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## ORIGINAL ARTICLE

# Proteomic profiling of sweat in patients with cystic fibrosis provides new insights into epidermal homoeostasis

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## Abstract

**Background:** A high proportion of patients with Cystic Fibrosis (CF) also present the rare skin disease aquagenic palmoplantar keratoderma. A possible link between this condition and absence of a functional CF Transmembrane conductance Regulator protein in the sweat acinus and collecting duct remains unknown.

**Methods:** In-depth characterization of sweat proteome profiles was performed in 25 CF patients compared to 12 healthy controls. A 20 µL sweat sample was collected after pilocarpine iontophoresis and liquid chromatography tandem mass spectrometry (LC-MS/MS) proteomic analysis was performed.

**Results:** Sweat proteome profile of CF patients was significantly different from that of healthy subjects with 57 differentially expressed proteins. Cystic Fibrosis sweat proteome was characterized by an increase in 25 proteins including proteases (Kallikrein 7 and 13, Phospholipase B domain containing 1, Cathepsin A L2 and B, Lysosomal Pro-X carboxypeptidase); proinflammatory proteins (Annexin A2, Chitinase-3-like protein 1); cytochrome c and transglutaminases. Thirty-two proteins were downregulated in CF sweat including proteases (Elastase 2), antioxidative protein FAM129 B; membrane-bound transporter SLC6A14 and regulator protein Sodium-hydrogen antiporter 3 regulator 1.

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**Conclusion:** This study is the first to report in-depth characterization of endogenous peptides in CF sweat and could help understand the complex physiology of the sweat gland. The proteome profile highlights the unbalanced proteolytic and proinflammatory activity of sweat in CF. These results also suggest a defect in pathways involved in skin barrier integrity in CF patients. Sweat proteome profile could prove to be a useful tool in the context of personalized medicine in CF.

## 1 | INTRODUCTION

One of the hallmarks of Cystic Fibrosis (CF) is high Chloride ( $\text{Cl}^-$ ) sweat concentration due to the absence of the CF Transmembrane conductance Regulator (CFTR) protein in the sweat acinus and collecting duct.<sup>1</sup> Patients with CF (pwCF) can display aquagenic palmoplantar keratoderma (AKP), characterized by exaggerated and early wrinkling of the skin after brief immersion in water associated with tingling and pain sensation.<sup>2</sup> This condition is probably underestimated as AKP is observed in 41%–84% of CF patients.<sup>3,4</sup> The physiopathology of AKP and its relationship to CFTR dysregulation is not yet fully elucidated. This condition has been recently reported to be improved by CFTR modulators in CF patients which relates this skin phenotype to CFTR defects.<sup>5</sup> To gain further insight into the pathophysiology of this rare skin disorder, we performed in-depth analysis of the sweat proteome of pwCF compared to non-CF individuals.

## 2 | PATIENTS AND METHODS

### 2.1 | Patients

We enrolled 25 pwCF, and 12 non-CF individuals (WT). Patients' genotype was classified according to genetic mechanism of the variant (class 1: nonsense, deletion or severe splicing; class 2: misfolding; class 3: activation defect; class 4: conductance defect; class 5: splicing with residual function).<sup>1</sup> All participants signed an informed consent form for sweat collection and analysis (no. Eudract 2016-A00309-42; Comité de Protection des Personnes IDF2).

Clinical status of the patient at the study visit was assessed by pancreatic status, microbiology of sputum and Respiratory Volume in 1 s (FEV1), expressed as percentage predicted (ppFEV1). Sweat induction was performed by pilocarpine iontophoresis, as previously reported.<sup>6</sup> Sweat was collected using the Wescor Macroduct (ELITech, Puteaux, France) and chloride concentration was assessed by coulometry (chloridometer 926S Sherwood, Servilab, Le Mans, France). Twenty  $\mu\text{L}$  of remaining sweat sample was used for proteomic analysis.

### What is already known about this topic?

- Although sweat is a reliable, non-invasive, and easy to collect biofluid, sweat proteome profiles as a source of biomarkers has been studied in relatively few diseases.

### What does this study add?

- Our study is the first to attempt a comparative analysis of sweat proteome profiles between Cystic Fibrosis (CF) patients and controls and reveals a unique subset of differentially expressed proteins. The results provide new insights to epidermal homeostasis in CF.

### What is the translational message?

- Sweat proteome profiles could serve as convenient tools in CF for diagnosis or personalized therapeutic interventions.

