SWEAT GLAND BIOELECTRICS DIFFER IN CYSTIC FIBROSIS: A NEW CONCEPT FOR POTENTIAL DIAGNOSIS AND ASSESSMENT OF CFTR FUNCTION IN CYSTIC FIBROSIS

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SUMMARY

Background: For nearly 50 years, the diagnosis of Cystic Fibrosis (CF) has depended on measurements of sweat Cl– concentration. While the validity of this test is universally accepted, increasing diagnostic challenges and the search for adequate biomarker assays to support curative oriented clinical drug trials have created a new demand for accurate, reliable and more practical CF tests. Herein, we propose a novel concept that may provide a more efficient, real time method to assess CFTR function in vivo.

Methods: Cholinergic and β-adrenergic agonists were iontophoresed to stimulate sweating. The bioelectric potential from stimulated sweat glands (SPD) was measured in vivo using a standard electrocardiogram (ECG) electrode applied to the skin surface. SPD and sweat chloride concentrations were compared in cohorts predicted to express a range of CFTR function as presented by healthy controls (HC), heterozygotes (Hz), pancreatic sufficient (CFPS) and pancreatic insufficient CF patients (CFPI).

Results: The median SPD was hyperpolarized in CF compared to control subjects (-47.4 mV vs. -14.5 mV, p<0.0001). In distinguishing between control and CF subjects, SPD (Area under Receiver-Operator Curve, AUC = 0.997) was similar to sweat [Cl–] (AUC = 0.986). Sequential cholinergic/β-adrenergic sweat stimulation dramatically depolarized the SPD in CF (p<0.001), but had no effect in control subjects (p=0.6) or on sweat [Cl–] in either group (p>0.5). Further, the positive SPD response was larger in CFPI than in CFPS subjects (p=0.04).

Interpretation: These results support the concept that skin surface voltages arising from stimulated sweat glands can be exploited to assess expressed CFTR function in vivo and may prove to be a useful diagnostic tool.
INTRODUCTION

Cystic fibrosis (CF) is the most common, inherited and life-shortening disorder in the Caucasian population. Defective epithelial chloride channel function due to mutations in the Cystic Fibrosis Transmembrane Regulator (CFTR) gene affects secretory and absorptive organs leading to progressive lung disease as a major factor for morbidity and mortality, pancreatic insufficiency, gastrointestinal complications and infertility. The pathognomic hallmark of high NaCl in sweat from CF patients formed the basis of the classic sweat test, which was developed 50 years ago.

The defect in CFTR Cl- channels also results in an altered bioelectrical potential in the CF sweat duct that is ~10 fold more negative than normal. Similar, though less pronounced, observations in airway epithelia led to the introduction of measurements of nasal potential differences (NPD) as an alternative functional diagnostic test for CF. However, performance of the NPD is technically demanding, as well as negatively influenced by inflammation and by exposure to irritants such as cigarette smoke.

Not only are sweat glands one of the richest sources of CFTR Cl- channels, but they are arguably the most assessable of affected CF organs. Moreover, in contrast to most other affected organs, sweat glands are not altered by secondary pathologies associated with ductal plugging and inflammation. We therefore explored the possibility of assessing the difference in the bioelectric properties of sweat glands in vivo by designing a novel diagnostic assay for Cystic Fibrosis and other diseases that may offer advantages in economy and simplicity to facilitate diagnoses in less developed settings. We also note the present need for biomarkers to assay new drugs, some of which are now being tested in early-phase clinical trials. The need for real time, easily executed outcome measures of the function of CFTR seems likely to increase as multiple clinical sites and larger numbers of subjects enter later phases of trials that require definitive markers of molecular function.

