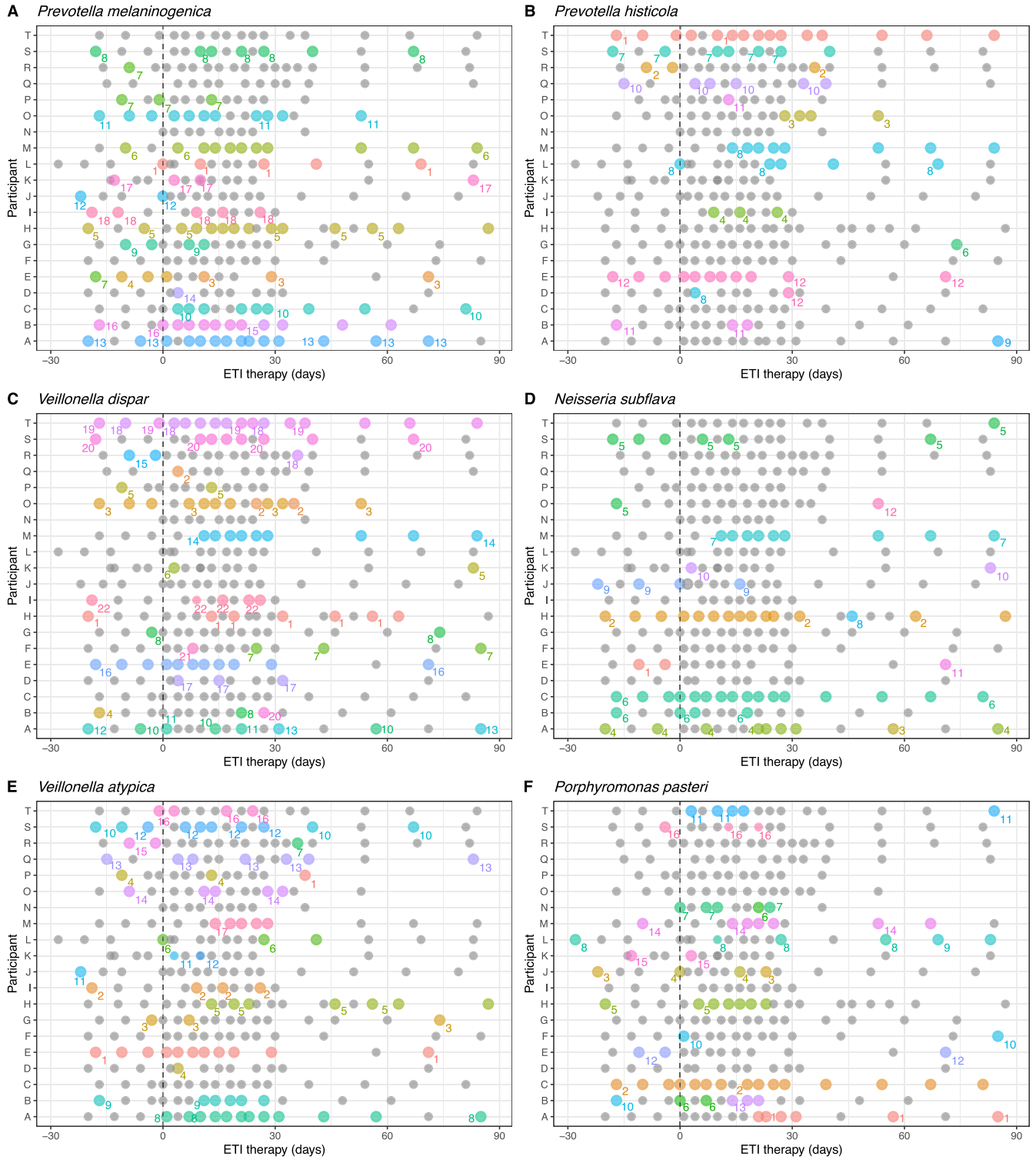

Fig. 4. Longitudinal display of clustering for six bacterial species detected on strain-level. (A-F) The x-axes display the time of ETI therapy in days and the vertical dashed line the start of therapy. Each study participant is displayed on the y-axes. The different colors show the clusters of detected strains based on a dissimilarity matrix. Numbers are displayed when the strain changes in consecutive swabs, or after a strain was not detected in the previous swab (grey color). ETI: Triple modulator therapy elexacaftor/tezacaftor/ivacaftor.

traditional CF pathogens declined, but an increase in commensal bacteria as observed in our study, was not reported [12]. Differences may relate to sampling methods, since we analyzed deep throat swabs while Nichols et al. analyzed sputum samples, as well as a different abundance of CF pathogens before ETI start. Pallenberg et al. studied pwCF with mild lung disease with a median age of 16 (range 12–45 years), including samples from divergent sources (induced sputum and deep cough samples) [14]. In their study of 31 pwCF, classical CF pathogens decreased while commensal bacteria e.g. *Prevotella* increased after 14 weeks ETI therapy, similarly as observed in our study. In summary, under ETI therapy the abundance of CF pathogens seems to decrease and commensal bacteria increase. Our study adds new knowledge since we

describe for the temporal dynamics of bacterial abundance development, which starts already after 2 weeks ETI therapy.