### 2.2 | Sample preparation for liquid chromatography mass spectrometry (LC-MS/MS) analysis

Twenty  $\mu\text{L}$  of sweat were processed in the S-Trap<sup>TM</sup> micro spin column (Protifi, Hutington, USA) for digestion according to manufacturer's instructions. Briefly, sodium dodecyl sulphate (SDS) was added to a final concentration of 5% and samples were reduced with 20 mM tris(2-carboxyethyl)phosphine (TCEP) and alkylated with 50 mM chloroacetamide (CAA) for 15 min at room temperature. Aqueous phosphoric acid was then added to a final concentration of 1.2% followed by the addition of six volumes of S-Trap binding buffer (90% aqueous methanol, 100 mM triethylammonium bicarbonate buffer (TEAB), pH 7.1). Mixtures were then loaded on S-Trap columns. Six washing steps were performed for thorough SDS elimination. Samples were digested with 0.8  $\mu\text{g}$  of trypsin (Promega) at 47°C for 1h30. After elution, peptides were vacuum dried and resuspended in 30  $\mu\text{L}$  of 2% acetonitrile (ACN), 0.1% formic acid (FA) in HPLC-grade water prior to mass

spectrometry (MS) analysis. The concentration of the peptide mixtures was determined using a Nanodrop 2000 from Labtech France.

## 2.3 | LC-MS/MS analysis

For each sample, 400 ng of peptides were injected on a nanoelute (Bruker Daltonics, Germany) high-performance liquid chromatography system coupled to a timsTOF Pro (Bruker Daltonics, Germany) mass spectrometer. High-performance liquid chromatography separation (Solvent A: 0.1% FA in water, 2% ACN; Solvent B: 0.1% FA in ACN) was carried out at 250 nL/min using a packed emitter column (C18, 25 cm × 75 μm 1.6 μm) (Ion Optics, Australia) with a gradient elution (2%–11% solvent B during 19 min; 11%–16% for 7 min; 16%–25% for 4 min; 25%–80% for 3 min and finally 80% for 7 min to wash the column). Mass-spectrometric data were acquired using the parallel accumulation serial fragmentation (PASEF) acquisition method. The measurements were carried out over the *m/z* range from 100 to 1700 Th. The ion mobilities values ranged from 0.8 to 1.3 V s/cm<sup>2</sup> (1/k0). The total cycle time was set to 1.16 s and the number of PASEF MS/MS scans was set to 10. For low sample amounts, the total cycle time was set to 1.88 s. QCs, consisting in 10 ng of HeLa total cell lysates were run before, after and during the acquisition of the series.

## 2.4 | Data analysis

The data were analyzed using MaxQuant version 2.0.17.0 and searched with Andromeda search engine against the UniProtKB/Swiss-Prot Homo sapiens database (release 02-2021, 20 396 entries). To search parent mass and fragment ions, we set a mass deviation of 3 and 20 ppm respectively. The minimum peptide length was set to 7 amino acids and strict specificity for trypsin cleavage was required, allowing up to two missed cleavage sites. Carbamidomethylation (Cys) was set as fixed modification, whereas oxidation (Met) and N-term acetylation were set as variable modifications. The false discovery rates at the protein and peptide level were set to 1%. Scores were calculated in MaxQuant as described previously.<sup>7</sup> The reverse and common contaminants hits were removed from MaxQuant output. Proteins were quantified according to the MaxQuant label-free algorithm using label free quantification intensities, providing a label-free normalization; protein quantification was obtained using at least 2 peptides per protein. Match between runs was allowed.

Data filtering and imputation was performed using the Prostar Software. Proteins were retained in the analysis if they were detected in at least 70% of the patients in at least one group. Statistical analyses were

conducted in R. Data in pwCF and WT conditions were compared by Student *t*-test. Benjamini-Hochberg corrections were applied to account for multiple testing. A *q*-value <0.01 combined with a log<sub>2</sub> fold change >0.5 was considered statistically significant.

## 3 | RESULTS

### 3.1 | Population

We enrolled 25 pwCF, mean age 9.1(6.3) years, all carrying 2 CF causing variants of the *CFTR* gene (11 homozygous p.Phe508del (F508del thereafter); 7 F508del compound heterozygotes; 7 carrying nonsense, splicing mutations or large deletion variants) (Table 1). Patient 13 carried the rare missense variant p.Phe191Val (F191 V) in *trans* of F508del and had a normal chloride sweat concentration at 22 mmol/L. However, β-adrenergic sweat secretion rate was in the CF range, providing insight for a genotype with minimal *CFTR* activity.<sup>8</sup> Patient 20 carried the 2789 + 5G > A splice site mutation, known to be associated with a small amount of normally spliced transcripts.<sup>9</sup> All patients were at steady state at the study visit. Three patients (patient 13, 20, 25) were pancreatic sufficient. Patients 2, 3, and 21 had chronic sputum colonization with *Pseudomonas.aeruginosa* or *Alcaligenes.spp* and were treated with inhaled antibiotics. The 12 non-CF individuals (WT) had a mean age of 12.5 (10.8) years and a normal sweat test.