In this proof of concept study, we introduce a novel technique to assess CFTR function in vivo by measuring the transductal voltage of sweat glands at the skin surface (SPD) as a new diagnostic parameter as well as a possible assay for monitoring changing levels of CFTR function in therapeutic trials. We first define the ability of cholinergically stimulated SPDs to segregate cohorts of healthy controls (HC) and heterozygote carriers (Hz) from pancreatic sufficient (CFPS) and pancreatic insufficient (CFPI) patients with CF. We then present evidence that additional β-adrenergic stimulation might further improve the diagnostic power of this novel method among cohorts predicted to express a range of CFTR function.

METHODS

Study participants

The ethical boards of the Hospital for Sick Children and St. Michael’s Hospital in Toronto, Canada, as well as Health Canada, approved the study. Between July 2006 and December 2007, we prospectively and consecutively ascertained 10 healthy controls (HC), 10 obligate heterozygotes (Hz), 10 pancreatic sufficient CF (CFPS), and 8 pancreatic insufficient CF patients (CFPI). The diagnosis for CF as well as the exocrine pancreas status was previously established according to current diagnostic criteria. Study subjects were selected based on...
their previous participation in a large diagnostic study, including HC, providing us with extended clinical, functional and genetic information. All study participants signed informed consent and underwent test protocols on at least two occasions.

**Sweat stimulation**

Sweat secretion was stimulated using the cholinergic agent pilocarpine 1% (USP grade, Spectrum, Gardena, USA) applied by iontophoresis for 5 minutes to the volar surface of the forearm (Protocol 1). In a second assay at an adjacent skin site, we iontophoresed pilocarpine (1%) as before, but immediately followed this cholinergic stimulation by iontophoresing into this same area a combination of the β-adrenergic agent, isoproterenol (1%) and the phosphodiesterase inhibitor, aminophylline (0.8%; USP grade, Spectrum, Gardena, USA and AC&C, Montreal, Canada) (Protocol 2). Drugs were dissolved in distilled water immediately before use. The β-adrenergic solution was acidified to pH 3.0 with HCl to protonate the drugs for maximal transdermal iontophoretic delivery.

To minimize hyperaemia of the skin often seen with the standard QPIT (50µA/cm² is recommended as standard operating procedure for the QPIT) and possible trauma to the underlying sweat glands, we first determined the iontophoresis current needed for a maximized SPD response in 15 additional control subjects (mean age 34.6 years, range 22-60 years). Maximal SPD and sufficient sweat volumes for chemical analysis were obtained with an applied iontophoresis current of 30µA/cm², which was therefore used in all subsequent experiments (figure 1).

**Sweat Cl⁻ concentration**

Sweat [Cl⁻] was determined by digital chloridometer (LabConco Corporation, Kansas City, Missouri, USA) from sweat collected in the Macroduct® collector cup system (Wescor Inc, Logan, USA) with a minimum volume of 20µl.

**Sweat gland potential difference measurements**

After stimulation, the skin area was gently cleaned with distilled water and carefully blown dry. Water saturated mineral oil was applied to the stimulated area to maximize SPDs by minimizing transdermal current shunts. An ECG electrode (ConMed, Utica, US), pre-soaked in 3M KCl for 30 minutes, was taped securely over the prepared area. An angiocath (G22) was inserted subcutaneously by needle puncture. The steel needle was removed and the plastic infusion line was filled with sterile 0.9% NaCl solution. The infusion line was then bridged via plastic tubing filled with 3M KCl to an Ag/AgCl reference electrode. The sweat gland potential difference was measured as the voltage between the ECG electrode and the subcutaneous reference electrode using a voltmeter (Digital Multimeter, ET1039, input impedance 10⁶ ohms). The SPD was recorded every minute for 30 minutes after sweat stimulation. As shown in figure 2, SPD measurements hyperpolarized with time, reaching a plateau 20 to 30 minutes after stimulation. An average of the last five minute SPD recordings (minute 26-30) was used for data analysis.
Electrode asymmetry and drift

Before and after each test, the asymmetry between the reference and the ECG electrode for SPD measurements was assessed by applying the ECG electrode and the infusion line to a 0.9% NaCl soaked sponge. Property differences between the ECG and reference electrode, as well as variability between each ECG electrode, apparently resulted in some electrode asymmetry and drift. We excluded data from any electrodes with > ±5.0 mV absolute asymmetry or drift. The final asymmetry was subtracted from each SPD before further analysis.

