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Early View

Original Research Article

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Changes in Sputum Viscoelastic Properties and Airway Inflammation in Primary Ciliary Dyskinesia are Comparable to Cystic Fibrosis on Elexacaftor/Tezacaftor/Ivacaftor Therapy

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Take home message (256 characters incl. spaces): Changes in sputum viscoelastic properties, inflammation markers and proteome in primary ciliary dyskinesia are less severe than in cystic fibrosis at baseline, but comparable to cystic fibrosis after starting elexacaftor/tezacaftor/ivacaftor therapy.

Abstract

Background: Primary ciliary dyskinesia (PCD) and cystic fibrosis (CF) are muco-obstructive lung diseases that are caused by distinct genetically determined defects in mucociliary clearance, however, knowledge on the relative severity of airway mucus dysfunction and chronic inflammation remains limited. The aim of this study was therefore to compare sputum viscoelastic properties, inflammation markers and the proteome between patients with PCD and patients with CF before and under elexacaftor/tezacaftor/ivacaftor (ETI) therapy.

Methods: We compared sputum rheology, inflammation markers and the proteome in 42 clinically stable adolescent and adult patients with PCD, 40 patients with CF with at least one *F508del* allele before (baseline) and 3 months after initiation of ETI and 15 age-matched healthy controls.

Results: The elastic modulus (G') and viscous modulus (G'') of PCD sputum was increased compared to healthy controls ($P<0.001$), lower than in CF at baseline ($P<0.001$) and similar to CF on ETI. Inflammation markers in PCD sputum including neutrophil elastase (NE), free DNA, myeloperoxidase (MPO), interleukin (IL)-1β and IL-8 were also increased compared to healthy controls (all $P<0.001$), lower than in CF at baseline ($P<0.05$ to $P<0.001$) and comparable to CF on ETI. Similar, changes in the sputum proteome were less pronounced in PCD compared to CF at baseline, but comparable between PCD and CF on ETI.

Conclusions: Clinically stable patients with PCD show changes in sputum viscoelastic properties, inflammation markers and the proteome that are less severe than in patients with CF at baseline, but comparable to CF patients on ETI therapy.

Word count: 247

Keywords: Airway inflammation, cystic fibrosis, elexacaftor/tezacaftor/ivacaftor, primary ciliary dyskinesia, proteome, sputum rheology

Word count: 3945

Introduction

Primary ciliary dyskinesia (PCD) and cystic fibrosis (CF) are rare genetic lung diseases caused by distinct molecular and cellular defects [1, 2]. PCD is caused by mutations in more than 50 genes that are essential for normal structure and function of respiratory cilia, thus leading to primary defects in ciliary function [2-5]. CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene encoding an epithelial chloride channel essential for chloride/fluid secretion and airway surface hydration, thus leading to increased concentration and viscoelasticity of airway mucus [6]. Primary defects in ciliary function in PCD, as well as primary abnormalities in mucus function in CF cause impaired mucociliary clearance that triggers a pathogenetic cascade that evolves into a 'vicious vortex' of airway mucus plugging, chronic polymicrobial infection, neutrophilic inflammation and structural airway damage [5, 7-9]. The secretion of the neutrophil serine proteases (NSP) neutrophil elastase (NE), cathepsin G (CatG) and proteinase 3 (PR3) by activated neutrophils causes proteolytic damage that plays a key role in the onset and progression of bronchiectasis characteristic of lung disease in PCD and CF [9-14]. Recent studies of patients with CF treated with the triple combination CFTR modulator therapy elexacaftor/tezacaftor/ivacaftor (ETI) showed that pharmacological restoration of CFTR chloride channel function reduces neutrophilic airway inflammation including NSP activity, however, without reaching levels close to healthy [15, 16]. Of note, markers of neutrophilic inflammation including NE and interleukin (IL)-1β were reported to induce goblet cell metaplasia and mucus hypersecretion, which may also cause increased viscoelasticity of airway mucus that may aggravate mucociliary dysfunction in PCD [6, 17, 18]. While previous studies demonstrated neutrophilic airway inflammation with elevated NE activity in CF and PCD, the viscoelastic properties of sputum from patients with PCD have not been studied and a comparison of the relative levels of NSP and other inflammation markers in PCD *vs*. CF patients without and after initiation of CFTR modulator therapy has not been performed.

The aim of this study was therefore to compare sputum viscoelastic properties and airway inflammation markers in clinically stable adolescent and adult patients with PCD and age-matched patients with CF at baseline and under triple combination therapy with ETI. To achieve this goal, we investigated sputum viscoelastic properties by rheology, determined levels of key inflammation markers including NSP activity, DNA, MPO, IL-1β and IL-8, and conducted proteomic analyses in 42 patients with PCD, 40 patients with CF at baseline and after initiation of ETI therapy and 15 age-matched healthy controls.

Methods

Additional details on methods are provided in the online supplementary material.

Study design and participants

This prospective observational study was approved by the ethics committee of Charité - Universitätsmedizin Berlin (EA2/220/18, EA2/143/19). Written informed consent was obtained from all participants, their parents or legal guardians. Patients with PCD had to be clinically stable at the time of sputum collection. Age-matched patients with CF were at least 12 years old, eligible for CFTR modulator therapy with ETI (homozygous for *F508del* or compound heterozygous for *F508del* and a minimal function mutation) and provided paired sputum samples before and after initiation of ETI therapy. CF patients were a subgroup of the previously described MODULATE-CF cohort (clinicaltrials.gov Identifier: NCT04732910) [16] and were matched with PCD patients for age, gender and the existence of matched sputum samples at baseline and at 3 months after initiation of ETI therapy for paired analysis. A complete list of inclusion and exclusion criteria is detailed in the online supplement. Healthy controls were age- and sex-matched non-smoking volunteers without any medical history of respiratory disease. Sputum samples from CF patients were analyzed at baseline and 3 months after initiation of therapy with the approved dose of elexacaftor 200 mg and tezacaftor 100 mg every 24 hours in combination with ivacaftor 150 mg every 12 hours. Due to limited amount of sputum in some patients not all measurements could be performed with every sputum sample.

Sputum collection

Sputum samples from patients with PCD and patients with CF were obtained after spontaneous expectoration. In healthy subjects, sputum expectoration was induced by hypertonic saline inhalation (NaCl 3 to 6%). Rheology measurements were performed on fresh sputum samples

that were immediately placed on ice. For all other analyses remaining samples were stored at -80°C.

Rheology

Rheological measurements were conducted using a rheometer with a cone-plate geometry (Kinexus Pro+, Netzsch GmbH, Selb, Germany) as previously described [19]. The elastic modulus (storage modulus, G') and viscous modulus (loss modulus, G") were derived from the linear viscoelastic region of the amplitude sweep.

Inflammation markers

IL-1β, IL-6, IL-8 and tumor necrosis factor alpha (TNF-α) concentrations in cell-free sputum supernatants were determined by cytometric bead array kits (BD Biosciences, San Diego, CA, USA), myeloperoxidase (MPO) concentration was measured by enzyme-linked immunosorbent assay (Enzo Life Sciences, Farmingdale, NY, USA) and DNA concentration was measured using the Quant-iT PicoGreen dsDNA assay kit (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions. Free NE, CatG and PR3 activity was measured as previously described [10, 20-22].

Proteomics

Sputum samples were solubilized in 2% SDS buffer at 95°C for 10 minutes. Proteins were reduced, alkylated and treated with Benzonase. Samples were cleaned-up using a single-pot solid-phase enhanced sample preparation protocol, followed by PNGase F and trypsin treatment overnight. Peptide samples were desalted and measured on an EASY-nLC 1200 System coupled to an Orbitrap HF-X mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) running on data-dependent acquisition mode as previously described [23].

Magnetic resonance imaging

Magnetic resonance imaging (MRI) of the lungs was performed in a subgroup of patients with PCD and patients with CF and images were analyzed for abnormalities in lung morphology and perfusion including the presence and extent of bronchiectasis using an established morpho-functional MRI score [24, 25] as detailed in the online supplement.

Statistical analysis

Data were analyzed using GraphPad Prism, version 9.5.1., or R (V 4.2.2.). Clinical data are presented as mean ± standard deviation and tested by Student's *t*-test. Data from measurements in sputum samples from healthy subjects, PCD patients and CF patients are presented as median with interquartile range and were compared by Mann-Whitney test. Changes in CF sputum between baseline and 3 months after initiation of ETI were tested with Wilcoxon matched-pairs signed rank test. For proteomics analysis limma moderated t-test or F-test was used. The Benjamini-Hochberg method was used for multiple comparisons correction. $P < 0.05$ was accepted to indicate statistical significance.

Results

Characteristics of study population

In total, 42 clinically stable adolescent and adult patients with PCD, 40 age-matched patients with CF and 15 healthy controls were included in this study and their demographics and clinical characteristics are summarized in table 1. Patients with PCD had a mean age of 27.2 ±14.7 years at sputum collection. Outer dynein arm (ODA) defects with homozygous or compound heterozygous mutations in the dynein axonemal heavy chain 5 (*DNAH5*) (19.1%) and the dynein axonemal intermediate chain 1 (*DNAI1*) (12.0%) gene were the most frequent PCD-causing genotypes [2]. PCD genotypes are provided in supplementary table S1. CF patients from the MODULATE-CF cohort had a mean age of 29.9 ± 9.5 years at baseline sputum collection and 50.0% were *F508del* homozygous and 50% compound heterozygous for *F508del* and a minimal function mutation (table 1). *CFTR* genotypes are indicated in supplementary table S2. As expected from previous studies, FEV_1 % predicted, body mass index (BMI) and sweat chloride concentration were improved in CF patients by ETI therapy (table 1) [16, 26]. The prevalence of bronchiectasis detected by MRI was 97.1% in PCD patients and 100% in CF patients. The extent of bronchiectasis and airway mucus plugging, as determined by the MRI bronchiectasis/wall thickening subscore and mucus plugging subscore, respectively, was lower in PCD compared to CF at baseline, but comparable to CF on ETI (supplementary table S4).

Comparison of sputum viscoelastic properties in PCD and CF at baseline and on ETI therapy

To determine disease-specific differences in sputum viscoelasticity we performed rheological measurements and determined the elastic modulus (G'), viscous modulus (G'') and the resulting effective rheological mesh size (ξ) in sputum samples of 39 patients with PCD, 40

patients with CF at baseline and 3 months after initiation of ETI, and 10 healthy controls. In all three groups, G' predominated over G'' indicating a gel-like behavior of sputum (figures 1a and 1b). G' and G'' of induced sputum from healthy controls was similar to G' and G'' of mucus collected from endotracheal tubes of healthy individuals without hypertonic saline induction [27]. In PCD, G' and G'' were increased and the mesh size was decreased compared to healthy controls (figure 1). In CF sputum prior to ETI, G' and G'' were increased and the mesh size was decreased compared to sputum from PCD patients. On ETI therapy, G' and G'' of CF sputum was reduced and the mesh size increased to levels comparable to values observed for PCD patients, however, rheological parameters remained significantly changed compared to healthy controls (figure 1). To determine the potential impact of inhaled hypertonic saline on sputum rheology, we compared viscoelastic properties of spontaneously expectorated *vs*. induced sputum from patients with PCD and patients with CF and did not observe differences in G' or G'' (supplementary figure S1). Further, hypertonic saline as standard care for PCD and CF, as well as rhDNase treatment in CF had no effect on sputum viscoelastic properties (supplementary figure S2).

Comparison of airway inflammation markers in PCD and CF at baseline and on ETI therapy

To study disease-specific differences in airway inflammation, we measured the concentration of free DNA, MPO, IL-1β, IL-6, IL-8 and TNF-α, i.e. key inflammation markers implicated in lung disease progression [18, 28-30], in sputum supernatants from 40 patients with PCD, 40 patients with CF at baseline and after 3 months on ETI, and 12 healthy controls (figure 2). In PCD, DNA, MPO, IL-1β, IL-8 and TNF-α levels were elevated compared to healthy controls, but lower compared to CF at baseline (figure 2a-d and 2f). In CF patients on ETI therapy DNA, MPO, IL-1β and IL-8 were reduced to levels similar to those observed in PCD, but remained elevated compared to healthy controls (figures 2a-d). TNF-α showed a trend towards a

decrease on ETI therapy, but was still increased compared to PCD patients and healthy controls (figure 2f). Sputum IL-6 was not changed in PCD compared to healthy controls (figure 2e). CF patients showed elevated sputum levels of IL-6 at baseline, which increased after 3 months of ETI therapy.

Comparison of airway protease burden in PCD and CF at baseline and on ETI therapy

An imbalance between proteases and anti-proteases, particularly an elevated NSP activity, constitutes a key risk factor for the initiation and progression of lung disease in both PCD and CF [9-11, 31]. To assess disease-specific differences in protease burden, we measured free activity of NE, CatG and PR3 in sputum supernatants from 42 PCD patients, 40 CF patients at baseline and after 3 months on ETI, and 12 healthy controls (figure 3). Free NE, CatG and PR3 activity was increased in PCD compared to healthy controls and showed a further increase in CF at baseline (figures 3a-c). On ETI therapy, free NE, CatG and PR3 activity in CF sputum was similar to that observed in PCD, but remained increased compared to healthy controls (figure 3a-c).

Relationship between changes in viscoelastic properties and airway inflammation markers

To determine the relationship between the changes in sputum viscoelastic properties and airway inflammation, we correlated G' and G'' with individual inflammation markers (supplementary figure S3 and S4). These analyses show a direct correlation of G' and G'' with DNA, IL-1β, IL-8, TNF-α and free NSP activities indicating that abnormal mucus is associated with more severe airway inflammation. On the other hand, lung disease severity determined by FEV_1 % predicted and the presence or absence of specific pathogens, such as *Haemophilus influenzae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa,* had a relatively small impact on changes in sputum viscoelasticity and inflammation markers in PCD and CF, suggesting that the

observed variability in sputum outcome measures may also be linked to sputum as a biospecimen that may originate from a more or less severely affected region of the airways (supplementary table S5 and S6).

Comparison of sputum proteome in PCD and CF at baseline and on ETI therapy

For an unbiased comparison of the sputum proteome, we conducted label-free shotgun proteomics of sputum samples from 29 patients with PCD, 35 patients with CF before and on ETI therapy, and 15 healthy controls. In total, 1149 proteins were detected, with 690 (60.1%) proteins being significantly different between all four groups (figure 4a). The complete list of all identified proteins is included in the online supplementary material. Hierarchical cluster analysis identified two major clusters (figure 4a). Cluster 1 contains proteins that were detectable at higher levels in PCD and CF sputum compared to healthy individuals, and were associated with inflammatory processes, e.g. leukocyte/myeloid-mediated immunity and phagocytosis (figure 4a). Cluster 2 includes proteins with overall decreased abundance in sputum from patients with PCD and patients with CF compared to healthy controls and is enriched in proteins involved in biological processes such as protein localization to membrane/organelle and epithelial cell differentiation (figure 4a).

Comparative analysis of protein expression revealed that 598 (52.0%) proteins were significantly different between healthy controls and patients with PCD, of which 388 showed a higher and 210 a lower abundance in PCD compared to healthy controls (supplementary figure S5a). Single sample gene set enrichment analysis (ssGSEA) of gene ontology (GO) terms showed a high enrichment of the GO terms leukocyte mediated immunity, leukocyte/myeloid activation and activation/positive regulation of immune response in PCD, whereas GO terms linked to epithelium development, protein targeting and localization to membrane were enriched in healthy controls (figure 4b). The comparison between the PCD and CF sputum proteome at

baseline revealed 447 (38.9%) proteins to be significantly different (supplementary figure S5b). Enrichment of both inflammation-associated GO terms, leukocyte mediated immunity and leukocyte/myeloid activation, was significantly higher in CF than in PCD sputum (figure 4b, supplementary figure S6d). The complete list of significantly different GO terms is included in the online supplement (supplementary figure S7). Protein expression profiles in PCD sputa were similar to CF on ETI therapy with only 63 (5.5%) significantly different proteins between PCD and CF (supplementary figure S5c). Correspondingly, log2 fold change analysis of protein abundances detected proteins with a greater fold change in the comparisons of PCD versus healthy controls and PCD versus CF at baseline (supplementary figure S5a and S5c) than in the comparison of PCD with CF on ETI (supplementary figure S5b). Taken together, these data show that the sputum proteome of PCD patients is rather similar to that of patients with CF on ETI therapy.