### 3.2 | Proteomic analysis

Protein concentration ranged between 0.25 and 0.70 μg/μL of sweat with no difference between pwCF and WT. Nine hundred ninety-one proteins were retained for the analysis. Data are available via ProteomeXchange with identifier PXD032894.

Fifty-seven proteins were found differentially abundant between pwCF and WT subjects: 25 were upregulated in CF sweat, and 32 were downregulated (Figure 1, Table 2). Hierarchical analysis confirmed that these proteins clearly separated the pwCF from the WT as shown in Figure 2.

Sweat proteome profile of F508del homozygous patients was not significantly different from that of compound F508del heterozygotes or patients with other genotypes, as shown in the dendrogram (Figure 2). Furthermore, patients carrying 2 class 1 variants had a similar profile to those carrying at least one class 2 variant. Neither age, nor sex influenced clustering, including in the infants. We thus performed statistical analysis on the overall pwCF cohort to increase the significance of our results.

Cystic Fibrosis sweat proteome was characterized by an increased level in (i) proteases (Kallikrein 7 and

TABLE 1 Patients characteristics at the moment of sweat collection

|                         | Age (years) | Sex | Genotype      | Genotype classification | Sweat test (mmol/L) | Pancreatic status | FEV1 pp | Bronchial colonization |                                                 |
|-------------------------|-------------|-----|---------------|-------------------------|---------------------|-------------------|---------|------------------------|-------------------------------------------------|
| Patients                |             |     |               |                         |                     |                   |         |                        |                                                 |
| 1                       | 13          | M   | F508del (a)   | F508del (a)             | 2/2                 | 81                | PI      | 104                    | <i>S.aureus</i>                                 |
| 2                       | 16          | M   | 444delA       | 444delA                 | 1/1                 | 107               | PI      | ND                     | <i>S.aureus</i> ,<br><i>Alcaligenes</i>         |
| 3                       | 13          | M   | F508del(a)    | L558S                   | 2/2                 | 105               | PI      | 33                     | <i>P.aeruginosa</i> ,<br><i>Alcaligenes</i> spp |
| 4                       | 12          | F   | F508del(a)    | F508del(a)              | 2/2                 | 110               | PI      | 116                    | <i>S.aureus</i>                                 |
| 5                       | 13          | F   | G542X(c)      | G542X(c)                | 1/1                 | 118               | PI      | 90                     | <i>S.aureus</i> ,<br><i>Alcaligenes</i> spp     |
| 6                       | 3           | M   | G542X(c)      | G542X(c)                | 1/1                 | 114               | PI      | ND                     | <i>P.aeruginosa</i>                             |
| 7                       | 17          | M   | F508del(a)    | F508del(a)              | 2/2                 | 120               | PI      | 129                    | <i>S.aureus</i>                                 |
| 8                       | 19          | F   | F508del(a)    | F508del(a)              | 2/2                 | 69                | PI      | 96                     | <i>S.aureus</i>                                 |
| 9                       | 0.1         | M   | F508del(a)    | W1282X(c)               | 2/1                 | 95                | PI      | ND                     | None detected                                   |
| 10                      | 3           | M   | Y275X(c)      | S466X(c)                | 1/1                 | 126               | PI      | ND                     | <i>S.aureus</i>                                 |
| 11                      | 0.1         | F   | F508del(a)    | del exons 2-3(b)        | 2/1                 | 102               | PI      | ND                     | None detected                                   |
| 12                      | 13          | F   | F508del(a)    | H199Y(a)                | 2/2                 | 101               | PI      | 109                    | <i>S.aureus</i>                                 |
| 13                      | 4           | M   | F508del(a)    | F191 V(a)               | 2/2                 | 22                | PS      | 100                    | None detected                                   |
| 14                      | 3           | F   | 1717-1G>A(d)  | 1717-1G>A(d)            | 1/1                 | 109               | PI      | ND                     | None detected                                   |
| 15                      | 5           | F   | F508del(a)    | F508del(a)              | 2/2                 | 110               | PI      | ND                     | None detected                                   |
| 16                      | 13          | M   | F508del(a)    | F508del(a)              | 2/2                 | 105               | PI      | 93                     | <i>S.aureus</i>                                 |
| 17                      | 10          | M   | F508del(a)    | F508del(a)              | 2/2                 | 104               | PI      | 129                    | <i>S.Maltophilia</i>                            |
| 18                      | 1.2         | M   | I507del(a)    | Del Exon 17B (b)        | 2/1                 | 111               | PI      | ND                     | None detected                                   |
| 19                      | 0.1         | M   | F508del(a)    | F508del(a)              | 2/2                 | 96                | PI      | ND                     | None detected                                   |
| 20                      | 15          | M   | 2789 + 5G > A | 3120 + 1G > A           | 5/1                 | 103               | PS      | 59                     | <i>S.aureus</i>                                 |
| 21                      | 17          | M   | F508del(a)    | 2143delT                | 2/1                 | 106               | PI      | 41                     | <i>P.aeruginosa</i> ,<br><i>Alcaligenes</i> spp |
| 22                      | 0.1         | M   | F508del(a)    | F508del(a)              | 2/2                 | 98                | PI      | ND                     | <i>S.aureus</i>                                 |
| 23                      | 8           | F   | F508del(a)    | F508del(a)              | 2/2                 | 114               | PI      | 116                    | None detected                                   |
| 24                      | 14          | F   | F508del(a)    | F508del(a)              | 2/2                 | 92                | PI      | 108                    | <i>S.aureus</i>                                 |
| 25                      | 13          | F   | F508del(a)    | R347H(b)                | 2/2                 | 49                | PS      | 100                    | None detected                                   |
| Non-CF control subjects |             |     |               |                         |                     |                   |         |                        |                                                 |
| 1                       | 25          | M   | NA            | NA                      | NA                  | 25                | NA      | NA                     | NA                                              |
| 2                       | 0.8         | M   | NA            | NA                      | NA                  | 11                | NA      | NA                     | NA                                              |
| 3                       | 14          | M   | NA            | NA                      | NA                  | <10               | NA      | NA                     | NA                                              |
| 4                       | 24          | F   | NA            | NA                      | NA                  | 10                | NA      | NA                     | NA                                              |
| 5                       | 0.4         | F   | NA            | NA                      | NA                  | <10               | NA      | NA                     | NA                                              |
| 6                       | 8           | F   | NA            | NA                      | NA                  | 17                | NA      | NA                     | NA                                              |
| 7                       | 6           | F   | NA            | NA                      | NA                  | 17                | NA      | NA                     | NA                                              |
| 8                       | 27          | F   | NA            | NA                      | NA                  | 12                | NA      | NA                     | NA                                              |
| 9                       | 25          | M   | NA            | NA                      | NA                  | 19                | NA      | NA                     | NA                                              |
| 10                      | 18          | M   | NA            | NA                      | NA                  | 12                | NA      | NA                     | NA                                              |
| 11                      | 0.8         | M   | NA            | NA                      | NA                  | 11                | NA      | NA                     | NA                                              |
| 12                      | 3           | M   | NA            | NA                      | NA                  | 12                | NA      | NA                     | NA                                              |