Statistics

Data were summarized as medians with interquartile ranges (IQR, 25th to 75th percentile). Missing data are explained as exclusion of SPD measurements due to asymmetry >±5.0 mV or unavailable sweat [Cl−] due to insufficient sweat volume. Nonparametric tests (Wilcoxon rank-sum test and Kruskal-Wallis test) were used for comparison between the groups. Analysis for diagnostic test accuracy was performed by determining the area under (AUC) a Receiver-Operator Characteristic (ROC) curve with its corresponding 95% confidence interval (CI). An AUC of over 0.7 was considered as indicative of ‘fair’ discriminative ability, 0.8 as ‘good’, and over 0.9 as ‘excellent’. For reliability analysis, two repeated tests in each individual were compared using the 95% limits of agreement, described by Bland and Altman.21 For all comparisons, two tail tests for a p-value of <0.05 were applied to determine significant differences. Graphs and analysis were performed with Prism (Version 4.0, GraphPad Software Inc, 2003) and SPSS (16.0, Graduate Student version, SPSS Inc, 2007).

RESULTS

The study subjects are described in Table 1. The groups are not sex matched. The mean (±SD) ages of the HC (29.7±8.5 years) and of the CFPI (29.9±4.7 years) are less than of the Hz (41.3±13.9 years) and CFPS (41.9±9.6 years) cohorts. However, there is no statistically significant age difference between the combined groups of HC/Hz and CFPS/CFPI (p=0.8).

Table 1: Summary of study subjects

<table>
<thead>
<tr>
<th>ID</th>
<th>Category</th>
<th>Sex</th>
<th>Age</th>
<th>Genotype</th>
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<th>Category</th>
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<th>Age</th>
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<td>+/-</td>
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<td>deltaF508/L1335P</td>
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<tr>
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<td>HC</td>
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<td>26</td>
<td>+/-</td>
<td>26</td>
<td>CFPS</td>
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<td>deltaF508/+</td>
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</table>
Legend – HC-healthy controls, Hz-heterozygotes, CFPS-pancreatic sufficient CF patients, CFPI – pancreatic insufficient CF patients. CFTR gene sequencing was performed for all subjects\textsuperscript{22,23}.

**SPD in CF patients and healthy controls**

In Protocol 1, sweat was stimulated only with the cholinergic drug pilocarpine (1%). Considering all HC and Hz individuals as a control group and all CFPS and CFPI patients as a CF group, we found that sweat gland SPDs were highly statistically significantly hyperpolarized in the CF group compared to the control group (-47.4 mV vs. -14.5 mV, respectively, p<0.0001). In an area immediately adjacent to the SPD measurement site, sweat was collected for determination of chloride concentration. The sweat [Cl] was also highly statistically significantly higher in the CF than in the control group (98.5 mmol/l versus 21.5 mmol/l, p<0.0001). Table 2 shows the SPD and sweat [Cl] data for all groups.
### Table 2: Comparison of SPD and Sweat [Cl-] for all groups