In line with Martin et al. and Sosinski et al., we observed compositional changes (β-diversity) after initiation of ETI therapy (Table 2) [13,15]. In the study by Sosinski et al., the time between two consecutive samples was longer compared to our study: 202 days (+/−108 days) between samples and mean 150 days (+/−81 days) since ETI prescription [13]. Using frequent microbiome sampling during three months after ETI therapy, we were able to add on to previous studies with less frequent but longer sampling times [13] that compositional changes start already two weeks after ETI therapy, and gradually increase over time. In the study by Martin et al., diversity was compared between pwCF on ETI and pwCF not prescribed with ETI therapy [15]. While the study lacked baseline microbiome samples, comparison between ETI treated and non-treated pwCF allowed to conclude that ETI increased both α- and β-diversity. To summarize, while previous studies with longer observation times similarly found persistent compositional differences upon ETI treatment over approximately one year [10,14], we complement previous findings showing that these changes occur already two weeks after ETI therapy.

After initiation of ETI therapy, we detected changed abundances of different bacteria. For example, we observed a relative decrease of *Haemophilus* and an increase of *Alloprevotella* and *Peptostreptococcus*. A biological explanation of changing abundances after initiation of ETI therapy in our study (but not in all previous studies) might be that pwCF were older and had more advanced lung disease associated with a more rigid microbiome composition, indicated by a higher abundance of *Pseudomonas aeruginosa.* For example, Schaupp et al., and Nichols et al. collected samples in pwCF aged 25–30 years, collected one sample before ETI therapy and compared with one to three samples after ETI therapy using 16S rRNA sequencing [10,12] . *Pseudomonas aeruginosa* was one of the most abundant bacteria present in 44 % to 66 % at baseline, decreasing during ETI therapy to 34 % [12] and 44 % after one to 12 months [10], respectively. In our study, only 2 % of the study participants were colonized with *Pseudomonas aeruginosa*, indicating a more diverse bacterial composition, which may influence the changes of the microbiome composition during ETI treatment.

In strain-level analysis, we observed strain-retention for the pwCF independent of ETI therapy. This is in line with our observation that within β-diversity is smaller than between β-diversity before and after start of ETI therapy. Both findings indicate that the microbial composition is highly personalized and responds within a personal range to ETI mediated changes. While we are the first to analyze the effect of ETI therapy at a strain-level, our observation time is limited to three months.

Our study has several strengths. Compared to other studies [10,13,14], we included more samples from each individual following a fixed schedule. Compared to previous studies collecting fewer samples over a longer observation time, the here performed high-frequency sampling at ETI therapy initiation allowed us to assess systematically the temporal dynamics of the changing microbiome after therapy initiation. Using frequent sampling over a fixed time frame, we were also able to apply more advanced statistical methods and control for known fluctuations of the microbiome composition and the individual variability independent of ETI therapy. Sequencing of the bacterial metagenome allowed analysis at strain-level, which we performed with state-of-the-art recently tools for comprehensive taxonomic profiling [20].

Our analysis is limited by the current lack of internationally accepted standards for metagenomic analysis of low-biomass samples. The presence of high amounts of host DNA in these samples complicated the analysis, resulting in fewer microbial reads. Consequently, viral and fungal analyses were not feasible due to limitations in sequencing depth and sample processing methods. Additionally, we were unable to analyze lower airway samples, which is challenging under ETI therapy due to minimal sputum production and ethical concerns related to more invasive sampling methods, such as bronchoscopy. We also acknowledge that the small number of participants may reduce the statistical power of the analysis potentially affecting the significance of observed changes in α-diversity and β-diversity. The short observation period further limits our ability to capture long-term changes. Furthermore, the absence of a control group, including individuals not affected by CF or those not undergoing ETI therapy, is a limitation of the. Although our aim was to include younger individuals with CF compared to previous studies, we were unable to include children younger than 12 due to ETI not being approved for this age group at the time of the study's initiation.

Besides the known beneficial effect ETI therapy has on lung function and structural lung changes in pwCF [4,5,24], ETI also influences the microbiome composition in younger pwCF. Initiation of ETI therapy may shape the microbiome to a healthier composition, as shown in our study, which may prevent subsequent infections and recurrent antibiotic therapies. However, more longitudinal data is needed to understand whether early ETI therapy may prevent colonization with typical CF pathogens and whether there is a long-lasting effect.

Data availability

Sequencing data generated during this study have been stored accessible for the public in the NCBI bioproject repository (https://www.ncbi.nlm.nih.gov/bioproject/ with the accession code "PRJNA1101448"). Study participant and further metadata is available upon reasonable request.

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Credit author statement

AM, MH, and JU conceptualized the study. AG and JU collected samples and performed clinical data and metadata curation, RS and NM performed bioinformatics analyses, RS and AG performed the formal analysis. MH, RS, and JU interpreted the data. RS and JU wrote the manuscript. All authors reviewed, edited and approved the final manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Alexander Moeller reports financial support was provided by Vertex Pharmaceuticals Incorporated. Jakob Usemann reports a relationship with Swiss Lung Foundation that includes: funding grants. Jakob Usemann reports a relationship with Palatin Foundation that includes: funding grants. Jakob Usemann reports a relationship with Swiss Group for Clinical Cancer Research that includes: funding grants. Jakob Usemann reports a relationship with Vertex Pharmaceuticals Incorporated that includes: speaking and lecture fees and travel reimbursement. Jakob Usemann reports a relationship with LUNGE ZURICH that includes: funding grants and speaking and lecture fees. Ruth Steinberg reports a relationship with Cystic Fibrosis Switzerland that includes: funding grants. Ruth Steinberg reports a relationship with Swiss National Science Foundation that includes: funding grants. Ruth Steinberg reports a relationship with Society of Paediatric Pneumology (GPP) that includes: funding grants & travel reimbursement. Ruth Steinberg reports a relationship with Vertex Pharmaceuticals Incorporated that includes: speaking and lecture fees and travel reimbursement. Markus Hilty reports a relationship with Lindenhof Bern Foundation that includes:

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2024.10.001.

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