Abbreviations: CF, Cystic Fibrosis; NA, not adapted; ND, not determined; PI, pancreatic insufficient; PS, pancreatic sufficient.



**FIGURE 1** Volcano plot of differentially expressed proteins between patients with cystic Fibrosis (CF) (patients with CF (pwCF)) and non-CF individuals (WT). Thresholds of 0.5 for log<sub>2</sub> Fold Change (FC) in abscissa, and of 0.01 for *q* values (expressed as -log<sub>10</sub>) in ordinate, are used for significancy and plotted in green. Differentially expressed proteins are plotted in colour (Proteases: green; proinflammatory proteins: orange; transglutaminases: purple; antioxydative proteins: pink; others: blue)

13, Phospholipase B domain containing 1, Cathepsin A L2 and B, Lysosomal Pro-X carboxypeptidase, neuraminidase); (ii) proinflammatory proteins (Annexin A2, Chitinase-3-like protein 1); (iii) cytochrome c; (iv) Transglutaminases 3 and 5; and (v) Toll-interacting protein (TOLLIP). This contrasted with decrease of Elastase 2, an epidermal protease, FAM129 B, an antioxydative protein and Desmoglein-3. Finally, 2 proteins known to interfere with CFTR biology were underexpressed (Sodium-hydrogen antiporter 3 regulator 1 [NHERF1] and Solute Carrier Family 6 Member 14, SLC6A14).

The severity of the disease, based on a ppFEV1 below 50%, and/or chronic colonization with *Pseudomonas.aeruginosa* or *Alcaligenes spp* did not modify the proteome profile.

## 4 | DISCUSSION

This study is to our knowledge the first report of CF sweat proteome. We were able to yield nearly 1000 proteins, in as low as 20  $\mu$ L of sweat thanks to an optimized protocol with high-resolution LC-MS/MS acquisition. To our knowledge, a similar depth of proteome coverage was

only reported in 2 previous studies in healthy adults and using larger volumes of sweat. Yu et al performed the analysis on 10 ml pooled samples collected from absorbing tissue pads at different body sites after exercise while Burat et al used a mean volume of 70  $\mu$ L of sweat.<sup>10,11</sup> We used 20  $\mu$ L of leftover sweat sample collected for the sweat test. Usually at least 50  $\mu$ L of sweat are collected for sweat test and around 20  $\mu$ L are used for sweat Cl<sup>-</sup> concentration evaluation. Our results demonstrate that the remainder aliquot of a diagnostic sweat test can be used reliably for sweat proteomics, including in infants as young as 1 month of age.