Discussion

PCD and CF are caused by distinct genetic defects in the mucociliary clearance system. However, our understanding of the relative severity of mucus dysfunction and chronic airway inflammation in these two muco-obstructive lung diseases remains limited. This is the first study to compare changes in sputum viscoelastic properties, inflammation markers and the sputum proteome in clinically stable adolescent and adult patients with PCD with age-matched patients with CF before and after initiation of ETI therapy. This study shows that compared to healthy controls, sputum from patients with PCD is characterized by increased viscoelasticity, increased markers of neutrophilic inflammation and protease burden, and an altered sputum proteome. Overall, the changes in mucus properties, inflammation markers and the proteome profile found in PCD sputum were less severe than in patients with CF at baseline, but comparable to residual abnormalities observed in CF after initiation of ETI therapy. Collectively, these data provide novel information on relative abnormalities in mucus properties and severity of neutrophilic inflammation and protease burden in PCD *vs*. CF, including patients with CF in whom CFTR chloride channel function is pharmacologically restored by highly effective CFTR modulators.

In CF, increased viscoelasticity of airway mucus is primarily caused by deficient CFTR-mediated chloride and fluid secretion leading to mucus hyperconcentration, which is well established as a key mechanism of mucociliary dysfunction in CF [32-35]. In addition to mucus hyperconcentration, increased cross-linking of mucins via disulfide bonds caused by oxidation of free thiols under conditions of neutrophilic inflammation was identified as an independent mechanism leading to pathologic mucus in CF [36, 37]. Conversely, mucociliary dysfunction in PCD has been primarily related to ciliary dysfunction [2]. Our data show for the first time that viscoelastic properties of airway mucus are also fundamentally altered in PCD with a

characteristic increase in the elastic modulus G' and viscous modulus G'', and a decrease in mesh size of the mucus gel. Similar to prior reports in CF, we found that G' is consistently higher than G'' in PCD. Since this G' dominance is a hallmark of a cross-linked gel, our data indicate that increased mucin cross-linking also contributes to mucus dysfunction in PCD. Further, several factors released in neutrophilic inflammation including NE and IL-1β were shown to induce mucin hypersecretion [9, 17, 18, 38] and thus may increase mucus concentration and contribute to increased viscoelasticity of airway mucus in PCD. Our data indicate that in addition to ciliary dysfunction, mucus dysfunction may also contribute to the pathogenesis of impaired mucociliary clearance in PCD, where increased viscoelasticity may impede back-up mechanisms of mucus clearance such as cough clearance and cilia-independent gas liquid transport [39, 40]. The relevance of mucus dysfunction in PCD is also supported by the high prevalence of airway mucus plugging detected by MRI. As stagnant mucus causes airflow limitation and hypoxia, and provides a constant nidus for chronic airway infection and inflammation, our results also provide a rationale for therapeutic targeting of mucus dysfunction as a treatable trait in PCD [39, 41]. Current strategies in this direction include treatment with inhaled idrevloride, a long-acting inhibitor of the epithelial sodium channel ENaC in combination with hypertonic saline to improve mucus hydration [42], and the development of novel mucolytics that reduce mucus cross-linking [33, 36]. Finally, our data show that the residual abnormalities in viscoelastic properties as well as residual mucus plugging detected by MRI in adolescent and adult CF patients treated with ETI are comparable to those observed in PCD. These data suggest that these novel muco-active and mucolytic strategies remain relevant for CF, not only in the ~10 % of patients who are genetically ineligible or do not tolerate CFTR modulators, but also in the much larger group of CF patients who can benefit from CFTR modulators, but have irreversible lung damage with bronchiectasis.

Neutrophilic inflammation with increased protease burden due to release of NE and other NSP has been identified as a key risk factor of the development of bronchiectasis and lung function decline in the broader non-CF bronchiectasis population as well as in patients with CF [9, 10, 31, 43, 44]. Our comparative studies of sputum inflammation markers and proteome profiles indicate that the severity of neutrophilic inflammation in clinically stable PCD patients is lower than in CF patients at baseline, but comparable to CF patients on CFTR modulator therapy with ETI. This pattern is observed for key proinflammatory cytokines that orchestrate neutrophilic airway inflammation such as IL-1β, IL-8 and TNF-α [18, 28], as well as all three NSP, namely NE, CatG and PR3 that play a key role in the pathogenesis of protease-antiprotease imbalance leading to proteolytic airway damage, progressive bronchiectasis and lung function decline [9-11, 31, 43-45]. Of note, IL-6 levels in sputum were not different in PCD *vs*. CF at baseline, but increased in CF patients on ETI therapy. Based on recent work in the gastrointestinal tract, where IL-6 has been linked to epithelial homeostasis [46, 47], we therefore speculate that IL-6 may represent a biomarker of airway epithelial regeneration on ETI therapy. A similar pattern was also observed in our proteomics analyses as an unbiased approach to study airways disease at the molecular level. As shown by comparative analysis of protein abundances, as well as single sample gene set enrichment analysis of GO terms, changes in PCD sputum including proteins related to leukocyte mediated immunity and myeloid leukocyte activation were less pronounced than in CF sputum at baseline, but comparable to CF after initiation of ETI therapy. These findings are in line with a previous report that compared these inflammation markers and activities of NE, CatG and PR3 in CF patients before and after initiation of ETI with patients with non-CF bronchiectasis [15]. Notably, the proteomic signature of a subset of CF patients at baseline was more similar to healthy individuals. Those CF patients tended to have a lower sputum elastic modulus (G') and viscous modulus (G'') and exhibited lower levels of airway inflammation markers supporting the sensitivity of proteomics to detect changes and commonalities between different disease and treatment conditions (supplementary table S8).

Collectively, these studies suggest that with pharmacological restoration of CFTR function, the airway pathobiology in CF may resemble more closely that in non-CF bronchiectasis, where the disease process is perpetuated by irreversible airway damage rather than CFTR dysfunction. This notion supports that novel anti-inflammatory therapies such as oral inhibitors of dipeptidyl peptidase 1 (DPP-1, also known as cathepsin C) that inhibit the activity of all three NSP and were recently shown to reduce pulmonary exacerbations in patients with non-CF bronchiectasis including PCD [31, 45, 48-50] should also be trialed in patients with CF including those that are treated with CFTR modulators.

This study has limitations. Our studies of ETI effects were limited to a 3 month timepoint and studies with a longer follow-up will be needed to determine when optimal benefits are reached and when disease progression may be observed in CF patients due to persistent bronchiectasis and residual mucus dysfunction, inflammation and infection on CFTR modulator therapy. We only included adolescent and adult patients with PCD or CF with chronic lung disease that were able to expectorate sputum and it remains unknown whether the abnormalities and disease-specific differences detected in sputum viscoelasticity, inflammation markers and the proteome in PCD and CF are also present in patients with milder lung disease including children. Therefore, future studies in younger patients with less severe lung disease, ideally prior to the onset of irreversible structural airway damage, are needed to clarify the extent to which airway mucus dysfunction and inflammation in PCD and CF are early consequences of the underlying genetic defects or secondary changes related to an independent pathogenetic mechanisms (i.e. the ‘vicious vortex’) driven by bronchiectasis. Such studies will have to rely on induced sputum that can be obtained from early childhood [51]. In this context, our data on viscoelastic properties of induced sputum in healthy individuals was comparable to mucus collected from endotracheal tubes from healthy individuals without prior

induction with hypertonic saline [27]. Further, viscoelastic properties and inflammation markers were comparable in spontaneously expectorated *vs*. induced sputum from PCD patients and CF patients, which may be explained by rapid absorption of the added fluid by the airway epithelium [52] and supports the suitability of induced sputum for such studies. Further, it will be important to determine how mucus properties and inflammation markers change over time including episodes of pulmonary exacerbations, and how these changes are related to patient reported outcome measures of health-related quality of life.

In summary, our data support that changes in mucus properties and neutrophilic airway inflammation in PCD are less severe than in CF patients not treated with CFTR modulators, but comparable to CF patients on ETI therapy. These results provide novel information on the relative severity of key contributors to the vicious vortex underlying the complex in vivo pathogenesis of chronic airways disease in PCD and CF, and support therapeutic targeting of mucus plugging and neutrophilic inflammation in both diseases, including CF patients who can benefit from CFTR modulators.

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Conflicts of interest

J.D. reports grants from the German Research Foundation (DFG) with payments made to the institution. M.S. reports grants or contracts from the German Research Foundation (DFG) and Vertex Pharmaceuticals with payments made to the institution, payment or honoraria for lectures from Vertex Pharmaceuticals and fees for participation on an advisory board from Vertex Pharmaceuticals. M.S. is an unpaid chairman in the German Cystic Fibrosis Association (FGM), member of the secretary group CF within the European Respiratory Society (ERS) and the treasurer in the German Society for Pediatric Pneumology (GPP). S.Y.G. reports grants from the Christiane Herzog Foundation, the German Cystic Fibrosis Association (FGM), and Vertex Pharmaceuticals, lecture honoraria from Chiesi and Vertex Pharmaceuticals, and advisory board participation for Chiesi and Vertex Pharmaceuticals outside the submitted work. J.R. reports payment or honoraria for lectures from Vertex Pharmaceuticals, Insmed, Chiesi and Pari, and is part of the BEAT-PCD Management Committee. M.G. reports grants from the German Research Foundation (DFG) and the German Federal Ministry for Education and Research (BMBF) with payments made to the institution. M.G. is a board member of the Colloid Society and the managing director and treasurer of the Berlin-Brandenburg Association for Polymer Research. P.M. reports grants from the German Research Foundation (DFG) and the German Federal Ministry for Education and Research (BMBF) with payments made to the

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Tables

Table 1

Clinical characteristics of healthy controls, patients with primary ciliary dyskinesia and patients with cystic fibrosis at baseline and after initiation of elexacaftor/tezacaftor/ivacaftor.

Clinical characteristic		**Healthy controls**	**PCD patients**	**CF patients at baseline**	**CF patients with 3 months ETI**
Number of individuals		15	42	40	40
Age (years)	Mean ± SD	32.5 ± 5.7	27.2 ± 14.9	29.9 ± 9.5	30.2 ± 9.5
Sex (female)	n (%)	13 (87.0%)	21 (51.2%)	25 (62.5%)	25 (62.5%)
Organ laterality defect	n (%)	---	18 (42.9%)	---	---
PCD mutation subgroups (ultrastructural defect category)					
ODA	n (%)	---	17 (40.5%)	---	---
IDA		---	1 (2.4%)	---	---
ODA + IDA		---	6 (14.3%)	---	---
CC/RS		---	5 (12.0%)	---	---
MTD		---	6 (14.3%)	---	---
Normal ciliary ultrastructure		---	4 (9.5%)	---	---
*Unknown genotype**		---	1 (2.4%)	---	---
CF genotype					
F508del/F508del		---	---	20 (50.0%)	20 (50.0%)
F508del/MF		---	---	20 (50.0%)	20 (50.0%)
No prior CFTR modulator	n (%)	---	---	24 (60.0%)	---
TEZ/IVA therapy		---	---	12 (30.0%)	---
LUM/IVA therapy		---	---	4 (10.0%)	---
Sweat chloride (mmol/L)	Mean (±SD)	---	---	95.8 (±13.3)	44.2 (±20.4)
	Change (±SD)	---	---	---	-51.6 (±17.3)
	P value	---	---	---	< 0.001

FEV_1 % predicted	Mean (±SD)	---	78.9 (±23.4)	41.7 (±20.4)	51.2 (±23.8)
	Change (±SD)	---	---	---	9.5 (±8.3)
	P value	---	---	---	< 0.001
BMI (kg/m²)	Mean (±SD)	24.2 (±3.2)	23.0 (±5.1)	19.3 (±2.4)	20.5 (±2.2)
	Change (±SD)	---	---	---	1.2 (±0.9)
	P value	---	---	---	< 0.001
Respiratory culture					
Haemophilus influenzae		---	11 (26.8%)	0 (0.0%)	1 (2.5%)
Staphylococcus aureus		---	13 (31.0%)	21 (52.5%)	19 (47.5%)
Streptococcus pneumoniae	n (%)	---	2 (4.8%)	0 (0.0%)	0 (0.0%)
Pseudomonas aeruginosa		---	19 (45.2%)	30 (75.0%)	30 (75.0%)
Mycobacteria (MOTT)		---	1 (2.4%)	0 (0.0%)	0 (0.0%)

Definition of abbreviations: BMI = body mass index; CF = cystic fibrosis; CC/RS = central complex/radial spokes defect; ETI = elexacaftor/tezacaftor/ivacaftor; FEV1 = forced expiratory volume in one second; IDA = inner dynein arm; IVA = ivacaftor; LUM = lumacaftor; MF = minimal function mutation; MTD = microtubular disorganization; ODA = outer dynein arm; PCD = primary ciliary dyskinesia; SD = standard deviation; TEZ = tezacaftor. *P* values describe the changes between paired measurements from baseline and elexacaftor/tezacaftor/ivacaftor therapy. * PCD diagnosis according to American Thoracic Society Criteria.

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Figure legends

Figure 1. Changes in viscoelastic properties of sputum of patients with primary ciliary dyskinesia (PCD) are comparable to changes in patients with cystic fibrosis (CF) on elexacaftor/tezacaftor/ivacaftor (ETI) therapy. Viscoelastic properties of sputum samples were determined by a cone and plate rheometer. (a-c) Measurements of the elastic modulus G' (a), the viscous modulus G'' (b), and the mesh size ξ (c) of sputum from healthy controls (n = 10), patients with PCD (n = 39) and patients with CF at baseline (BL) and at 3 months after initiation of ETI therapy (n = 40). Bars represent the group median, error bars represent the 25th and 75th percentile. *P < 0.001 compared with PCD; $^{\dagger}P$ < 0.05 and $^{\dagger\dagger}P$ < 0.01 compared with baseline; $^{\#}P$ < 0.01 and $^{\#\#}P$ < 0.001 compared with healthy.

Figure 2. Inflammation markers in sputum of patients with primary ciliary dyskinesia (PCD) are comparable to patients with cystic fibrosis (CF) on elexacaftor/tezacaftor/ivacaftor (ETI) therapy. (a-f) Measurements of DNA (a), myeloperoxidase (MPO) (b), interleukin (IL)-1β (c), IL-8 (d), IL-6 (e) and tumor necrosis factor alpha (TNF-α) (f) in sputum supernatant from healthy controls (n = 10-12), patients with PCD (n = 36-41) and patients with CF at baseline (BL) and at 3 months after initiation of ETI therapy (n = 36-40). Bars represent the group median, error bars represent the 25th and 75th percentile. *P < 0.05, **P < 0.01 and ***P < 0.001 compared with PCD; $^{\dagger}P$ < 0.05 and $^{\dagger\dagger}P$ < 0.001 compared with baseline; $^{\#}P$ < 0.05, $^{\#\#}P$ < 0.01 and $^{\#\#\#}P$ < 0.001 compared with healthy.

Figure 3. Neutrophil serine protease activity in sputum of patients with primary ciliary dyskinesia (PCD) is comparable to patients with cystic fibrosis (CF) on elexacaftor/tezacaftor/ivacaftor (ETI) therapy. (a-b) Measurement of free activity of neutrophil elastase (NE) (a), cathepsin G (CatG) (b) and proteinase 3 (PR3) (c) in cell-free sputum supernatants from healthy controls (n = 12-

13), patients with PCD (n = 40-42) and patients with CF at baseline (BL) and at 3 months after initiation of ETI therapy (n = 40). Bars represent the group median, error bars represent the 25th and 75th percentile. *P < 0.001 compared with PCD; $^{\dagger}P$ < 0.001 compared with baseline; $^{\#}P$ < 0.001 compared with healthy.