<table>
<thead>
<tr>
<th></th>
<th>SPD - cholinergic (Protocol 1)</th>
<th>ΔSPDchol/β-adren* (Protocol 2)</th>
<th>Sweat Cl'-cholinergic</th>
<th>p-value</th>
<th>p-value</th>
<th>p-value</th>
<th>p-value</th>
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<tr>
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<td>2. Test</td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
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<td>HC</td>
<td>-14.4 0.6 -16.6 to -8.1 (9)</td>
<td>-17.6 0.5 -27.2 to -13.4 (9)</td>
<td>-0.1 0.5 -3.6 to 5.2 (8)</td>
<td>23.5 0.7 11 to 42.4 (10)</td>
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<tr>
<td>Hz</td>
<td>-16.0 0.5 -20.5 to -6.7 (10)</td>
<td>-16.8 0.5 -19.6 to -8.3 (10)</td>
<td>1.8 0.5 -0.6 to 6.4 (7)</td>
<td>18 0.2 13.5 to 28.5 (10)</td>
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<td>CFPS</td>
<td>-41.3 0.5 -55.9 to -37.4 (10)</td>
<td>-40.3 0.07 -51.2 to -28.5 (5)</td>
<td>17.1 0.04 12.3 to 19.8 (8)</td>
<td>91 0.2 68 to 103.5 (8)</td>
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<td>CFPI</td>
<td>-48.8 0.5 -56.8 to -43.5 (8)</td>
<td>-55.2 0.07 -63.8 to -43.3 (7)</td>
<td>24.0 0.04 19.6 to 29.1 (7)</td>
<td>104 0.2 92.5 to 119.5 (8)</td>
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<tr>
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<td>-17.6 &lt;0.0001 -19.7 to -11.3 (19)</td>
<td>1.5 &lt;0.0001 -2.2 to 6.4 (15)</td>
<td>21.5 &lt;0.0001 13.5 to 28.5 (20)</td>
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<tr>
<td>CFPS + CFPI</td>
<td>-47.4 &lt;0.0001 -56.1 to -39.1 (18)</td>
<td>-46.4 &lt;0.0001 -60.8 to -38.1 (12)</td>
<td>19.8 &lt;0.0001 16.1 to 25.4 (15)</td>
<td>98.5 &lt;0.0001 78.5 to 112 (16)</td>
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</table>

Legend – All data are medians, IQR and number of subjects (n). *Wilcoxon rank-sum test
**ΔSPDchol/β-adren** indicates SPD recorded after cholinergic followed immediately by β-adrenergic stimulation minus SPD recorded after cholinergic stimulation alone.

### SPD compared to sweat [Cl-]

The power of the SPD assay to distinguish between different groups was comparable to that of the sweat [Cl-] assay (table 2; figure 3). Both assays were plotted in relation to each other, revealing good correlation of SPD to sweat [Cl-] obtained simultaneously (r=-0.7, 95% CI: -0.84 to -0.48, P<0.0001) (figure 4). Further, using ROC as an analytical tool, we demonstrated similar accuracy for SPD in identifying CF patients as for sweat [Cl-] with equal, excellent AUC of 0.997 (95% CI: 0.988 to 1.007) for SPD and 0.986 (95% CI: 0.958 to 1.014) for sweat [Cl-] (figure 4).

Neither sweat [Cl-] nor cholinergically stimulated SPD resulted in a significant difference between HC and Hz (p=0.7 and p=0.6, respectively) or between CFPS and CFPI (p=0.2 and p=0.5, respectively).
For 31 of 37 study subjects, repeated SPD and sweat [Cl-] measurements were available (figure 4). Analyzing the differences between test and re-test results by the Bland-Altman method, sweat [Cl-] showed better repeatability for mostly CF patients (values > 40 mmol/l), whereas equal, but higher variability was seen for SPD throughout the whole functional spectrum. However, there was no fixed or proportional bias for either test and the 95% confidence limits of agreement were comparable. Despite the wider difference between two tests seen for SPD in some study subjects, the second SPD test did not alter the classification of individuals in their subgroup (table 2).