A large range of proteases and their respective inhibitors, antimicrobial peptides and effectors of skin innate immunity have already been reported in sweat of healthy subjects<sup>10–14</sup> or in specific diseases such as schizophrenia or tuberculosis.<sup>15,16</sup> Detection of other proteins such as cytoskeletal proteins or proteins involved in oxidative stress, unfolded protein response, Endoplasmic Reticulum stress, proteasome and cancellous metabolism pathways points to the diversity of sweat biology.<sup>11,17–20</sup> Although some studies report a correlation between blood versus sweat concentration for specific biomarkers such as glucose,<sup>16,21</sup> the literature shows mixed results for others<sup>22</sup> and relatively

TABLE 2 List of differentially expressed proteins in cystic fibrosis (CF) and WT sweat

| Protein        | P                    | Q (Benjamini-Hochberg) | Log fold change (CF - WT) | Expression in CF sweat |
|----------------|----------------------|------------------------|---------------------------|------------------------|
| TGM3           | 1.58170503179399e-09 | 1.56746968650784e-06   | 1.48299833333333          | Overexpressed          |
| PLBD1          | 2.19449916269758e-08 | 1.08737433511665e-05   | 3.19911266666667          | Overexpressed          |
| TGM5           | 6.43668943899884e-08 | 1.96835309247718e-05   | 2.09593033333334          | Overexpressed          |
| TOLLIP         | 1.71984520823241e-06 | 0.000284061100226386   | 2.593015                  | Overexpressed          |
| KLK7           | 1.99882588608743e-05 | 0.00132055763540843    | 2.80988066666667          | Overexpressed          |
| KLK13          | 1.97919945229845e-05 | 0.00132055763540843    | 1.48745466666667          | Overexpressed          |
| CHI3L1         | 3.01262586508373e-05 | 0.00157132222752525    | 2.6340845                 | Overexpressed          |
| ANXA2; ANXA2P2 | 3.88823861049168e-05 | 0.00183487831571298    | 1.92317566666667          | Overexpressed          |
| CTSB           | 4.28375399575546e-05 | 0.00192963645899712    | 1.05712433333333          | Overexpressed          |
| CYCS           | 5.0216366740295e-05  | 0.00207351747665135    | 2.224024                  | Overexpressed          |
| CNP            | 6.03328049753096e-05 | 0.00239159238922127    | 1.86662933333333          | Overexpressed          |
| VCL            | 9.66636492911453e-05 | 0.00309011859508145    | 1.04174433333333          | Overexpressed          |
| CTBS           | 0.000100169383365191 | 0.00310212059109076    | 0.788042000000001         | Overexpressed          |
| CTSV           | 0.000169602967955179 | 0.00400182241056147    | 1.95409066666667          | Overexpressed          |
| GGH            | 0.00020565187231108  | 0.00463184103318819    | 0.690882000000002         | Overexpressed          |
| SERPINB6       | 0.000218078527698848 | 0.00480257379887907    | 0.856213666666665         | Overexpressed          |
| WDR5           | 0.000259717676076137 | 0.00547617482960537    | 2.56654883333333          | Overexpressed          |
| CHAD           | 0.000265784565267186 | 0.00548734383707879    | 2.12672166666667          | Overexpressed          |
| SNRPN; SNRPB   | 0.000275203341900658 | 0.0055658471800725     | 2.27634633333333          | Overexpressed          |
| CTSA           | 0.000357792614031708 | 0.0069524015785377     | 0.989661000000002         | Overexpressed          |
| LYZ            | 0.000355199902106944 | 0.0069524015785377     | 2.700541                  | Overexpressed          |
| SLUR2          | 0.000419516866382318 | 0.00784417386009203    | 2.11938466666667          | Overexpressed          |
| PRCP           | 0.000415002068705056 | 0.00784417386009203    | 0.955500666666666         | Overexpressed          |
| QPCT           | 0.000493990855610998 | 0.00906564699834257    | 1.365012                  | Overexpressed          |
| ACP5           | 0.000527198325269628 | 0.00932952750611073    | 1.02944833333333          | Overexpressed          |
| FAM129B        | 7.94491661948408e-08 | 1.96835309247718e-05   | -2.0791504                | Underexpressed         |
| RHOA; RHOC     | 2.04192488811702e-07 | 4.04709512824794e-05   | -2.41087553333333         | Underexpressed         |
| VPS4B          | 9.60588941329161e-06 | 0.00119159328624749    | -2.144275                 | Underexpressed         |
| MPO            | 1.08217351929641e-05 | 0.00119159328624749    | -3.42752986666667         | Underexpressed         |
| TFG            | 9.78268215062519e-06 | 0.00119159328624749    | -1.835101                 | Underexpressed         |
| ELANE          | 1.51021002126614e-05 | 0.00127553183077237    | -2.48330053333333         | Underexpressed         |
| GRIPAP1        | 1.47731114847263e-05 | 0.00127553183077237    | -4.4502426                | Underexpressed         |
| CCT7           | 1.54453904836211e-05 | 0.00127553183077237    | -2.04755293333333         | Underexpressed         |
| SLC6A14        | 1.70723579325206e-05 | 0.00130143897777907    | -2.0631152                | Underexpressed         |
| SLC3A2         | 2.25999565002928e-05 | 0.00139978480573689    | -0.881463                 | Underexpressed         |
| RPL11          | 2.66865246950978e-05 | 0.00155566741016717    | -1.91789953333333         | Underexpressed         |
| P4HB           | 2.84976534262211e-05 | 0.00156895414141028    | -0.849515333333335        | Underexpressed         |
| G6PD           | 3.29256052077666e-05 | 0.00163146373804484    | -3.14162133333333         | Underexpressed         |
| TMED9          | 4.5015504560557e-05  | 0.00193958108780487    | -1.8657086                | Underexpressed         |
| HSP90AB1       | 6.36883655989668e-05 | 0.00242750655032985    | -2.41141233333333         | Underexpressed         |