Figure 4. Changes in the sputum proteome of patients with primary ciliary dyskinesia (PCD) are comparable to changes in patients with cystic fibrosis (CF) on elexacaftor/tezacaftor/ivacaftor (ETI) therapy. (a) Heatmap of significantly differentially expressed proteins (cutoff P < 0.01) in sputum from healthy controls (n = 15), patients with PCD (n = 29) and patients with CF at baseline and at 3 months (n = 35) after initiation of ETI therapy (n = 35). Hierarchical clustering identified two major clusters with upregulated (cluster 1) and downregulated proteins (cluster 2) in PCD and CF compared to healthy controls. Proteins contributing to the most representative enriched gene ontology (GO) terms are highlighted in the first column. (b) Single sample gene set enrichment analysis of GO terms in PCD sputum compared to healthy controls (first column), CF baseline (second column) and CF after 3 months ETI treatment (third column) ordered by change in normalized enrichment score (NES) (FDR < 0.01).

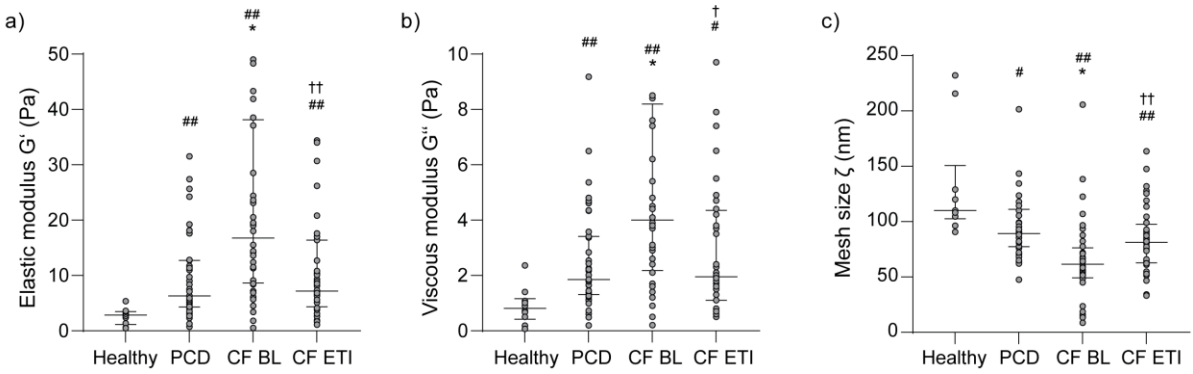
a)
Elastic modulus G' (Pa)
0
10
20
30
40
50
Healthy
PCD
CF BL
CF ETI
##
##
*
††
##
b)
Viscous modulus G'' (Pa)
0
2
4
6
8
10
##
##
*
†
#
c)
Mesh size ζ (nm)
0
50
100
150
200
250
#
##
*
††
##

Figure 1

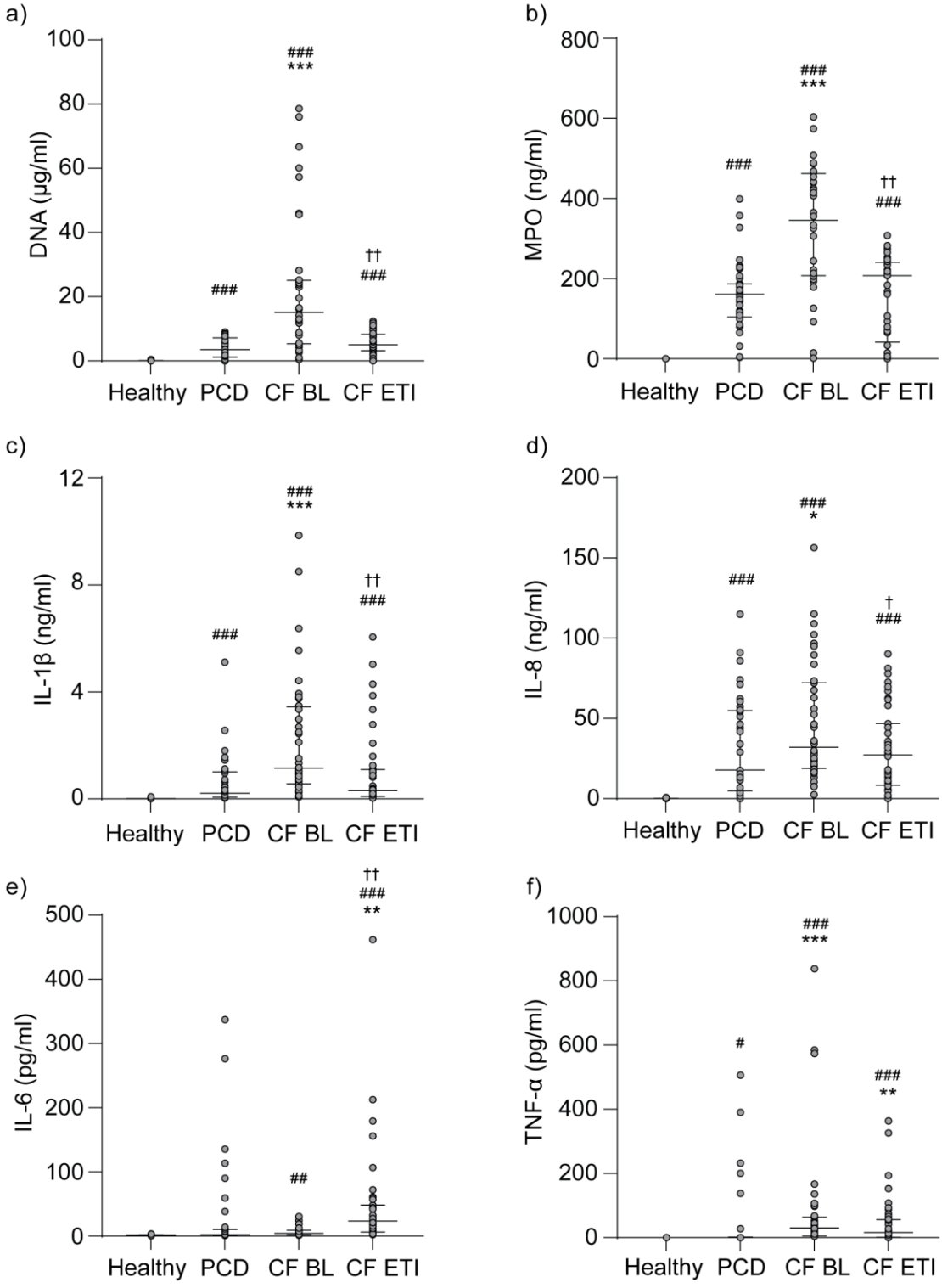
a)
DNA (µg/ml)
100
80
60
40
20
0
###
***
###
††
Healthy
PCD
CF BL
CF ETI
b)
MPO (ng/ml)
800
600
400
200
0
###
***
###
††
Healthy
PCD
CF BL
CF ETI
c)
IL-1β (ng/ml)
12
8
4
0
###
***
###
###
††
Healthy
PCD
CF BL
CF ETI
d)
IL-8 (ng/ml)
200
150
100
50
0
###
*
###
†
Healthy
PCD
CF BL
CF ETI
e)
IL-6 (pg/ml)
500
400
300
200
100
0
##
††
###
**
Healthy
PCD
CF BL
CF ETI
f)
TNF-α (pg/ml)
1000
800
600
400
200
0
#
###
***
###
**
Healthy
PCD
CF BL
CF ETI

Figure 2

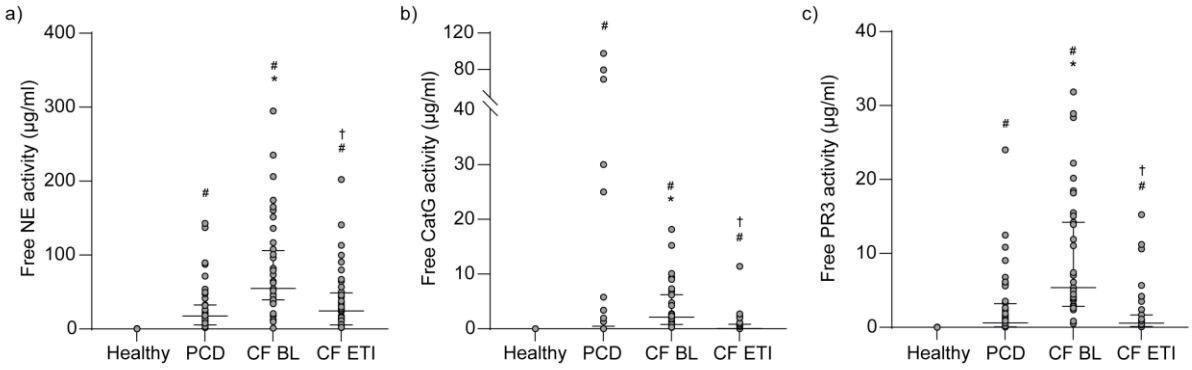
a)
Free NE activity (µg/ml)
400
300
200
100
0
Healthy
PCD
CF BL
CF ETI
b)
Free CatG activity (µg/ml)
120
80
40
30
20
10
0
Healthy
PCD
CF BL
CF ETI
c)
Free PR3 activity (µg/ml)
40
30
20
10
0
Healthy
PCD
CF BL
CF ETI

Figure 3

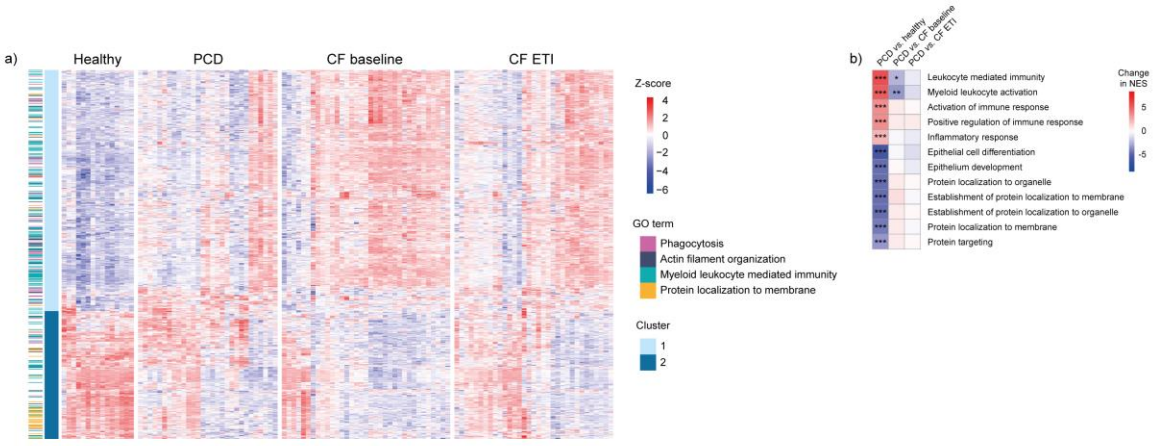
a)
Healthy
PCD
CF baseline
CF ETI
Z-score
4
2
0
−2
−4
−6
GO term
Phagocytosis
Actin filament organization
Myeloid leukocyte mediated immunity
Protein localization to membrane
Cluster
1
2
b)
PCD vs. healthy
PCD vs. CF baseline
PCD vs. CF ETI
Leukocyte mediated immunity
Myeloid leukocyte activation
Activation of immune response
Positive regulation of immune response
Inflammatory response
Epithelial cell differentiation
Epithelium development
Protein localization to organelle
Establishment of protein localization to membrane
Establishment of protein localization to organelle
Protein localization to membrane
Protein targeting
Change in NES
5
0
-5

Figure 4

Changes in Sputum Viscoelastic Properties and Airway Inflammation in Primary Ciliary Dyskinesia are Comparable to Cystic Fibrosis on Elexacaftor/Tezacaftor/Ivacaftor Therapy

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Online Supplementary Material

Supplementary methods

Study design and participants

Patients were eligible to participate if they had a confirmed PCD diagnosis according to the European Respiratory Society and American Thoracic Society guidelines [1, 2]. Patients with CF were included from the MODULATE-CF cohort and had at least one *F508del* allele and were aged 12 years and older as previously described [3]. CF patients from the MODULATE-CF cohort were matched with PCD patients for age, gender and the existence of matched sputum samples at baseline and at 3 months after initiation of treatment with ETI for paired analysis. Exclusion criteria were an acute respiratory infection or pulmonary exacerbation at the time of sputum collection [4]. Sputum rheology, inflammatory markers and proteomics were assessed in PCD patients that provided sputum at one of their routine visits to the outpatient clinic during the recruitment period, and in CF patients at baseline and 3 months after initiation of therapy with the approved dose of elexacaftor 200 mg and tezacaftor 100 mg every 24 hours in combination with ivacaftor 150 mg every 12 hours. For this subgroup of CF patients from the MODULATE-CF cohort, data on demographics, sputum rheology and inflammation markers were included in a previous study [3]. In the present study, additional inflammation markers such as DNA and MPO concentration and CatG and PR3 activity were determined and sputum proteomics was completely rerun and reanalyzed for comparison with PCD. Further, data of CF patients from MODULATE-CF were used for several additional analyses to assess the relationship between changes in viscoelastic properties and airway inflammation markers (supplementary figure S3 and S4), and the role of infection status and lung function impairment (supplementary tables S5 and S6).

Lung function

Spirometry was conducted and forced expiratory volume in one second (FEV1) was determined according to European Respiratory Society and American Thoracic Society

guidelines [5]. Percent predicted results were premised on equations of the global lung function initiative [6].

Sweat chloride concentration (SCC)

Sweat tests in patients with CF were conducted according to the German national diagnostic guideline [7] and the guidelines of the Clinical and Laboratory Standards Institute [8]. Sweat samples were collected after stimulation by pilocarpine iontophoresis with the Macroduct® system (Model 3700, Wescor, Logan UT, USA) and chloride concentration was determined in a minimum volume of 30 µl by using a chloridometer (KWM 20 Chloridometer, Kreienbaum, Langenfeld, Germany).

Sputum collection in healthy individuals

Collection of sputum samples from healthy controls was accomplished after inhalation of hypertonic saline (NaCl 3-6%) using a PARI boy classic and a LC sprint nebulizer (PARI GmbH, Starnberg, Germany). Before inhalation, the mouth was flushed with water to reduce saliva.

Sputum rheology

Sputum samples were immediately put on ice after expectoration or induction. Saliva and possible debris were removed by gentle aspiration with a pipette. Rheological measurements were subsequently conducted with a cone and plate rheometer (Kinexus Pro+, Netzsch GmbH, Selb, Germany) using a stainless-steel cone-plate geometry (cone-diameter 20 mm, cone-angle 1°). Samples were transferred onto the lower static plate of the rheometer with a non-electrostatic spatula. After sample loading and between sequences, temperature equilibration (5 min at 37°C) was conducted to ensure sufficient network relaxation. Each measurement comprised an amplitude sweep and a frequency sweep downwards. The amplitude sweep was performed at 1 Hz with a shear deformation γ between 0.01 - 100%. The frequency sweep was conducted at a shear deformation of 2% within a range from 10 to

0.1 Hz. The elastic modulus (storage modulus, G') and viscous modulus (loss modulus, G″) were determined directly from the linear viscoelastic region of the amplitude sweep. The effective mesh size ξ of the mucin network was calculated using the following formula as previously described [9]:

$$\xi = \frac{k_B T}{G}$$

with k_B being the Boltzmann constant, T the absolute temperature and G the shear modulus, that was approximated from the G' value of the frequency sweep at 1 Hz.