**SPD after sequential cholinergic/β-adrenergic stimulation**

In an attempt to directly activate CFTR Cl- channels and improve the segregation of subgroups, we modified the sweat stimulation protocol using sequential stimulation with cholinergic followed by β-adrenergic agonists (Protocol 2). Interestingly and unexpectedly, sequential stimulation with cholinergic and then β-adrenergic agonists had no effect on median SPD in HC and Hz as control group (p=0.6), but statistically significantly depolarized the SPD of all CF patients (p<0.0001) (figure 5). Sweat [Cl-] did not change in either group following sequential stimulation (HC/Hz: p=0.95 and CFPS/CPFI: p=0.94) (figure 5).

Calculating the difference of the SPD response between cholinergic and sequential cholinergic/β-adrenergic stimulation (ΔSPD_{chol/β-adren}), we find a statistically significant difference between controls and CF patients (p<0.001). After sequential stimulation, neither the SPD of HC nor Hz subjects changed significantly; whereas, the SPD of CFPS and CFPI patients was statistically significantly depolarized with both agonists. Thus, subgroup analysis showed no statistical difference between the response of HC from Hz (p=0.5). However, SPD was statistically significantly more positive in CFPI patients following sequential cholinergic/β-adrenergic sweat stimulation than in CFPS patients, so that ΔSPD_{chol/β-adren} between CFPS and CFPI was statistically significantly different (p=0.04) (table 2; figure 5).

**Adverse events**

No severe systemic or local adverse events were observed. About 20% of the study subjects showed very mild transient reddening of the skin following iontophoresis. One subject showed a moderate skin reaction (reddening), which resolved after 30 minutes.

**DISCUSSION**

A Novel Sweat Test: For over 50 years, the gold standard test in CF has been the QPIT which involves a multi-step procedure of 1) in vivo stimulation of the sweat glands; 2) collection of the sweat secretion, and 3) laboratory determination of the sweat [Cl]-. We have now explored the increased negative bioelectric potential arising directly from the basic CF defect to introduce a proof of concept for a new simple, practical, real time assay of sweat gland function, which appears to be as accurate as the traditional QPIT, and perhaps, with further technical improvement in electrode design, may prove to be more reproducible and sensitive.

The bioelectrical properties of isolated sweat ducts have been extensively studied. Moreover,
in vivo measurements in sweat droplets from single glands on the skin showed that the electrical potential of the sweat ducts can be recorded at the gland orifice on the skin surface^{20,25}. Thus, we surmised that placing an ECG electrode over an area of stimulated skin would measure the average of the electrical potentials from ducts of all secreting sweat glands (>1,000) under the electrode. This skin potential could, in turn, serve as a real time diagnostic measure without further procedures such as chemically analyzing sweat. Moreover, since the electrical potential from the sweat duct directly reflects the function of CFTR, this technique might assess the impact of drugs administered in clinical trials for correcting the defect in CFTR Cl\(^-\) conductance.

**The SPD as a biomarker assay for CF:** In order to establish the usefulness of the sweat gland SPD for assessing CFTR function, we measured phenotypically and genotypically well characterized cohorts of healthy controls (HC), heterozygotes (HZ), pancreatic sufficient (CFPS) and pancreatic insufficient CF patients (CFPI). We anticipate that these cohorts should serve as surrogates for distinct levels of CFTR function that might be induced by corrective therapies. We demonstrated that the SPD (figure 3) was statistically significantly more negative in CF patients than in control subjects (-47.4 mV vs. -14.5 mV; Table 2), which corresponded well to previous results on single glands (-66 mV vs. – 29 mV)^{25}\).

In our preselected cross-sectional cohort, SPD appeared to be as effective as the classic QPIT sweat [Cl\(^-\)] in segregating the different subgroups (figure 3) giving equal diagnostic accuracy in identifying CF patients (figure 4). This finding must now to be confirmed in a larger, unselected and more representative population in a future study. The high correlation between SPD and sweat [Cl\(^-\)] indicates that both methods measure phenomena related to the same defect (CFTR) in sweat glands. Repeatability analysis showed larger variability in the control group for sweat [Cl\(^-\)] and an overall larger, but similar variability in the controls and CF group for SPD measurements. However, the absence of any bias in the Bland Altman analysis, as well as comparable limits of agreement, suggest acceptable repeatability for SPD measurements. The larger variability of SPD measurements observed in some individuals may be due at least partly to reduced sensitivity caused by electrode asymmetries (discussed later) and should be reduced with improvements in electrode design.