TABLE 2 (Continued)

| Protein             | P                    | Q (Benjamini-Hochberg) | Log fold change (CF - WT) | Expression in CF sweat |
|---------------------|----------------------|------------------------|---------------------------|------------------------|
| MTHFD1              | 7.2379130902605e-05  | 0.0026565821749808     | -1.390594                 | Underexpressed         |
| CALR                | 8.12542575545831e-05 | 0.00277665411160662    | -0.987771333333333        | Underexpressed         |
| CCT8                | 7.98292581291651e-05 | 0.00277665411160662    | -2.119981333333333        | Underexpressed         |
| B3GNT2              | 8.49078785657414e-05 | 0.00280479025528832    | -1.879466                 | Underexpressed         |
| HSP90B1             | 0.000104409061495325 | 0.00313543575581414    | -2.160834                 | Underexpressed         |
| HSP90AA1            | 0.00011554029444987  | 0.00327144090856632    | -1.23706566666667         | Underexpressed         |
| SLC38A10            | 0.000113663277186564 | 0.00327144090856632    | -1.841559933333333        | Underexpressed         |
| RPS18               | 0.000120845307526837 | 0.00332660277108598    | -1.675564333333333        | Underexpressed         |
| SLC25A5; SLC25A6    | 0.000126964753433738 | 0.00340059650413066    | -1.8838592                | Underexpressed         |
| MFGE8               | 0.000134504290978555 | 0.00350773032525652    | -1.57676266666667         | Underexpressed         |
| RAB11A; RAB11B      | 0.000149194674859679 | 0.00379107494322929    | -1.074447333333333        | Underexpressed         |
| PSMD6               | 0.000154226015316499 | 0.00382094952946626    | -0.887891                 | Underexpressed         |
| DSG3                | 0.000169543652295849 | 0.00400182241056147    | -1.4409494                | Underexpressed         |
| SLC9A3R1            | 0.000191808840825127 | 0.00442052468041165    | -2.15198906666667         | Underexpressed         |
| MB                  | 0.000240247633526712 | 0.00517576967010808    | -1.530990333333333        | Underexpressed         |
| FAM129 A            | 0.000516733099906237 | 0.00931059094558329    | -1.828377333333333        | Underexpressed         |
| NCALD; HPCA; HPCAL1 | 0.000554437456083902 | 0.0096394301575289     | -1.301822                 | Underexpressed         |

Abbreviations: CALR, Calreticulin; CHAD, Chondroadherin; CNP, 2',3'-cyclic nucleotide 3' phosphodiesterase; CTBS, Chitinase; CTSA, Cathepsin A; CTSB, Cathepsin B; CTSV, Cathepsin V; CYCS, Cytochrome C; ELANE, Elastase, Neutrophil Expressed; GGH, Gamma-Glutamyl Hydrolase; HPCA, Hippocalcin; LYZ, Lysozyme C; MB, Myoglobin; MPO, Myeloperoxidase; NCALD, Neurocalcin Delta; PRCP, Prolylcarboxypeptidase; QPCT, Glutamyl-Peptide Cyclotransferase; RHOA, Ras Homolog Family Member A; RHOC, Ras Homolog Family Member C; SNRPN, small nuclear ribonucleoprotein polypeptide; TFG, Trafficking From ER To Golgi Regulator; VCL, Vinculin.

few proteins have yet been fully assessed for blood versus sweat concentration correlation.