Sputum inflammation markers and DNA concentration

Sputum samples were treated with 10% (v/v) sputolysin (Calbiochem, Darmstadt, Germany) to obtain single-cell solutions. In cell-free sputum supernatants concentrations of interleukin (IL)-1 β, IL-6, IL-8 and tumor necrosis factor alpha (TNF-α) were measured using cytometric bead array kits according to the manufacturer's instructions (BD Biosciences, San Diego, CA, USA). Myeloperoxidase (MPO) concentrations were determined in cell-free sputum supernatants by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Enzo Life Sciences, Farmingdale, NY, USA). Free NE activity was measured in cell-free sputum supernatants using the FRET reporter NEmo-1 (Sirius Fine Chemicals, Bremen, Germany). Free cathepsin G (CatG) and proteinase 3 (PR3) activity in cell-free sputum supernatants was determined using the fluorescence-quenching substrates Abz-Glu-Pro-Phe-Trp-Glu-Asp-Gln-EDDnp and Abz-Val-Ala-Asp-Nva-Arg-Asp-Arg-Gln-EDDnp (PeptaNova, Sandhausen, Germany), respectively. Kinetic assays were performed at 25°C using a fluorescence microplate reader (SpectraMax iD5, Molecular Devices, San Jose, CA, USA) and reporter cleavage was recorded over time (for NE: $\lambda_{Excitation}$ = 354 nm, $\lambda_{Emission\ Donor}$ = 400 nm, $\lambda_{Emission\ Acceptor}$ = 490 nm; for CatG and PR3: $\lambda_{Excitation}$ = 320 nm, $\lambda_{Emission}$ = 420 nm). Subsequently, neutrophil protease activity was determined by applying an enzyme standard curve as previously described [10-12]. DNA concentrations

were determined in cell-free sputum supernatants using the Quant-iT PicoGreen dsDNA assay kit according to the manufacturer's instructions (Invitrogen, Waltham, MA, USA).

Sputum proteomics

Sodium dodecyl sulfate (SDS) buffer (4% SDS, 100 mM Tris-HCl pH 8, 1 mM EDTA, 150 mM NaCl) was added to sputum samples in a 1:1 volume to volume ratio followed by an incubation at 95°C for 10 min to inactivate and solubilize proteins. Protein concentration was measured by a BCA assay and 100 µg protein were used for downstream analysis. Proteins were reduced and alkylated with 10 mM dithiothreitol and 40 mM chloroacetamide at 95°C for 10 min, followed by Benzonase® endonuclease (25U, Merck, Darmstadt, Germany) treatment for 15 min. Subsequently, a single-pot solid-phase-enhanced sample-preparation (SP3) protocol was applied for protein clean-up [13]. Protein containing beads were treated with 2 µg peptide-N-glycosidase F (NEB, Ipswich, MA, USA) in 50 mM ABC buffer for 1 hour at 37°C, followed by enzymatic digestion with trypsin (Promega, Madison, WI, USA) and lysyl endopeptidase (Fujifilm Wako Pure Chemical Corporation, Richmond, VA, USA) at a 1:50 enzyme:substrate ratio overnight at 37°C. The peptide containing supernatant was collected and desalted using C18 stage tips [14]. Peptide samples (2 µg) were subsequently measured on an EASY-nLC 1200 System coupled to an Orbitrap HF-X mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) running on data dependent acquisition (DDA) mode as previously described [15]. Raw data were analyzed using the MaxQuant software package (Ver. 2.0.3.0; Max Planck Institute of Biochemistry, Martinsried, Germany) and a decoy human UniProt database (2023-03) [16]. Variable modifications of N-terminal acetylation, deamidation (N, Q), oxidation (M) and fixed modification of carbamidomethyl cysteine were selected. For peptide and protein identification a false discovery rate (FDR) of 1% was chosen, and unique and razor peptides were considered for quantification. Further, "Match between runs" and label-free quantitation (LFQ) algorithm were applied. Data analysis was done in R (V 4.2.2). Data were filtered by removing reverse hits, proteins only identified by site and potential contaminants and proteins identified by at least two peptides or at least 5

MS/MS counts with an Andromeda score above 20. Outlier patient samples, defined by number of proteins identified (<550) and principal component analysis, were excluded and proteins identified in at least 25% of the remaining patient samples were included in further analysis. LFQ intensities were log2-transformed and missing values were substituted by random values deduced from a normal distribution with a width of 0.3 and a down shift of 1.8. Moderated t-testing and moderated F testing were applied for statistical analysis [17]. *P*-values were adjusted using the Benjamini-Hochberg method with cutoff values of 0.05 or 0.01. Single sample gene set enrichment analysis (ssGSEA) was conducted using the ssGSEA2.0 package by Broad Institute [18, 19] and over representation analysis of proteins/cluster analysis was performed using the clusterProfiler package [20]. For both analyses only gene ontology (GO) terms of biological processes by the MsigSB [19] with a minimum size of 50 genes were considered. Filtering for at least 50 genes in the gene sets was conducted to remove gene sets that are not well represented within the data. Filtering for similarity between gene sets was not performed to prevent introduction of a bias by a chosen score and to prevent a further reduction of the list of gene sets. Proteomics of sputum samples from CF patients included in the MODULATE-CF cohort [3] was completely rerun and reanalyzed for comparison with PCD. Raw data and a table with all identified proteins, including *p* and adjusted *q* values, are available on the Proteomics Identifications Database PRIDE [21] (https://www.ebi.ac.uk/pride/; project accession number: PXD061751).

Magnetic resonance imaging

T1-weighted sequences before and after intravenous contrast, T2-weighted sequences, and first-pass four-dimensional (4D) perfusion imaging were acquired using a clinical 1.5T MR scanner (Magnetom Avanto or Magnetom Aera, Siemens, Erlangen, Germany). Images were assessed by an experienced thoracic radiologist (FD) for abnormalities in lung morphology and perfusion using a dedicated morpho-functional MRI score as previously described [22-24]. Perfusion studies were performed with intravenous administration of macrocyclic gadolinium-based contrast medium. The MRI morphology score comprises subscores for (1) bronchial wall abnormalities (wall thickening and/or bronchiectasis), (2) mucus plugging, (3) sacculations and/or abscesses, (4) consolidations, and (5) pleural reaction including effusion. The extent of these structural abnormalities as well as abnormal perfusion are rated in each lobe as 0 (no abnormality), 1 (<50% of the lobe involved), or 2 (≥50% of the lobe involved). The MRI global score results from the sum of the MRI morphology and MRI perfusion score. In this real-world study, MRI studies were limited to 35 PCD patients and 24 CF patients, in part due to the limited availability of MRI scan time in the clinical setting. MRI scans of CF patients were obtained as part of the MODULATE-CF study [25], but reanalyzed for comparison with PCD.

Statistics

Data of subgroup analyses are presented as group median and interquartile ranges or mean with standard deviation and were compared by Mann-Whitney test, Wilcoxon matched-pairs signed rank test, one-way ANOVA or Kruskal-Wallis test, as appropriate. The Benjamini-Hochberg method was used for multiple comparisons correction. Correlations analysis was performed using Spearman correlation. $P < 0.05$ was accepted to indicate statistical significance.

Supplementary references

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Supplementary tables

Supplementary table S1. List of genotypes of included patients with primary ciliary dyskinesia.

Predicted Ciliary Ultrastructure	Gene	First allele	Second allele
ODA	*DNAI1 (NM_012144.4)*	*Exon 3, c.138del*	*Exon 3, c.138del*
ODA	*DNAI1 (NM_012144.4*	*Exon 1, c.48+2dupT*	*Exon 10, c.817-2A>T*
ODA	*DNAI1 (NM_012144.4*	*Exon 1, c.48+2dupT*	*Exon 1, c.48+2dupT*
ODA	*DNAI2 (NM_023036.6)*	*Exon 4, c.346-3T>G*	*Exon 4, c.346-3T>G*
ODA	*DNAH5 (NM_001369.3)*	*Exon 7, c974dupA*	*Exon 27, c.4348C>T*
ODA	*DNAH5 (NM_001369.3)*	*Exon 54, c.8998C>T*	*Exon 63, c.10815delT*
ODA	*DNAH5 (NM_001369.3)*	*Exon 50, c.8440_8447del*	*Exon 74, c.12753T>G*
ODA	*DNAH5 (NM_001369.3)*	*Exon 27, c.4236 C>T*	*Exon 57, c.9637del*
ODA	*DNAH5 (NM_001369.3)*	*Exon 50, c.8440_8447del*	*Exon 25, c.4053+1G>T/Exon 25, c.3880G>A, Exon 42 c.6970A>G, Exon 43 c.7223 T>C*
ODA	*DNAH5 (NM_001369.3)*	*Exon 61, c.10384C>T*	*Exon 72, c.12430C>T*
ODA	*DNAH5 (NM_001369.3)*	*Exon 50, c.8440_8447del*	*Exon 63, c.10815del/Exon 42 c.6970A>G, Exon 43 c.7223 T>C*
ODA	*ODAD2 (NM_018076.5)*	*Exon 15, c.2147T>G*	*Exon 19, c. 2825*
ODA	*ODAD2 (NM_018076.5)*	*Exon 18, c.2675C>A*	*Exon 18, c.2675C>A*
ODA	*ODAD1 (NM_001364171.2)*	*Exon 4, c.337C>T*	*Exon 7, c.742G>A*
IDA	*DNAH1 (NM_015512.5)*	*Exon 32, c.5140A>T*	*Exon, 69, c.11072C>T*
ODA + IDA	*CCDC103 (NM_213607.2)*	*Exon 4, c.568_569dupAG*	*Exon 4, c.568_569dupAG*
ODA + IDA	*DNAAF1 (NM_178452.6)*	*Exon 5 c.1349dupC*	*Exon 5 c.1349dupC*
ODA + IDA	*DNAAF1 (NM_178452.6*	*Exon 5, c.683C>T*	*Exon 5, c.683C>T*
ODA + IDA	*DNAAF11 (NM_012472.6)*	*Exon 5, c.436G>C*	*Exon 5, c.436G>C*
ODA + IDA	*SPAG1* (*NM_003114.5)*	*Exon 16, c.2014C>T*	*Exon 1 and 2, Del. Ex 1-2*
ODA + IDA	*DNAAF6 (NM_173494.2)*	*Exon 5, c.266G>A (X-linked)*	*- - -*
MTD	*CCDC40 (NM_017950.4)*	*Exon 3, c.248delC*	*Exon 3, c.248delC*

MTD	*CCDC39 (NM_181426.2)*	*Exon 19, c.2596G>T*	*Exon 19, c.2596G>T*
MTD	*CCDC65 (NM_033124.5)*	*Exon 2, c.268 C>T*	*Exon 2, c.268C>T*
CPD	*RSPH3 (NM_031924)*	*Exon 1, c45.73del*	*Exon 1, c45.73del*
CPD	*RSPH4A (NM_001010892.3)*	*Exon 3, c.1105G>C*	*Exon 3, c.1105G>C*
CPD	*RSPH4A (NM_001010892.3)*	*Exon 3, c.1453C>T*	*Exon 3, c.1453C>T*
CPD	*RSPH4A (NM_001010892.3)*	*Exon 3, c1453C>T*	*Exon 4, c.1707del*
NU/CPD	*HYDIN (NM_001270974.2)*	*Exon 18, c.23494C>A*	*Exon 64, c.10816G>T*
NU	*DNAH11 (NM_001277115.2)*	*Exon 75, c.12363C>G*	*Exon 82, c.13531_*36del*
NU	*DNAH11 (NM_001277115.2)*	*Exon 26, c.4669C>T*	*Exon 70, c.11374-18A>G*
NU	*DNAH11 (NM_001277115.2)*	*Exon 26, c.4904_4905delAT*	*Exon 77, c.12899G>A/Exon 79, c.13181C>G*

Definitions of abbreviations of ultrastructure defects and genes: CCDC39 = coiled-coil domain-containing protein 39; CCDC40 = coiled-coil domain-containing protein 40; CCDC103 = coiled-coil domain containing protein 103; CPD = central pair defect; DNAAF1 = dynein arm assembly factor 1; DNAAF11 = dynein arm assembly factor 11; DNAI1 = dynein axonemal intermediate chain 1; DNAI2 = dynein axonemal intermediate chain 2; DNAH 5 = dynein arm heavy chain 5; DNAH1 = dynein arm heavy chain 1; DNAH11 = dynein arm heavy chain 11; IDA = inner dynein arm; MTD = microtubular disorganization; NU = (near) normal ultrastructure; ODA = outer dynein arm; ODAD 1 = outer dynein arm docking complex subunit 1; ODAD 2 = outer dynein arm docking complex subunit 2; RSPH3 = radial spoke head protein 3 homolog; RSPH4A = radial spoke head protein 4 homolog A; SPAG1 = sperm associated antigen 1

Supplementary table S2. List of cystic fibrosis transmembrane conductance regulator (*CFTR*) genotypes of included patients with cystic fibrosis.

First allele	Second allele	Number of patients
F508del	*F508del*	21
F508del	*N1303K*	3
F508del	*R347P*	3
F508del	*G542X*	2
F508del	*CFTRdele2,3*	1
F508del	*3302T>A*	1
F508del	*R1158X*	1
F508del	*W1282X*	1
F508del	*R709X*	1
F508del	*1525-1G>A*	1
F508del	*2991del32*	1
F508del	*p.Leu863fs/p.iie1027Thr*	1
F508del	*CFTRdele17a,17b*	1
F508del	*1078delT*	1
F508del	*R1066C*	1

Supplementary table S3. Maintenance therapy in patients with primary ciliary dyskinesia and patients with cystic fibrosis at baseline and after initiation of elexacaftor/tezacaftor/ivacaftor.

Clinical characteristic		**PCD patients**	**CF patients at baseline**	**CF patients with 3 months ETI**
Number of individuals		42	40	40
Inhaled hypertonic saline	n (%)	33 (78.6%)	29 (72.5%)	27 (67.5%)
	Change (%)	---	---	-2 (5.0%)
	P value	---	---	0.626
rhDNase	n (%)	---	24 (60.0%)	23 (57.5%)
	Change (%)	---	---	-1 (2.5%)
	P value	---	---	0.820
Inhaled antibiotics	n (%)	6 (14.3%)	32 (80.0%)	32 (80.0%)
	Change (%)	---	---	0
	P value	---	---	---
Oral azitromycin	n (%)	18 (42.9%)	19 (47.5%)	19 (47.5%)
	Change (%)	---	---	0
	P value	---	---	---
Other systemic antibiotics	n (%)	6 (14.3%)	12 (30.0%)	8 (18.3%)
	Change (%)	---	---	-4 (10.0%)
	P value	---	---	0.302

Definitions of abbreviations: CF = cystic fibrosis; ETI = elexacaftor/tezacaftor/ivacaftor; PCD = primary ciliary dyskinesia; rhDNase = human recombinant deoxyribonuclease.

Supplementary table S4. Lung magnetic resonance imaging scores for patients with primary ciliary dyskinesia (PCD) and patients with cystic fibrosis (CF) before and after initiation of elexacaftor/tezacaftor/ivacaftor (ETI) therapy. Perfusion studies were obtained in 30 of 35 patients with PCD, 20 of 24 patients with CF at baseline and 21 of 24 patients with CF on ETI.