Previous studies in a larger cohort demonstrated a slight elevation of mean sweat [Cl\(^-\)] in heterozygotes compared to controls and a statistically lower mean sweat [Cl\(^-\)] for CFPS as compared to CFPI, although the overlap between the groups precluded segregation of individuals^{26}. Therefore, an assay that provides an increased ability to detect differences between subgroups, particular between CFPS and CFPI, based on small increments in CFTR function, may have significant value for assessing degrees of correction achieved by drug therapy to modify mutant CFTR function. However, neither sweat [Cl\(^-\)] nor cholinergically induced SPD testing distinguished between HC and HZ or between CFPS and CFPI in this small cohort (figure 3, table 1).

Sequential cholinergic/β-adrenergic sweat stimulations promise to improve diagnostic performance of SPD: We reasoned that maximally stimulating CFTR in all groups might enhance differences in SPD responses between the subgroups where theoretically, at least, CFTR function is twofold greater in HC than in HZ subjects and likely greater in CFPS than in
CFPI patients. Following cholinergic sweat stimulation, we iontophoresed a mixture of the β-adrenergic agonists isoproterenol and aminophylline to enhance cAMP levels and maximally stimulate CFTR activity. We expected maximal effects, if any, in control subjects, where CFTR is highly expressed. However, stimulation with β-agonists did not change the average SPD in either HC or HZ subjects, but surprisingly increased (depolarized) the SPD response in all CF patients, with the greatest effect in CFPI patients (figure 5). The differences between the SPD response following cholinergic sweat stimulation alone and sequential cholinergic/β-adrenergic stimulation (ΔSPD_{chol/β-adren}) was not only statistically significantly different between controls and CF patients (p<0.0001), but also between CFPS from CFPI (p=0.04). In fact, the ΔSPD_{chol/β-adren} linearly correlated with the measured baseline SPD (r=-0.8, 95% CI: -0.92 to -0.69, P<0.0001), suggesting that the β-adrenergic SPD response is directly related to the degree of CFTR dysfunction. The ΔSPD_{chol/β-adren} might support further segregation between different CF subgroups in the future. A range of ΔSPD_{chol/β-adren} in the CFPS and CFPI subjects is anticipated because different mutations in the CFTR gene express varying degrees of residual CFTR function.

Why does β-adrenergic sweat stimulation depolarize SPD in CF: While the β-adrenergically unaltered SPD response in controls may be explained by the presence of constitutively, fully activated CFTR in these glands, the dramatic depolarization in patients with CF seems puzzling. We expected that less (or no) CFTR expression in CF patients would result in smaller (or no), not larger changes in SPD. Two scenarios might possibly explain these results. First, β-adrenergic activation may stimulate, or insert, additional mutant CFTR in the membrane, which would shunt the electrogenic potentials in these ducts, thus leading to a more positive, "normalized" SPD. However, increased CFTR conductance should have decreased sweat [Cl-], which was not observed. Moreover, β-adrenergic stimulation is not known to increase the expression of ∆F508 mutations.

Second, and perhaps more likely, β-adrenergic stimulation appears to stimulate acid secretion by the sweat duct which should depolarize the lumen and hence the SPD. The presence of a small Cl- conductance as expected for some mutations would partially shunt this potential as seen in CFPS patients here and reduce, but not abolish, the depolarizing effect of proton secretion.