As in previous studies, we did not report any strong influence of sex or age, even in 1 month old babies. Nor did we observe any influence of disease severity based on genetics or lung disease pattern, although the very limited number of patients with severe disease precludes a definitive conclusion.

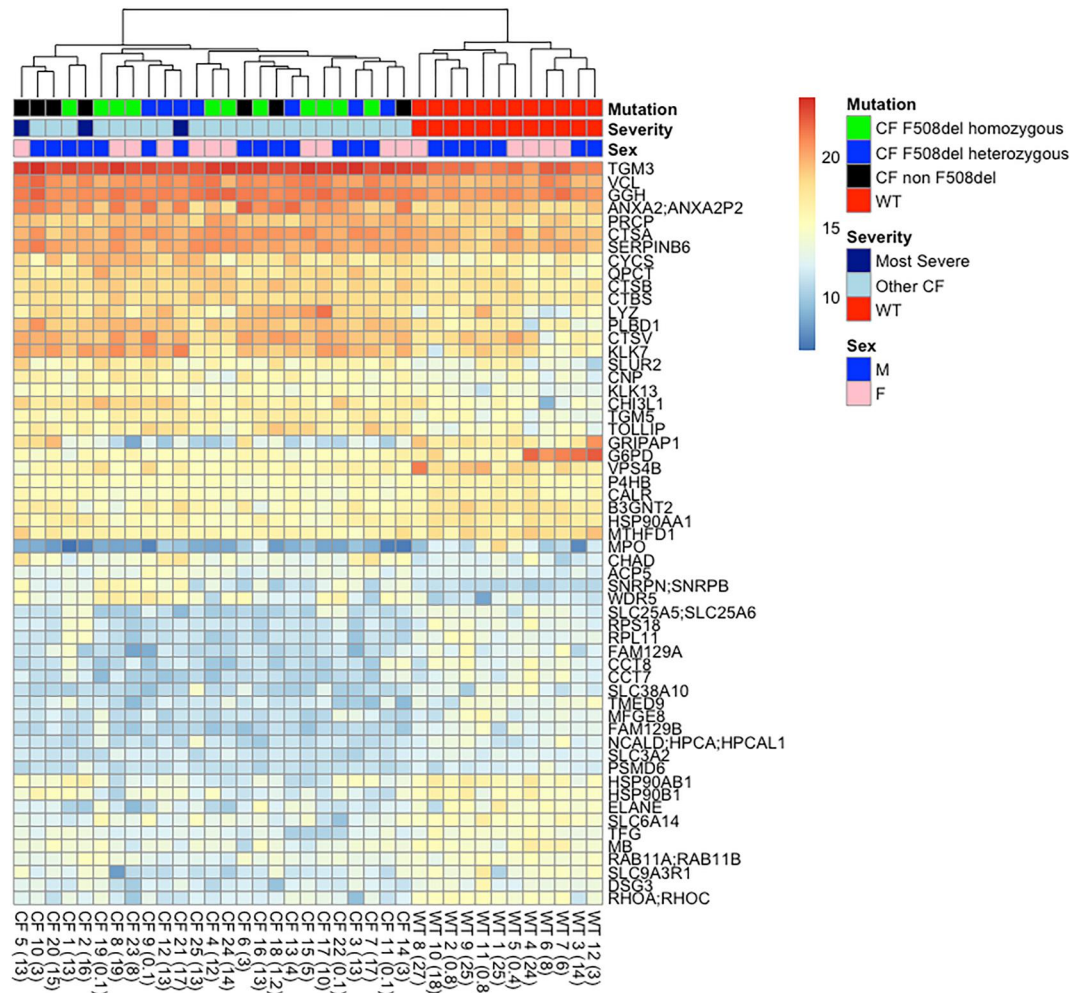
Our results add additional insights in sweat physiology by showing an unbalanced proteolytic and proinflammatory activity of this biofluid, in pwCF. A number of proteases were upregulated, including Kallikreins 7 and 13. These epidermal-specific serine proteases activate a proteolytic cascade, generating skin inflammation and ultimately skin physical barrier impairment, as already reported in the Netherton Syndrome or Atopic Dermatitis.<sup>23</sup> Annexin A2 forms a complex with S100A10 involved in a large variety of pathophysiological processes, including activation of metalloprotease induced inflammation.<sup>24</sup> Other regulators of innate immunity were also significantly upregulated, such as Chitinase-3-like protein (CHI3L1) and TOLLIP. CHI3L1 reflects induction of T-helper cell type 2, IL-13-induced inflammation, and macrophage activation.<sup>25</sup> Interestingly, this

protein is also elevated in sputum and serum of CF patients with lung disease.<sup>26</sup>