		PCD	**CF baseline**	**CF ETI**
Number of individuals	n	35	24	24
MRI global score	Prevalence n (%)	29 (96.7)	21 (100)	21 (100)
	Mean (±SD)	20.6 (±8.1)	31.2 (±5.9)***	26.1 (±9.2)*$^{\#}$
MRI morphology score	Prevalence n (%)	34 (97.1)	24 (100)	24 (100)
	Mean (±SD)	14.6 (±6.4)	22.4 (±6.7)**	18.6 (±7.2)*
MRI perfusion score	Prevalence n (%)	29 (96.7)	20 (100)	21 (100)
	Mean (±SD)	5.6 (±2.2)	9.4 (±1.4)***	7.9 (±2.5)**$^{\#}$
Bronchiectasis/wall thickening subscore	Prevalence n (%)	34 (97.1)	24 (100)	24 (100)
	Mean (±SD)	7.1 (±2.4)	10.4 (±1.8)***	9.9 (±2.4)***
Mucus plugging subscore	Prevalence n (%)	33 (94.3)	24 (100)	20 (83.3)
	Mean (±SD)	3.9 (±2.4)	6.6 (±2.7)**	4.6 (±3.2)$^{\#}$
Abscesses/sacculations subscore	Prevalence n (%)	0 (0.0)	5 (20.8)	3 (12.5)
	Mean (±SD)	0.0 (±0.0)	0.5 (±1.1)*	0.4 (±1.1)
Consolidation subscore	Prevalence n (%)	25 (71.4)	18 (75.0)	11 (45.8)
	Mean (±SD)	1.5 (±1.3)	1.8 (±1.7)	0.9 (±1.2)
Special findings subscore	Prevalence n (%)	27 (77.1)	17 (70.8)	18 (75.0)
	Mean (±SD)	2.0 (±1.9)	2.8 (±2.6)	2.7 (±2.4)

Definitions of abbreviations: CF = cystic fibrosis; ETI = elexacaftor/tezacaftor/ivacaftor; MRI = magnetic resonance imaging; PCD = primary ciliary dyskinesia; SD = standard deviation.

*P < 0.05 compared with PCD; **P < 0.001 compared with PCD; ***P < 0.0001 compared with PCD; $^{\#}P$ < 0.05 compared with CF baseline

Supplementary table S5. Subgroup analysis of patients with primary ciliary dyskinesia and patients with cystic fibrosis at baseline and after initiation of elexacaftor/tezacaftor/ivacaftor according to FEV_1 % predicted.

		PCD			CF baseline			CF ETI		
FEV_1 % predicted		<40%	40-70%	>70%	<40%	40-70%	>70%	<40%	40-70%	>70%
G‘ (Pa)	n	2	15	21	24	12	4	13	21	6
	Mean (±SD)	4.8 (±2.8)	7.4 (±7.1)$^{\#\#}$	10.1 (±8.2)	19.4 (±14.6)	29.6 (±30.5)	7.9 (±7.5)	12.0 (±11.0)	9.9 (±9.5)$^{\#\#}$	8.8 (±4.2)
G‘‘ (Pa)	n	2	15	21	24	12	4	13	21	6
	Mean (±SD)	1.9 (±0.9)	2.0 (±1.0)$^{\#}$	2.8 (±2.3)	4.5 (±3.3)	7.6 (±8.7)	2.2 (±1.9)*	3.1 (±2.9)	2.7 (±2.2)$^{\#}$	2.2 (±1.4)
DNA (µg/ml)	n	3	15	21	21	12	3	13	17	6
	Mean (±SD)	4.1 (±2.7)$^{\#}$	6.0 (±2.8)	2.8 (±2.6)*	26.1 (±24.1)	18.3 (±18.2)	7.6 (±8.0)	6.1 (±3.2)$^{\#\#}$	5.5 (±3.2)$^{\#}$	5.5 (±4.8)
MPO (ng/ml)	n	3	15	21	21	12	3	13	17	6
	Mean (±SD)	208.5 (±107.1)	165.7 (±95.1)$^{\#\#}$	133.7 (±86.1)	333.1 (±160.6)	334.3 (±155.4)	269.7 (±234.5)	140.8 (±110.7)$^{\#\#}$	166.2 (±101.3)$^{\#\#}$	150.4 (±114.5)
IL-1β (ng/ml)	n	3	15	22	24	12	4	13	21	6
	Mean (±SD)	0.5 (±0.4)	4.5 (±12.4)	0.4 (±0.5)	2.8 (±2.7)	1.3 (±1.3)	1.5 (±1.7)	1.3 (±1.9)	0.9 (±1.3)	1.0 (±1.5)
IL-8 (ng/ml)	n	3	15	22	24	12	4	13	21	6
	Mean (±SD)	66.7 (±53.0)	120.6 (±212.1)	45.6 (±73.9)	56.9 (±40.4)	37.0 (±26.8)	17.0 (±12.5)	36.3 (±31.4)	28.8 (±22.8)	25.6 (±27.3)
IL-6 (pg/ml)	n	3	15	22	24	12	4	13	21	6
	Mean (±SD)	15.7 (±18.1)	21.4 (±40.3)	31.3 (±67.7)§§	6.2 (±6.3)	6.8 (±7.2)	8.0 (±4.7)	22.7 (±21.1)$^{\#}$	41.8 (±98.2)	120.9 (±76.5)*†
TNF-α (pg/ml)	n	3	15	22	24	12	4	13	21	6
	Mean (±SD)	0.7 (±0.6)$^{\#}$	62.1 (±119.8)	18.4 (±57.3)§	124.7 (±221.9)	35.4 (±44.0)	11.7 (±16.0)	78.4 (±124.0)	30.9 (±44.6)	43.1 (±60.7)
Free NE activity (µg/ml)	n	3	15	22	24	12	4	13	21	6
	Mean (±SD)	42.6 (±41.4)	43.4 (±45.1)	16.9 (±17.9)$^{\#}$	96.1 (±76.5)	61.8 (±40.1)	23.3 (±21.0)	54.6 (±55.8)	33.1 (±35.9)$^{\#}$	17.1 (±20.9)
Free CatG activity (µg/ml)	n	3	15	22	24	12	4	13	20	6
	Mean (±SD)	32.6 (±56.3)	7.6 (±18.7)	3.7 (±17.0)**	4.7 (±4.9)	3.0 (±2.8)	0.6 (±0.3)	0.6 (±0.9)$^{\#\#\#}$	1.0 (±2.5)$^{\#\#}$	0.4 (±0.5)
Free PR3 activity (µg/ml)	n	3	15	22	24	12	4	13	20	6
	Mean	4.3	3.7	1.6	11.6	6.7	3.0	2.3	2.3	1.0

	(±SD)	(±7.1)	(±3.3)	(±5.1)*	(±8.3)	(±8.4)	(±2.9)	(±3.9)$^{\#\#\#}$	(±4.1)$^{\#\#}$	(±1.4)

Definitions of abbreviations: CatG = cathepsin G; CF = cystic fibrosis; DNA = deoxyribonucleic acid; ETI = elexacaftor/tezacaftor/ivacaftor; FEV1 = forced expiratory volume in one second; G' = elastic modulus; G'' = viscous modulus; IL = interleukin; MPO = myeloperoxidase; NE = neutrophil elastase; PCD = primary ciliary dyskinesia; PR3 = proteinase 3; SD = standard deviation; TNF-α = tumor necrosis factor α.

*P < 0.05 compared with ppFEV$_1$=40-70% within patient group; **P < 0.01 compared with ppFEV$_1$=40-70% within patient group; $^{\dagger}P$ < 0.05 compared with ppFEV$_1$<40% within patient group; $^{\#}P$ < 0.05 compared with CF baseline within ppFEV$_1$ group; $^{\#\#}P$ < 0.01 compared with CF baseline within ppFEV$_1$ group; $^{\#\#\#}P$ < 0.001 compared with CF baseline within ppFEV$_1$ group; $^{\S}P$ < 0.05 compared with CF ETI within ppFEV$_1$ group; $^{\S\S}P$ < 0.01 compared with CF ETI within ppFEV$_1$ group.

Supplementary table S6. Subgroup analysis of patients with primary ciliary dyskinesia and patients with cystic fibrosis at baseline and after initiation of elexacaftor/tezacaftor/ivacaftor according to microbial status.

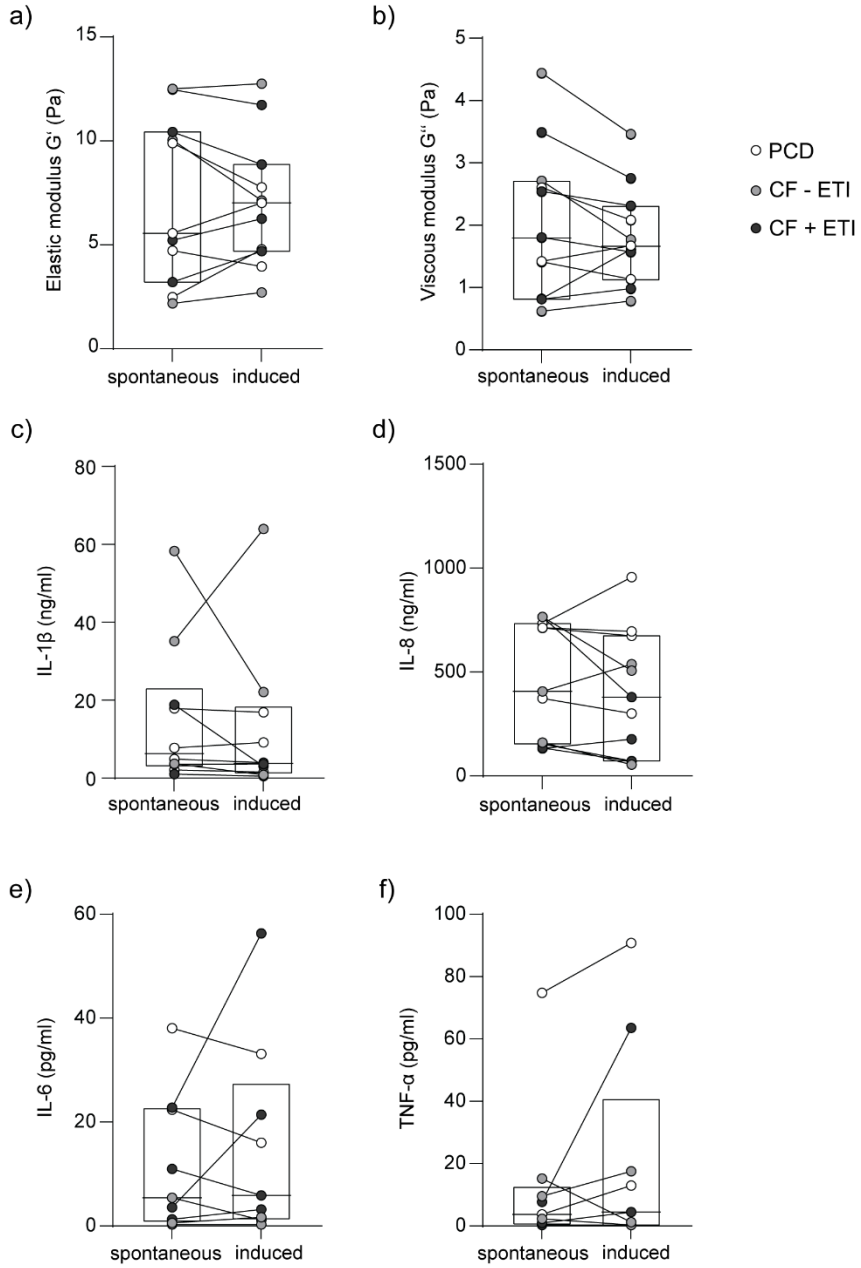
		PCD						CF baseline						CF ETI					
		H. influenzae		*S. aureus*		*P. aeruginosa*		*H. influenzae*		*S. aureus*		*P. aeruginosa*		*H. influenzae*		*S. aureus*		*P. aeruginosa*	
		-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
G‘ (Pa)	n	27	11	23	16	21	18	40	0	19	21	10	30	39	1	21	19	10	30
	Mean (±SD)	10.5 (±8.7)	5.7 (±2.9)	8.8 (±7.4)	9.7 (±8.3)	8.8 (±7.3)	9.6 (±8.4)	24.8 (±29.3)	-	20.9 (±25.4)	27.9 (±32.4)	16.9 (±12.8)	27.6 (±33.0)	13.1 (±16.3)	4.1 (±0.0)	3.2 (±2.8)	8.9 (±6.3)	7.7 (±4.5)	11.9 (±10.4)
	P value	0.251		0.899		0.967		---		0.690		0.438		---		0.002		0.523	
G“ (Pa)	n	27	11	23	16	21	18	40	0	19	21	10	30	39	1	21	19	10	30
	Mean (±SD)	2.7 (±2.1)	1.8 (±0.8)	2.4 (±1.9)	2.5 (±1.8)	2.4 (±1.6)	2.6 (±2.1)	6.2 (±8.0)	-	5.4 (±7.4)	5.2 (±4.1)	4.0 (±2.8)	5.7 (±6.5)	3.4 (±4.0)	1.1 (±0.0)	3.2 (±2.8)	3.6 (±4.9)	2.0 (±1.4)	3.9 (±4.5)
	P value	0.450		0.927		0.906		---		0.769		0.527		---		0.931		0.263	
DNA (µg/ml)	n	29	11	25	16	22	19	36	0	16	20	8	28	35	1	20	16	8	28
	Mean (±SD)	4.3 (±3.1)	4.0 (±2.9)	3.4 (±3.0)	5.2 (±2.9)	3.0 (±2.8)	5.4 (±2.9)	22.0 (±21.7)	-	16.4 (±11.6)	26.4 (±26.8)	22.4 (±27.2)	21.8 (±20.5)	5.8 (±3.3)	0.2 (±0.0)	6.0 (±2.9)	5.3 (±4.0)	4.0 (±2.8)	6.2 (±3.5)
	P value	0.550		0.055		0.005		---		0.765		0.695		---		0.459		0.193	
MPO (ng/ml)	n	29	11	25	16	22	19	36	0	16	20	8	28	35	1	20	16	8	28
	Mean (±SD)	168.6 (±92.7)	111.7 (±73.1)	140.4 (±91.0)	168.1 (±89.0)	128.4 (±95.4)	177.6 (±77.8)	337.5 (±152.8)	-	307.6 (±173.2)	362.7 (±132.7)	343.6 (±174.4)	335.7 (±149.4)	163.4 (±100.3)	0.3 (±0.0)	160.6 (±104.7)	156.4 (±130.3)	175.1 (±129.1)	153.9 (±95.7)
	P value	0.090		0.217		0.045		---		0.589		0.954		---		0.882		0.384	
IL-1β (ng/ml)	n	29	12	26	16	23	19	40	0	19	21	10	30	39	1	21	19	10	30
	Mean (±SD)	0.7 (±1.1)	0.9 (±1.3)	0.6 (±0.9)	1.1 (±1.4)	0.63 (±1.0)	0.9 (±1.3)	2.2 (±2.3)	-	2.5 (±2.7)	1.9 (±1.9)	2.5 (±3.1)	2.1 (±2.1)	1.0 (±1.5)	0.0 (±0.0)	1.0 (±1.6)	1.0 (±1.4)	0.3 (±0.4)	1.2 (±1.7)
	P value	0.487		0.180		0.389		---		0.344		0.981		---		0.825		0.145	
IL-8 (ng/ml)	n	29	12	26	16	23	19	40	0	19	21	10	30	39	1	21	19	10	30
	Mean (±SD)	32.0 (±28.8)	36.2 (±36.1)	32.1 (±35.4)	47.0 (±37.5)	31.0 (±36.7)	45.0 (±35.5)	47.0 (±36.5)	-	51.2 (±32.3)	43.1 (±40.3)	48.1 (±39.3)	46.6 (±36.3)	31.5 (±26.0)	0.3 (±0.0)	31.0 (±26.8)	30.5 (±26.0)	23.8 (±18.0)	32.8 (±28.0)
	P value	0.903		0.206		0.157		---		0.282		0.975		---		0.979		0.483	
IL-6 (pg/ml)	n	29	12	26	16	23	19	40	0	19	21	10	30	39	1	21	19	10	30
	Mean (±SD)	15.7 (±33.6)	49.5 (±83.4)	34.7 (±74.6)	31.3 (±71.8)	49.0 (±92.5)	15.5 (±33.4)	6.5 (±6.4)	-	7.8 (±8.4)	5.4 (±3.7)	8.3 (±9.0)	6.0 (±5.5)	48.4 (±83.8)	9.6 (±0.0)	19.1 (±18.3)	78.8 (±112.0)	62.5 (±77.6)	43.1 (±85.1)
	P value	0.139		0.589		0.027		---		0.789		0.463		---		0.022		0.2907	
TNF-α (pg/ml)	n	29	12	26	16	23	19	40	0	19	21	10	30	39	1	21	19	10	30
	Mean (±SD)	29.9 (±101.7)	123.7 (±194.3)	32.7 (±92.1)	93.9 (±188.6)	45.4 (±101.3)	68.9 (±175.0)	84.9 (±175.1)	-	120.2 (±216.0)	53.0 (±124.5)	194.4 (±303.6)	53.1 (±103.1)	49.4 (±82.5)	0.4 (±0.0)	48.7 (±79.8)	47.6 (±86.2)	50.0 (±105.1)	47.6 (±75.9)
	P value	0.084		0.278		0.759		---		0.083		0.371		---		0.867		0.649	

Free NE activity (µg/ml)	n	29	12	26	16	23	19	40	0	19	21	10	30	39	1	21	19	10	30
	Mean (±SD)	25.6 (±24.5)	33.8 (±50.6)	25.6 (±31.4)	30.4 (±37.8)	24.8 (±38.6)	32.0 (±26.8)	78.6 (±66.2)	-	96.5 (±71.6)	62.4 (±57.9)	78.0 (±73.0)	78.8 (±65.4)	38.6 (±43.2)	2.4 (±0.0)	47.1 (±52.4)	27.3 (±27.1)	23.2 (±31.3)	41.9 (±45.4)
	P value	0.543		0.848		0.065		---		0.069		0.874		---		0.320		0.177	
Free CatG activity (µg/ml)	n	29	12	26	16	23	19	39	0	19	20	9	29	38	1	18	21	9	29
	Mean (±SD)	10.6 (±26.0)	0.8 (±1.8)	3.0 (±13.6)	15.0 (±30.4)	3.8 (±16.6)	12.1 (±27.0)	3.8 (±4.2)	-	3.5 (±3.1)	4.1 (±5.2)	3.3 (±4.1)	3.9 (±4.3)	0.8 (±1.9)	0.0 (±0.0)	1.0 (±2.5)	0.4 (±0.8)	0.4 (±0.8)	0.8 (±2.1)
	P value	0.2163		0.045		0.006		---		0.647		0.670		---		0.3431		0.966	
Free PR3 activity (µg/ml)	n	29	12	26	16	23	19	39	0	19	20	9	29	38	1	18	21	9	29
	Mean (±SD)	2.8 (±5.1)	2.4 (±3.1)	1.7 (±2.8)	4.2 (±6.3)	2.2 (±5.3)	3.2 (±3.6)	9.1 (±8.4)	-	9.2 (±9.1)	9.1 (±8.0)	8.9 (±9.2)	9.2 (±8.4)	2.1 (±3.7)	0.0 (±0.0)	2.1 (±3.8)	2.0 (±3.6)	1.7 (±3.4)	2.2 (±3.8)
	P value	0.871		0.084		0.097		---		0.813		0.635		---		0.631		0.955	

Definitions of abbreviations: CatG = cathepsin G; CF = cystic fibrosis; DNA = deoxyribonucleic acid; ETI = elexacaftor/tezacaftor/ivacaftor; G' = elastic modulus; G'' = viscous modulus; *H. influenzae* = *Haemophilus influenzae*; IL = interleukin; MPO = myeloperoxidase; NE = neutrophil elastase; *P. aeruginosa* = *Pseudomonas aeruginosa*; PCD = primary ciliary dyskinesia; PR3 = proteinase 3; *S. aureus* = *Staphylococcus aureus*; SD = standard deviation; TNF-α = tumor necrosis factor α

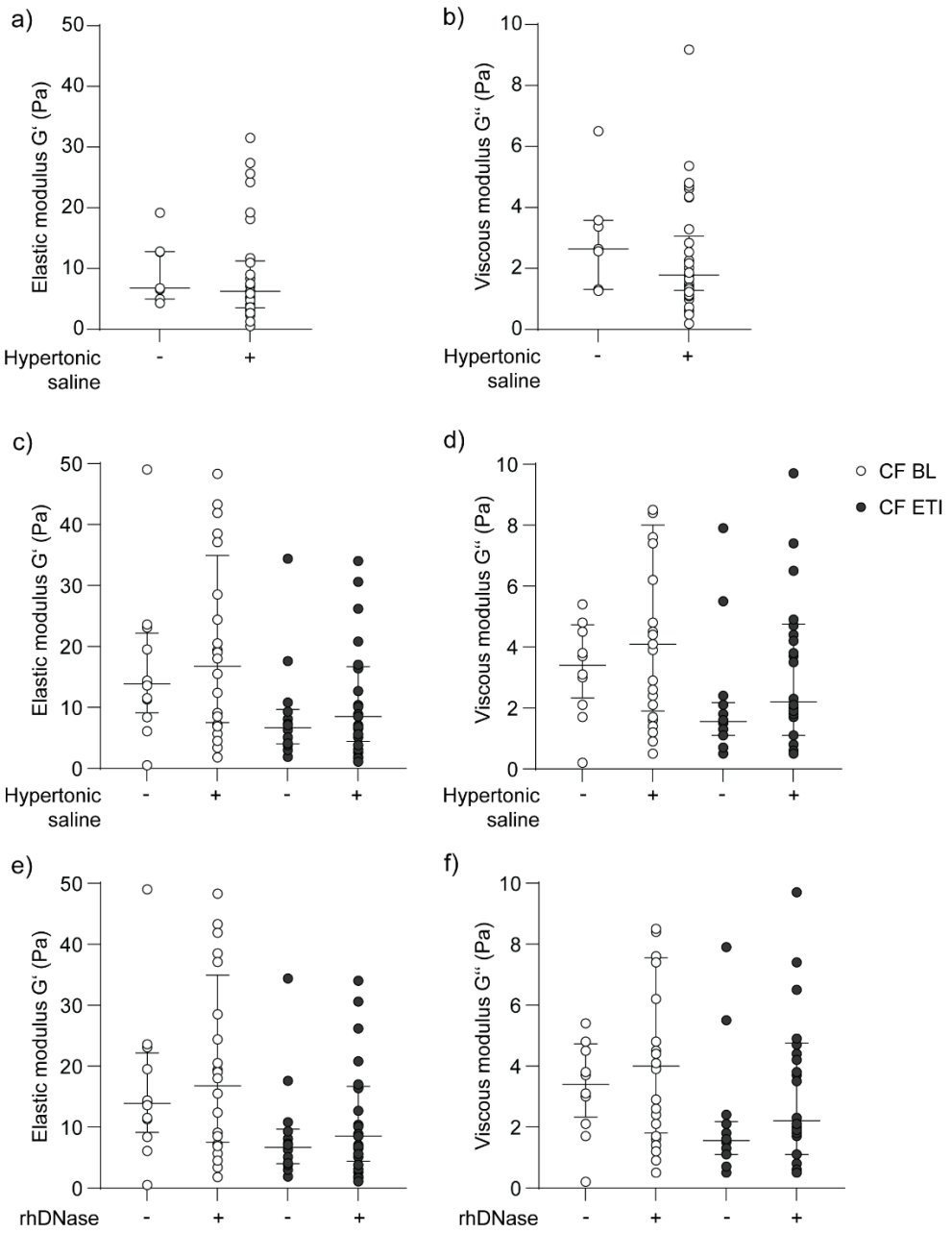
Supplementary figures

Supplementary figure S1



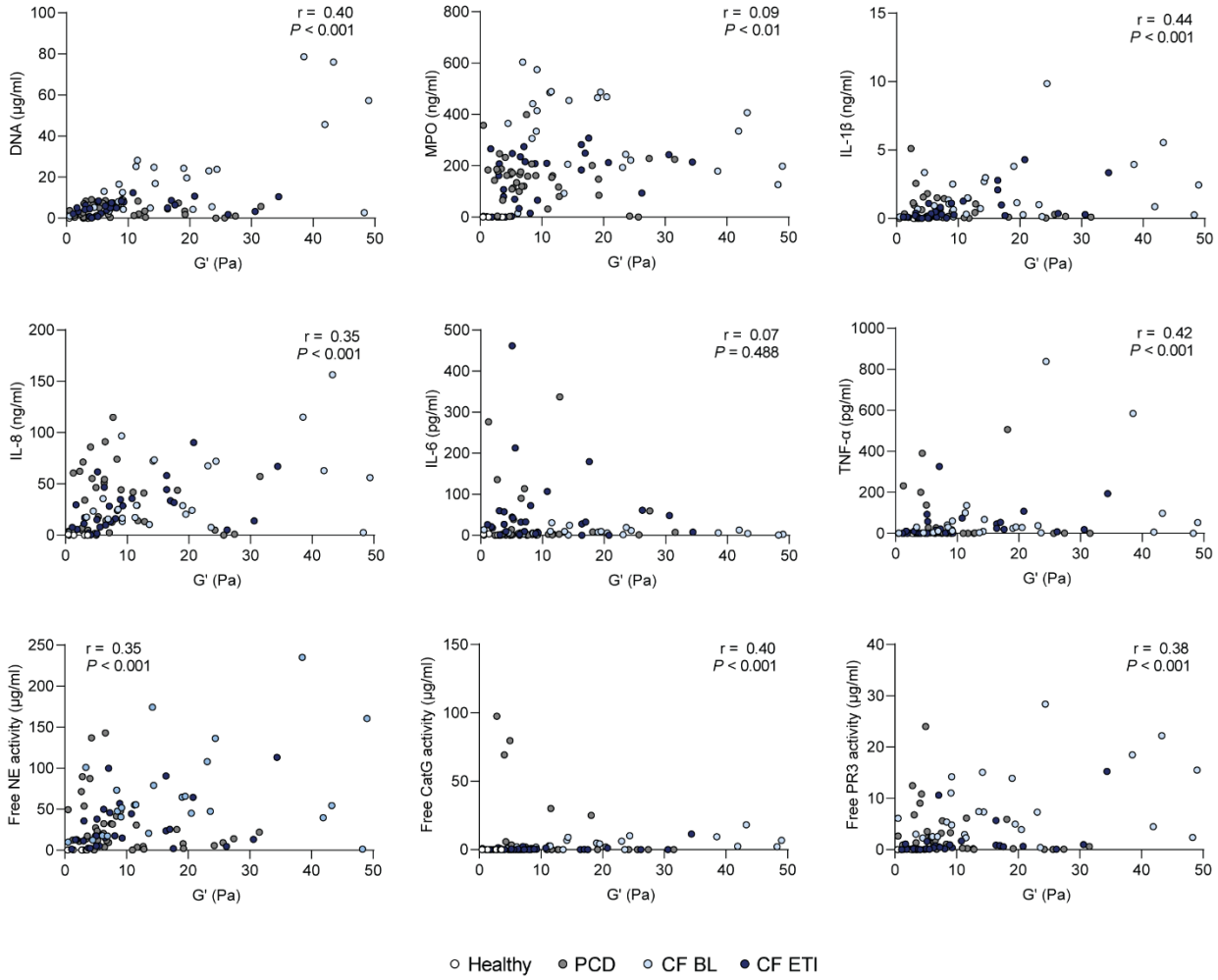
Supplementary figure S1. Comparison of viscoelastic properties and inflammation markers in spontaneously expectorated vs. induced sputum from patients with primary ciliary dyskinesia (PCD) (n = 4) and patients with cystic fibrosis (CF) without (- ETI; n = 3) or with elexacaftor/tezacaftor/ivacaftor (ETI) therapy (+ ETI; n = 4). Induced sputum was collected within 24 h after spontaneous sputum expectoration from the same patient. Elastic modulus G' (a), viscous modulus G'' (b). Interleukin (IL)-1β (c), IL-8 (d), IL-6 (e) and tumor necrosis factor (TNF)-α (f) levels in sputum supernatant. Boxes represent the 25th and 75th percentile with the group median (middle bar).

Supplementary figure S2



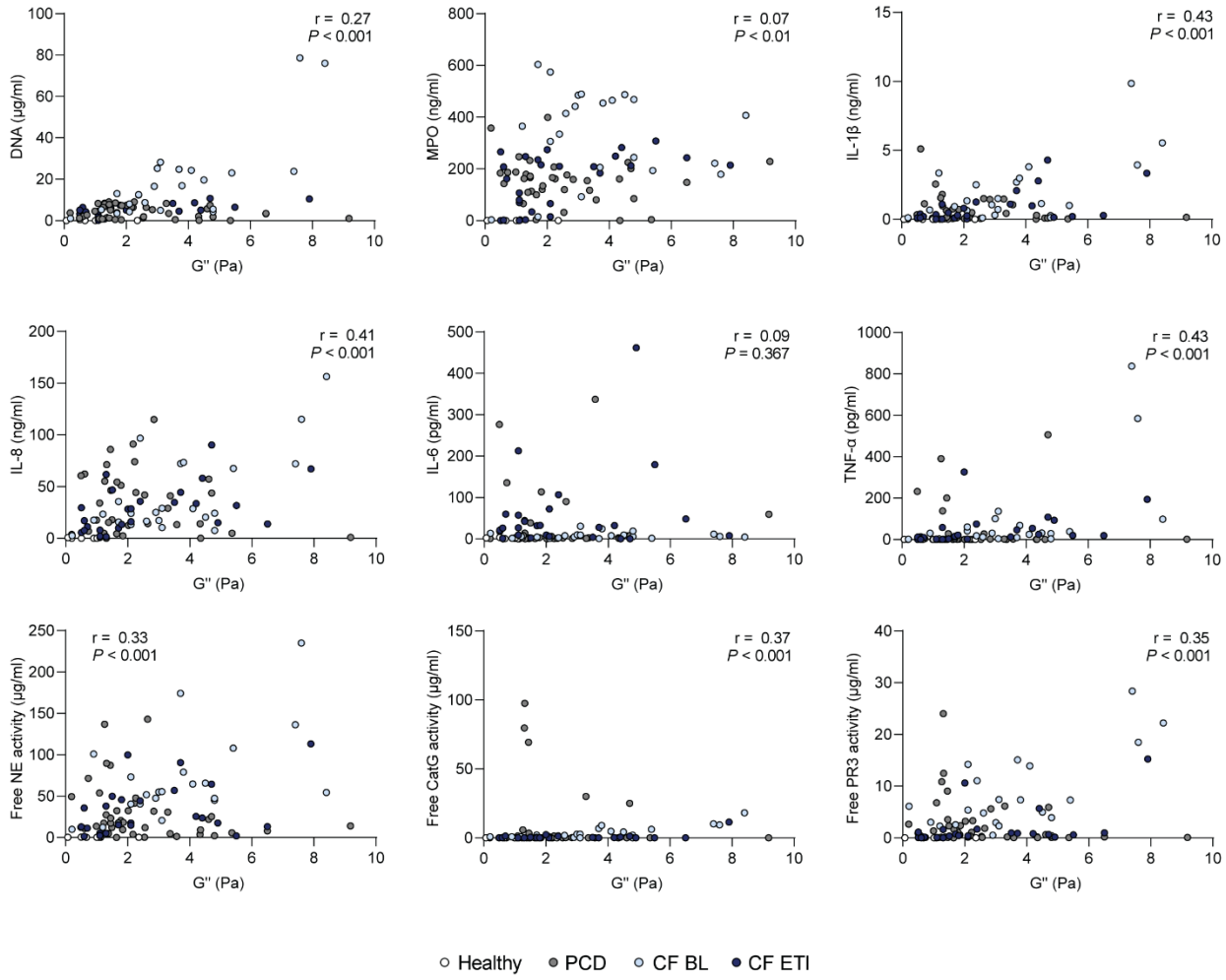
Supplementary figure S2. (a-b) Comparison of the elastic modulus G' (a) and viscous modulus G'' (b) of sputum from patients with primary ciliary dyskinesia (PCD) without (n = 7) or with (n = 32) inhaled hypertonic saline as maintenance therapy. (c-f) Sputum viscoelastic properties of patients with cystic fibrosis (CF) at baseline (BL) and at 3 months after initiation of elexacaftor/tezacaftor/ivacaftor (ETI) therapy without (BL: n = 11, ETI: n = 13) or with (BL: n = 29, ETI: n = 27) inhaled hypertonic saline (c-d) and without (BL: n = 16, ETI: n = 17) and with BL: n = 24, ETI: n = 23) inhalation therapy with rhDNase as maintenance therapy (e-f). Bars represent the group median, error bars represent the 25th and 75th percentile.