Toll-interacting protein is an intermediate in interleukin (IL)-1 signalling involved in the Toll-like receptor mediated inflammation and triggers the nuclear factor (NF)- $\kappa$ B and mitogen-activated protein kinase signalling.<sup>27</sup> All these proinflammatory signalling cascades may be associated with an increased oxidative status, as assessed by the increase in Cytochrome C, a mitochondrial protein involved in the electron transport system and oxidative phosphorylation. This may be potentiated by the decrease of the antioxidative protein FAM129B6.<sup>28</sup>

Our results also suggest a defect in the integrity of the skin barrier in pwCF. Indeed, downregulation of elastase 2 is anticipated to decrease the cleavage of filaggrin, a protein which plays a key role in the integrity of the epidermal skin barrier through its end products.<sup>29,30</sup> Absence of filaggrin in null carriers predisposes to ichthyosis vulgaris, and/or atopic dermatitis.<sup>31,32</sup> Downregulation of Desmoglein 3, a component of desmosomes, should reinforce the skin barrier defect by decreasing cell to cell junction.<sup>33</sup> Finally, transglutaminases overexpression may perturb the formation





**FIGURE 2** Heat map of differentially expressed proteins between patients with cystic fibrosis (CF) (patients with CF (pwCF)) and non-CF individuals (WT). Dendrogram is shown according to genetic status (mutation), severity (most severe: ppFEV1 < 50%, chronic sputum colonization with *P.aeruginosa* or *Alcaligenes* spp), and sex. Patients' age (in years) is presented after their assigned study number

of the outer layer of the epidermis by altering cross-linking of keratins and structural proteins.<sup>34</sup>

All these defects should result in altered skin barrier function and favour increased water content when the skin is immersed in water, one of the main features observed in AKP.<sup>35</sup> Indeed, although the pathogenesis of AKP is not fully understood, it is assumed to be related to hydropic changes of the horny layer and the excessive electrolyte content of CF sweat which moistens the epidermis obviously plays an additional role.<sup>34</sup> The observation that CFTR function restoration by ivacaftor, a highly efficient CFTR modulator correcting Cl<sup>-</sup> channel activity, improves AKP symptoms provides clinical evidence that this condition is related to CFTR dysfunction.<sup>5</sup> Interestingly, in our data set, we also show a defective expression of 2 proteins known to modulate the expression and function of CFTR at the apical membrane: NHERF, a protein known to anchor CFTR at the membrane and SLC26A14, an amino-acid transporter involved in cGMP-mediated F508del-CFTR channel activity.<sup>35,36</sup>

Our study is the first report of human sweat proteome in pwCF.

Altogether, our results, based on a deep proteomic evaluation, provide evidence for an unbalanced proteolytic and proinflammatory activity of CF sweat, and alteration of signalling pathways involved in the integrity of the epidermal skin barrier. This CF-specific profile provides novel insight for AKP physiopathology and CFTR biology and may be used in the context of personalized medicine in CF.

## AUTHOR CONTRIBUTIONS

**Mathieu Cornet:** Formal analysis (equal); Validation (equal); Visualization (equal); Writing – original draft (equal). **Thao Nguyen-Khoa:** Conceptualization (equal); Data curation (equal); Investigation (equal). **Mairiad Kelly-Aubert:** Investigation (equal); Writing – review & editing (equal). **Vincent Jung:** Data curation (equal); Formal analysis (equal); Investigation (equal). **Frederique Chedevert:** Data curation (equal); Investigation (equal). **Muriel Le Bourgeois:** Data

curation (equal); Investigation (equal). **Laura Aoust:** Data curation; Investigation (equal). **Kevin Roger:** Formal analysis; Visualization; Investigation (equal). **Chiara Guerrera:** Conceptualization (equal); Data curation; Investigation; Methodology; Project administration (equal); Resources (equal); Supervision (equal); Writing – original draft; Writing – review & editing (equal). **Isabelle Sermet-Gaudelus:** Conceptualization (equal); Funding acquisition (equal); Investigation; Methodology; Project administration (equal); Resources (equal); Supervision (equal); Writing – original draft; Writing – review & editing (equal).

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## CONFLICT OF INTEREST

Isabelle Sermet-Gaudelus has been awarded a grant from Vertex Therapeutics (Vertex Innovation Award). She participates in Vertex Therapeutics, Eloxx Therapeutics and Proteostasis Therapeutics scientific boards.

## DATA AVAILABILITY STATEMENT

Data available on request from the authors.

## ETHICS STATEMENT

All participants signed an informed consent form for sweat collection and analysis (no. Eudract 2016-A00309-42; Comité de Protection des Personnes IDF2).

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