Supplementary figure S3



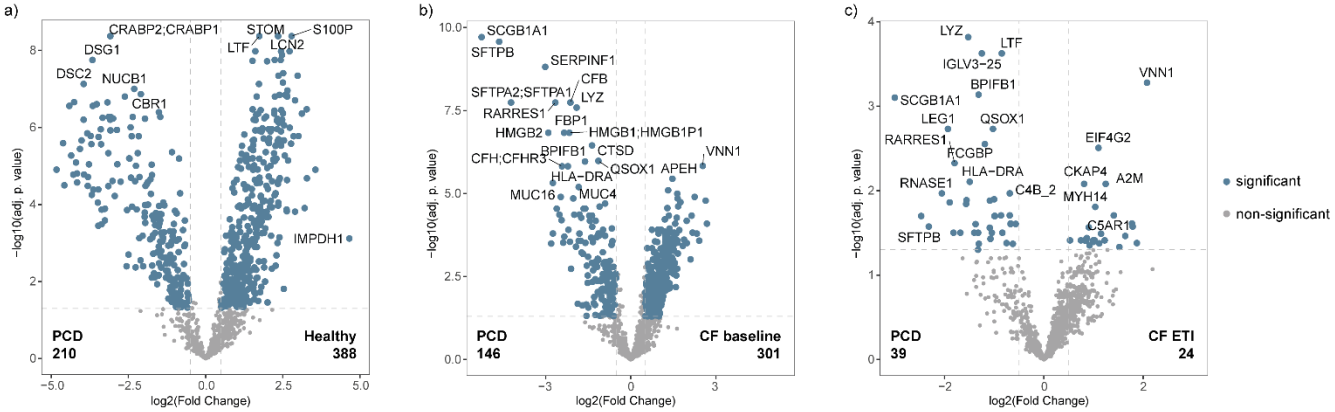
Supplementary figure S3. Relationship between the elastic modulus (G') and inflammation markers in sputum from the entire study population including healthy controls, patients with primary ciliary dyskinesia (PCD) and patients with cystic fibrosis (CF) at baseline (BL) and at 3 months after initiation of elexacaftor/tezacaftor/ivacaftor (ETI) therapy. Spearman correlation coefficients r and *P* values are provided for each correlation. Definitions of abbreviations: CatG = cathepsin G; DNA = deoxyribonucleic acid; IL = interleukin; MPO = myeloperoxidase; NE = neutrophil elastase; PR3 = proteinase 3; TNF-α = tumor necrosis factor α.

Supplementary figure S4



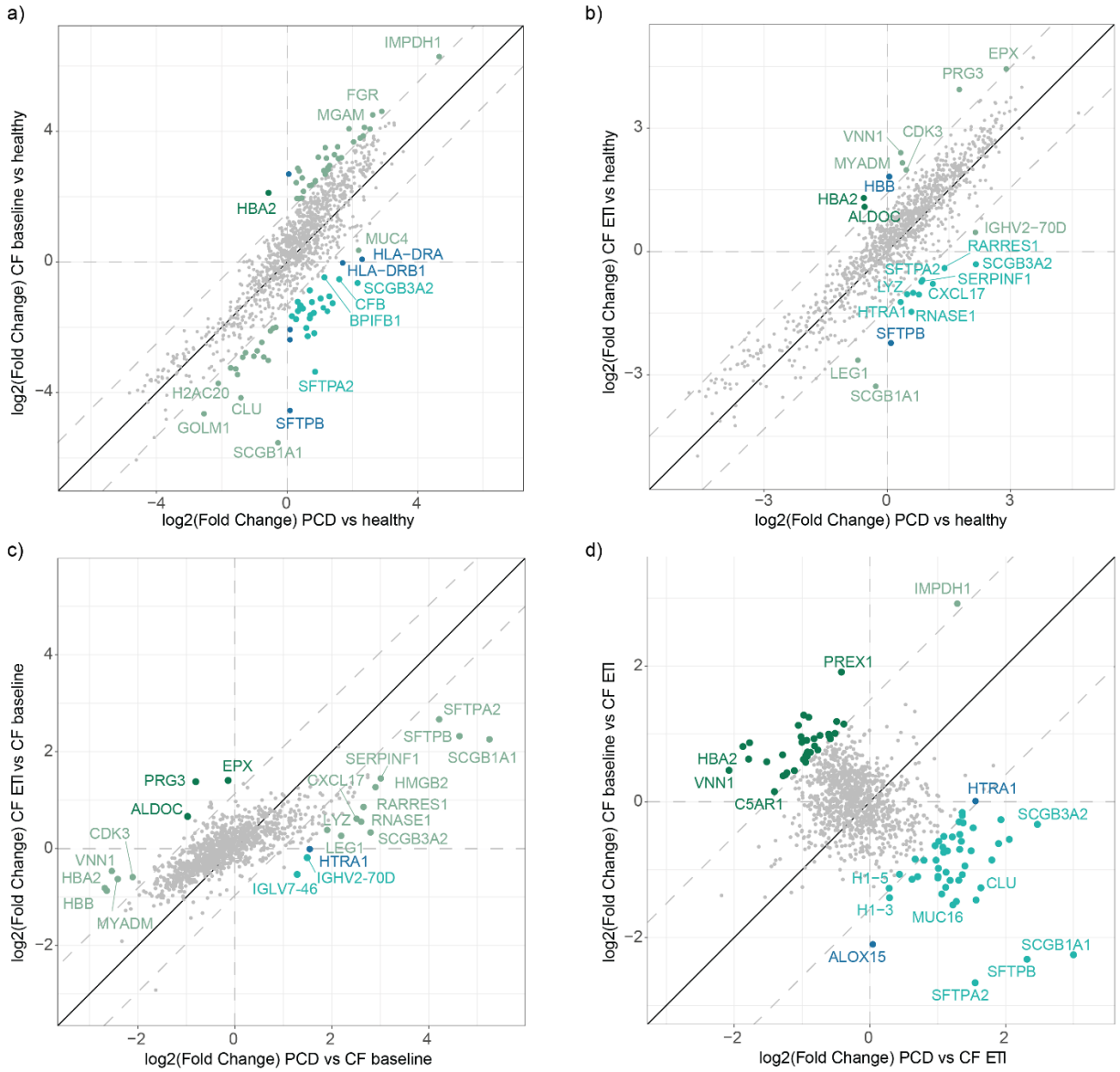
Supplementary figure S4. Relationship between the viscous modulus (G'') and inflammation markers in sputum from the entire study population including healthy controls, patients with primary ciliary dyskinesia (PCD) and patients with cystic fibrosis (CF) at baseline (BL) and at 3 months after initiation of elexacaftor/tezacaftor/ivacaftor (ETI) therapy. Spearman correlation coefficients r and *P* values are provided for each correlation. Definitions of abbreviations: CatG = cathepsin G; DNA = deoxyribonucleic acid; IL = interleukin; MPO = myeloperoxidase; NE = neutrophil elastase; PR3 = proteinase 3; TNF-α = tumor necrosis factor α.

Supplementary figure S5



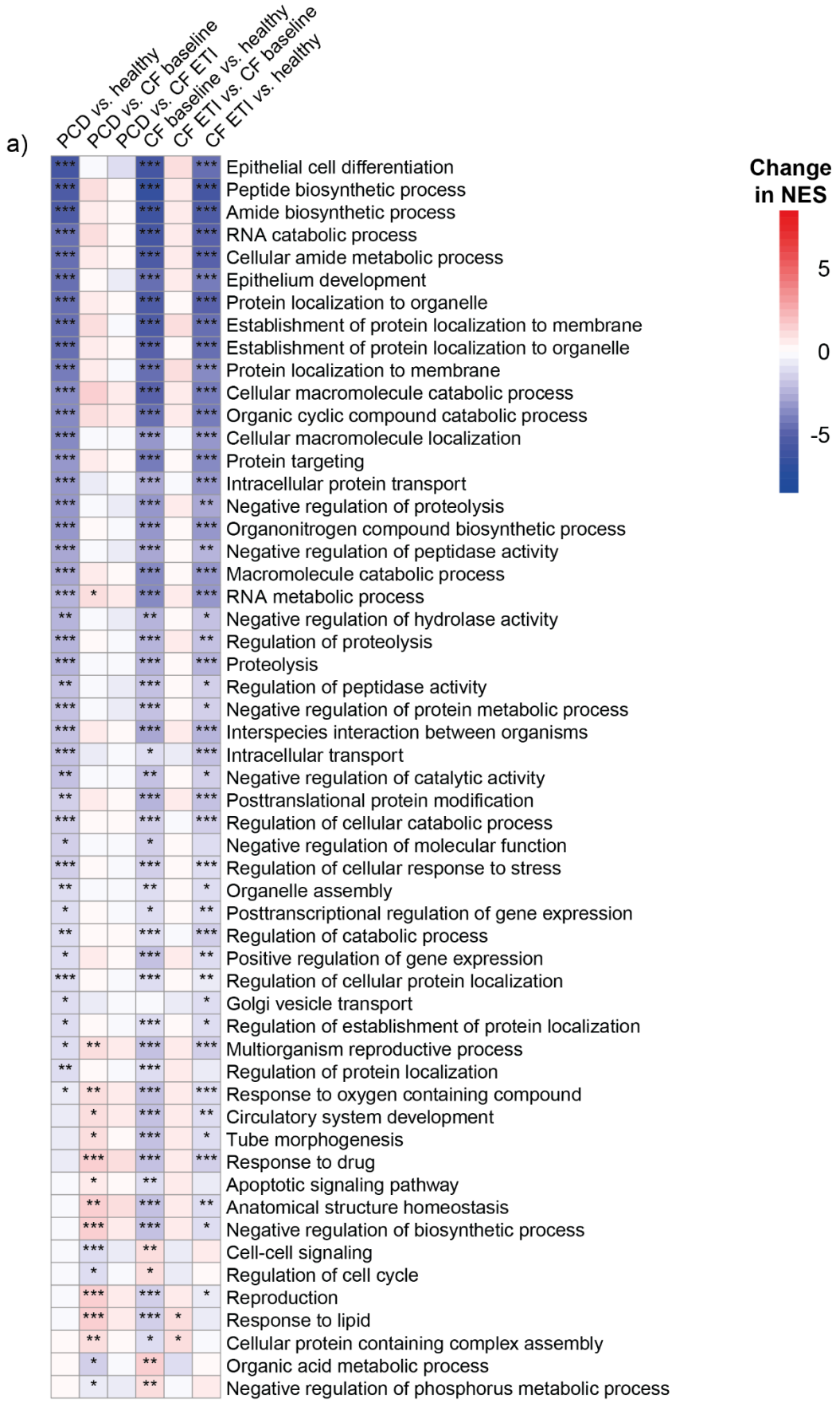
Supplementary figure S5. (a-c) Volcano plots demonstrating comparisons of protein expression in sputum from patients with primary ciliary dyskinesia (PCD) versus healthy individuals (598 differential proteins) (a), PCD patients versus patients with cystic fibrosis (CF) before elexacaftor/tezacaftor/ivacaftor (ETI) initiation (447 differential proteins) (b) and PCD patients versus CF patients at 3 months on ETI (63 differential proteins) (c). Moderated t-testing was used for statistical analysis and *P* values were adjusted by Benjamini-Hochberg correction with a cut-off value of 0.05 and a fold change cut-off of 0.5.

Supplementary figure S6

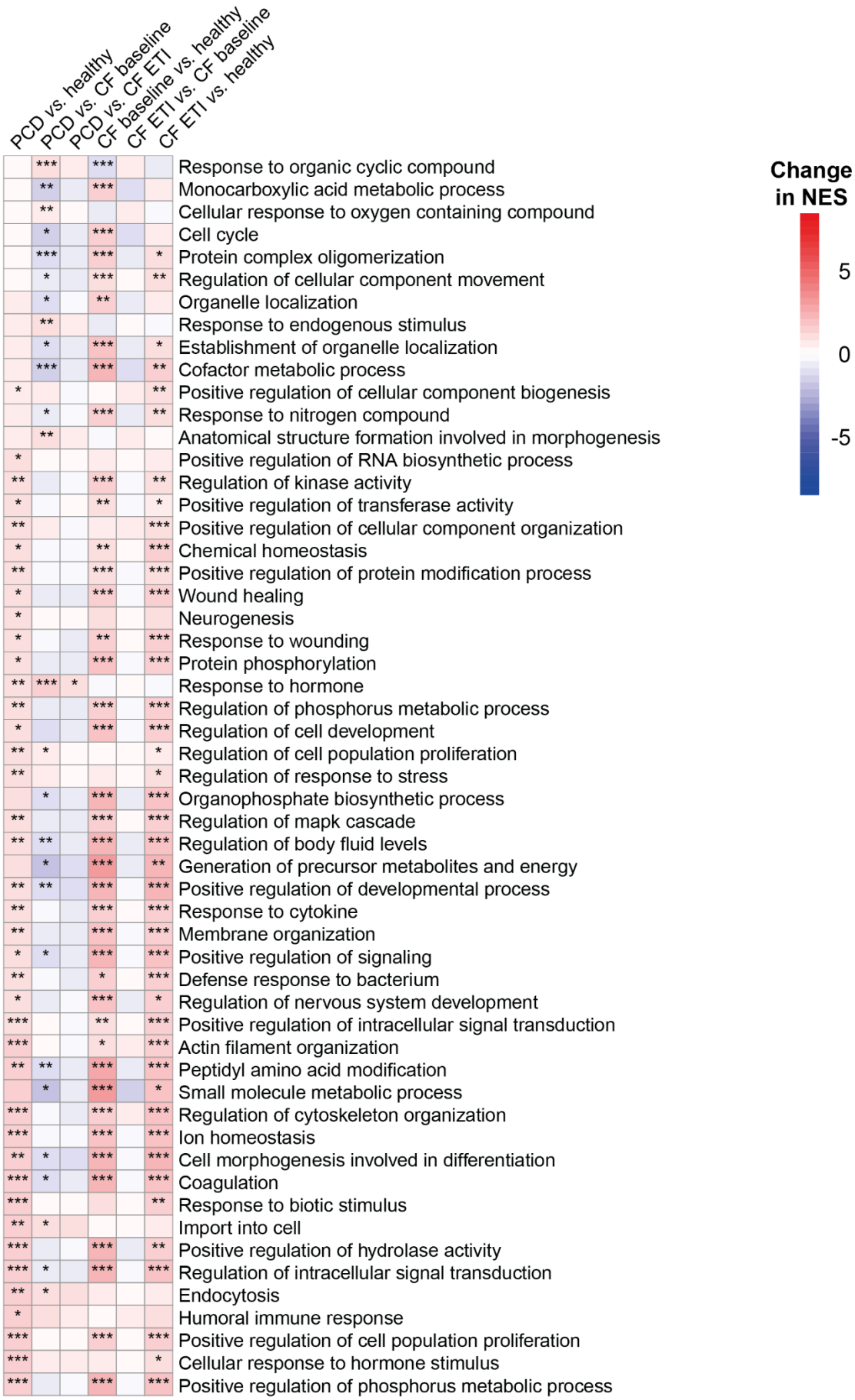


Supplementary figure S6. (a-d) Log2-fold changes of protein expression showing multiple comparisons of patients with primary ciliary dyskinesia (PCD) with patients with cystic fibrosis (CF) and healthy controls. Log2-fold protein expression was compared in PCD and CF patients before elexacaftor/tezacaftor/ivacaftor (ETI) treatment versus healthy individuals (a), in PCD and CF patients on 3 months ETI versus healthy individuals (b), in PCD patients and CF patients on 3 months ETI versus CF prior to ETI (c) and in PCD patients and CF patients at baseline versus CF patients at 3 months on ETI (d). Light green-colored proteins develop into the same direction, while certain proteins change only in one group (blue) or into opposite directions (dark green, cyan). A fold change cut-off value of 1.5 was chosen.

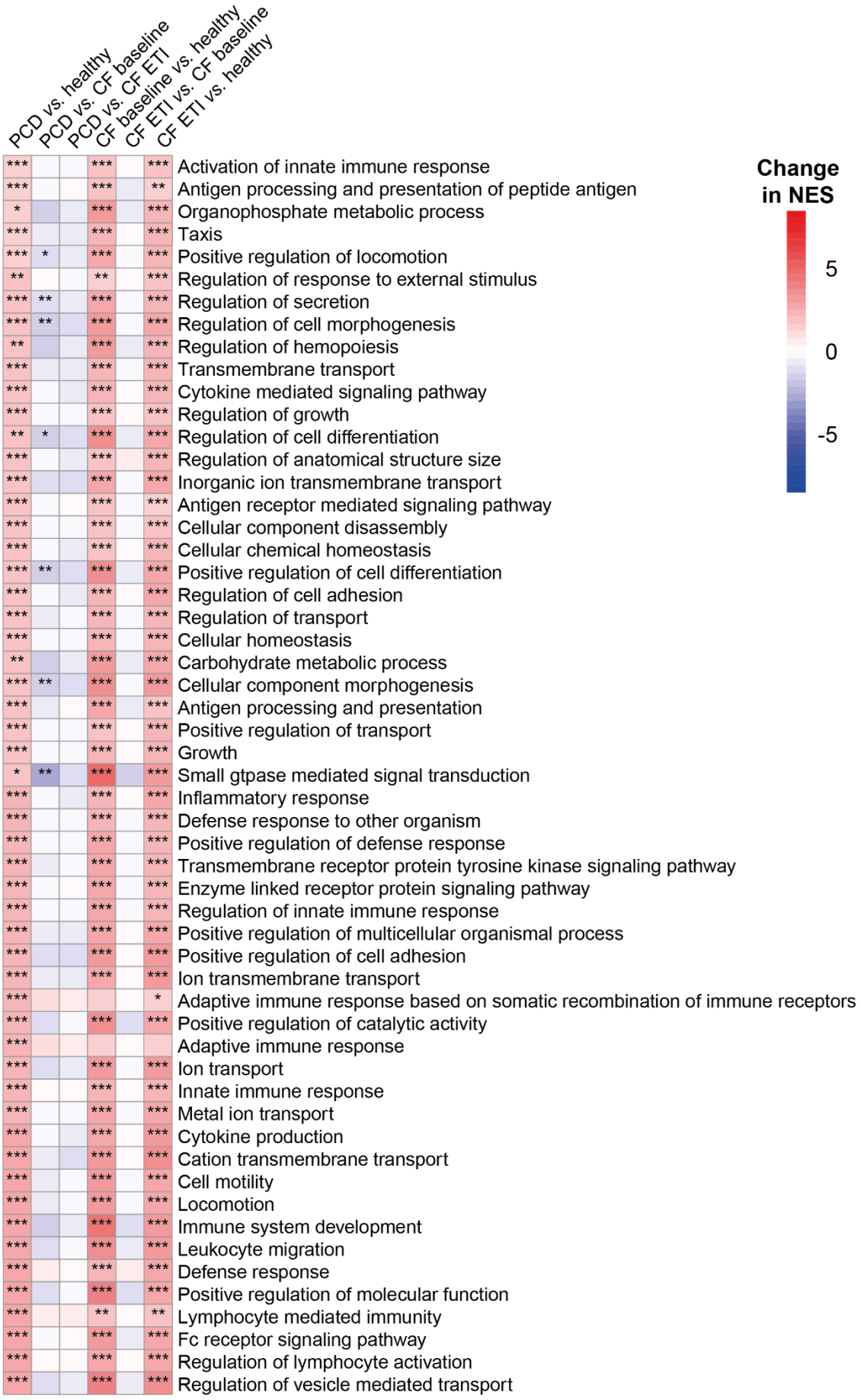
Supplementary figure S7

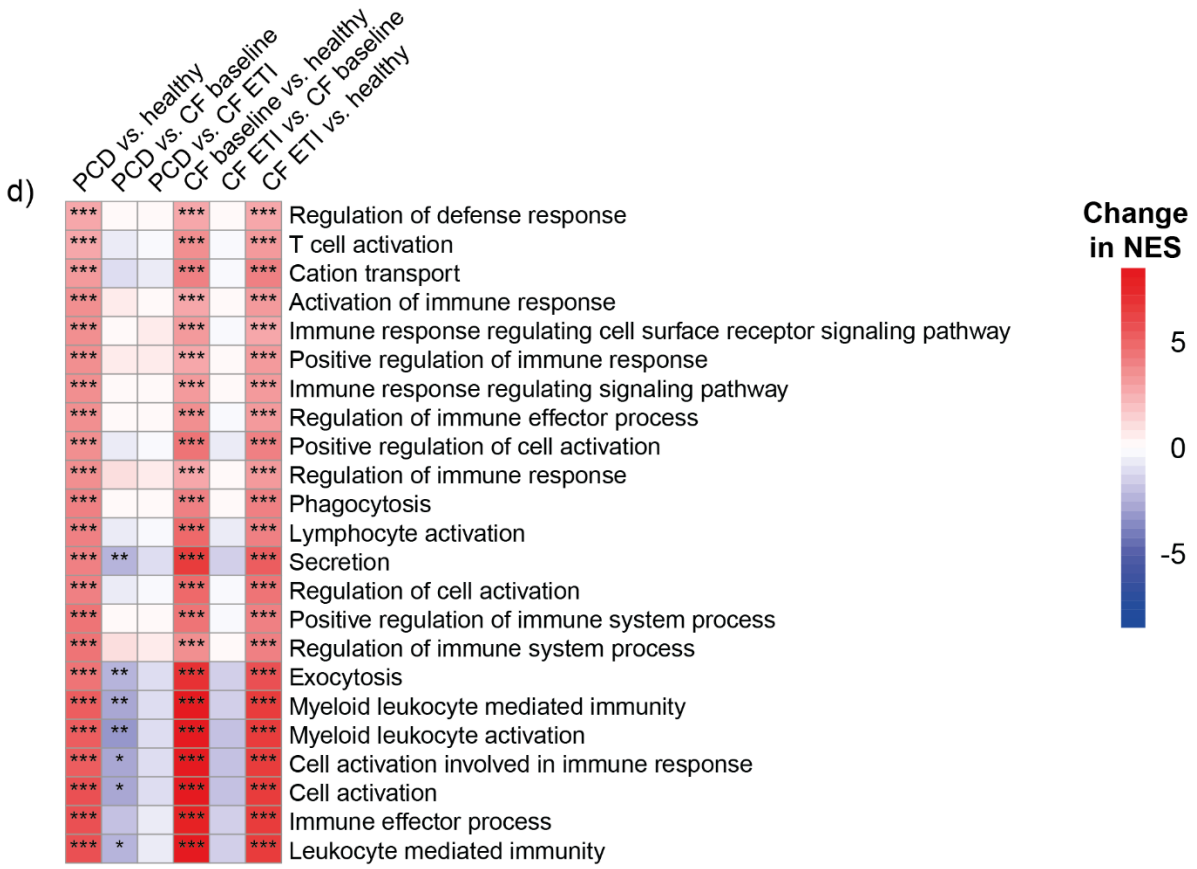


b)


PCD vs. healthy
PCD vs. CF baseline
PCD vs. CF ETI
CF baseline vs. healthy
CF ETI vs. CF baseline
CF ETI vs. healthy
Response to organic cyclic compound
Monocarboxylic acid metabolic process
Cellular response to oxygen containing compound
Cell cycle
Protein complex oligomerization
Regulation of cellular component movement
Organelle localization
Response to endogenous stimulus
Establishment of organelle localization
Cofactor metabolic process
Positive regulation of cellular component biogenesis
Response to nitrogen compound
Anatomical structure formation involved in morphogenesis
Positive regulation of RNA biosynthetic process
Regulation of kinase activity
Positive regulation of transferase activity
Positive regulation of cellular component organization
Chemical homeostasis
Positive regulation of protein modification process
Wound healing
Neurogenesis
Response to wounding
Protein phosphorylation
Response to hormone
Regulation of phosphorus metabolic process
Regulation of cell development
Regulation of cell population proliferation
Regulation of response to stress
Organophosphate biosynthetic process
Regulation of mapk cascade
Regulation of body fluid levels
Generation of precursor metabolites and energy
Positive regulation of developmental process
Response to cytokine
Membrane organization
Positive regulation of signaling
Defense response to bacterium
Regulation of nervous system development
Positive regulation of intracellular signal transduction
Actin filament organization
Peptidyl amino acid modification
Small molecule metabolic process
Regulation of cytoskeleton organization
Ion homeostasis
Cell morphogenesis involved in differentiation
Coagulation
Response to biotic stimulus
Import into cell
Positive regulation of hydrolase activity
Regulation of intracellular signal transduction
Endocytosis
Humoral immune response
Positive regulation of cell population proliferation
Cellular response to hormone stimulus
Positive regulation of phosphorus metabolic process
Change in NES
5
0
-5

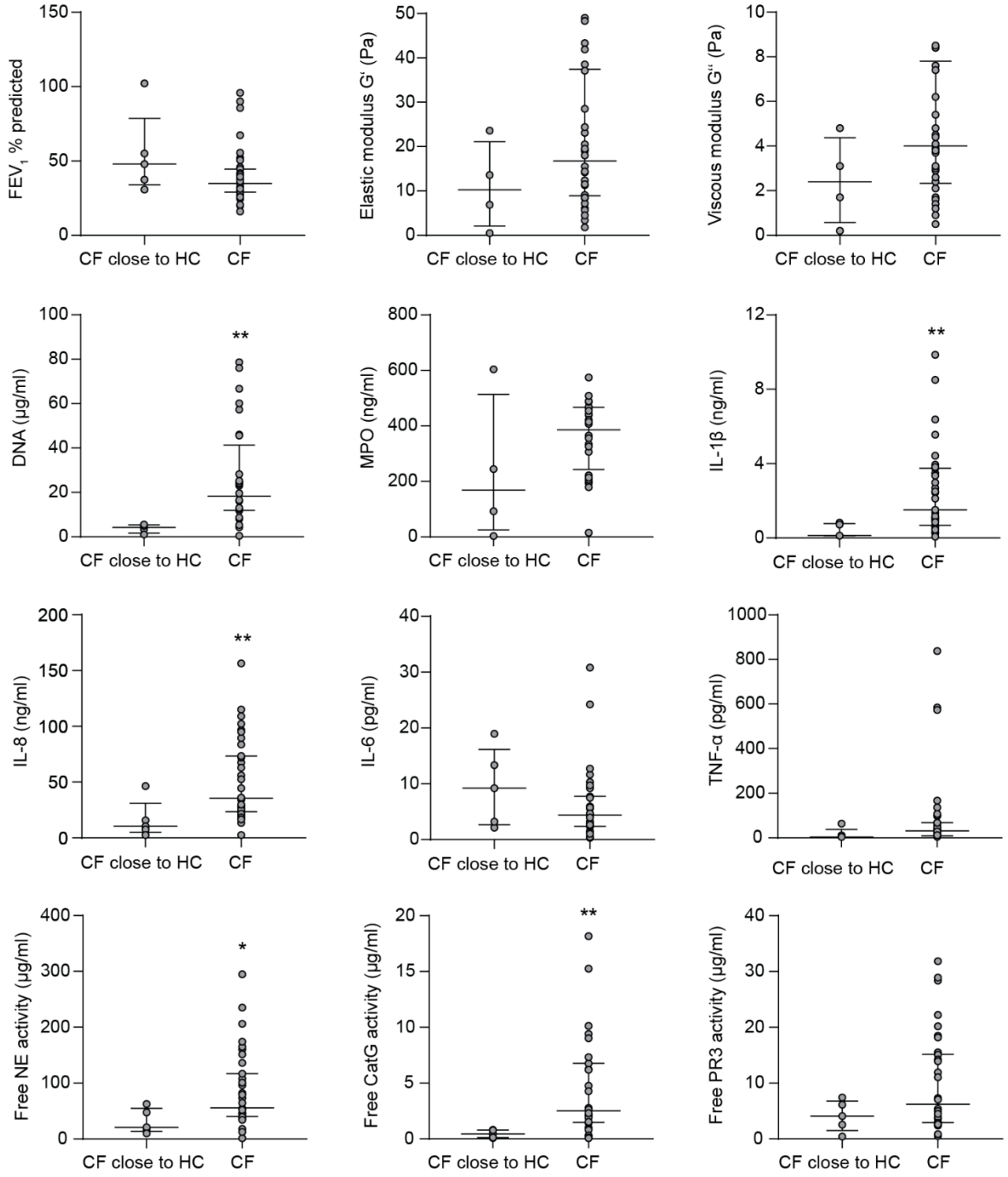
c)


PCD vs. healthy
PCD vs. CF baseline
PCD vs. CF ETI
CF baseline vs. healthy
CF ETI vs. CF baseline
CF ETI vs. healthy
Change in NES
5
0
-5
Activation of innate immune response
Antigen processing and presentation of peptide antigen
Organophosphate metabolic process
Taxis
Positive regulation of locomotion
Regulation of response to external stimulus
Regulation of secretion
Regulation of cell morphogenesis
Regulation of hemopoiesis
Transmembrane transport
Cytokine mediated signaling pathway
Regulation of growth
Regulation of cell differentiation
Regulation of anatomical structure size
Inorganic ion transmembrane transport
Antigen receptor mediated signaling pathway
Cellular component disassembly
Cellular chemical homeostasis
Positive regulation of cell differentiation
Regulation of cell adhesion
Regulation of transport
Cellular homeostasis
Carbohydrate metabolic process
Cellular component morphogenesis
Antigen processing and presentation
Positive regulation of transport
Growth
Small gtpase mediated signal transduction
Inflammatory response
Defense response to other organism
Positive regulation of defense response
Transmembrane receptor protein tyrosine kinase signaling pathway
Enzyme linked receptor protein signaling pathway
Regulation of innate immune response
Positive regulation of multicellular organismal process
Positive regulation of cell adhesion
Ion transmembrane transport
Adaptive immune response based on somatic recombination of immune receptors
Positive regulation of catalytic activity
Adaptive immune response
Ion transport
Innate immune response
Metal ion transport
Cytokine production
Cation transmembrane transport
Cell motility
Locomotion
Immune system development
Leukocyte migration
Defense response
Positive regulation of molecular function
Lymphocyte mediated immunity
Fc receptor signaling pathway
Regulation of lymphocyte activation
Regulation of vesicle mediated transport



Supplementary figure S7. (a-d) Heatmaps indicating enriched gene ontology (GO) terms of biological processes, ordered by change in normalized enrichment score (NES) (FDR < 0.01), in sputum of patients with primary ciliary dyskinesia (PCD) compared to healthy controls (first column), to patients with cystic fibrosis (CF) at baseline (second column) and to CF patients at 3 months after initiation of elexacaftor/tezacaftor/ivacaftor (ETI) (third column). In addition, GO terms were compared between patients with CF at baseline and healthy controls (fourth column), CF patients on ETI and at baseline (fifth column), and CF patients on ETI were compared to healthy controls (sixth column). Statistical significances are indicated as
*P < 0.05, **P < 0.01 and ***P < 0.001.

Supplementary figure S8



Supplementary figure S8. Comparison of indicated parameters between patients with cystic fibrosis (CF) at baseline that exhibit a proteomic signature more closely to healthy controls (n = 5) and the remaining CF patients (n = 35). Bars represent the group median, error bars represent the 25th and 75th percentile. *P < 0.05 and **P < 0.01. Definitions of abbreviations: CatG = cathepsin G; DNA = deoxyribonucleic acid; FEV1 = forced expiratory volume in one second; IL = interleukin; MPO = myeloperoxidase; NE = neutrophil elastase; PR3 = proteinase 3; TNF-α = tumor necrosis factor α.