Technical challenges of the SPD sweat test: Using a simple ECG set-up for SPD recordings, we have achieved excellent statistical separations. However, artefacts introduced by asymmetries and voltage drifts between the measuring and reference electrodes (>± 5 mV in up to 35% of the experiments) may account for some of the intra- and inter-individual variability. These errors may have been caused by 1) alterations of the composition of the gel of measuring ECG electrode due to exposure to different volumes and salt concentrations in secreted sweat, and 2) use of unmatched electrodes, ECG gel vs. agar Ag/AgCl. Improvements in the design of the electrode are expected to minimize these artefacts and increase accuracy of the SPD recording. Furthermore, to obviate the need for subcutaneous needles in paediatric subjects, other means of establishing an electrical reference with the serosal fluid, such as a minor skin abrasion may prove preferable.
Summary: The presented data show, for the first proof of concept that cholinergically stimulated sweat gland potentials (SPD) can be used as an immediate, simple, and accurate method to assess CFTR function \textit{in vivo}. Including a second protocol to compare cholinergically stimulated SPDs with combined cholinergically and \(\beta\)-adrenergically stimulated SPDs increased the power of the test to detect smaller differences in CFTR function, notably between subjects with CFPS and CFPI. Thus, these observations justify further development of SPD as an outcome parameter in clinical trials and as a diagnostic test for cystic fibrosis.

\textbf{Competing interest:} none

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FIGURE LEGENDS

Figure 1: **Sweat gland potential difference (SPD) and sweat volume depend on iontophoresis current**

Data show medians and IQR (error bars) of measurements on number (n) of healthy controls. Each of 15 healthy individuals underwent 2-6 sweat tests using cholinergic stimulation by iontophoresis with current ranging from 2.5 to 50µA/cm². The dose-effect curves were fitted using nonlinear regression based on a simple receptor-ligand kinetic \( Y = Y_0 + (Y_{max} - Y_0) \frac{X}{(K+X)} \). Since larger currents did not statistically significantly alter either SPD or sweat volumes, we used an iontophoresis current of 30µA/cm² as optimal for SPD results and sweat volume for sweat chloride analysis (> 20µl).

Figure 2: **Summary of recordings of sweat gland potential difference (SPD)**

SPD hyperpolarized over time, but a plateau was achieved 20-30 minutes after stimulation in all subjects. Therefore, the data acquired during the last 5 minutes of the 30 minutes post iontophoresis of pilocarpine were averaged for each subject and used as a single SPD value for that subject in further analysis. Each data point here is the median and IQR of the SPD at the time indicated in minutes following cholinergic sweat stimulation for each of the four groups: HC, healthy control (n=9); HZ, heterozygotes (n=10); CFPS, pancreatic sufficient CF (n=10); CFPI, pancreatic insufficient CF patients (n=8).

Figure 3: **Comparison of sweat gland potential difference (SPD) and sweat [Cl⁻] measurements**

Each subject underwent simultaneous SPD and sweat chloride measurements, (n) number of subjects. Scatter plots show the mean of SPDs recorded during the last 5 minutes post stimulation (A) and the sweat [Cl⁻] (B) for each individual in each group. SPD and sweat [Cl⁻] show statistically significantly different medians between the control and patient groups, p<0.0001. Groups are as in figure 2.

Figure 4: **Characteristics of SPD and sweat [Cl⁻] tests following cholinergic sweat stimulation**

Receiver Operator Curves (ROC), which plots the sensitivity (true positive rate) versus the 1-specificity (false positive rate), was used as an analytical tool to test for the ability of SPD compared to sweat [Cl⁻] to distinguish between control and disease (A). AUC under ROC is 0.997 (95% CI: 0.988 to 1.007) for SPD and 0.986 (95% CI: 0.958 to 1.014) for sweat [Cl⁻]. SPD showed good correlation to the simultaneous performed sweat [Cl⁻] \( r=-0.7, 95\% \) confidence interval: -0.84 to -0.48, P<0.0001 \( (B) \). Test and re-test analysis using Bland-Altman method of differences showed no fixed bias between 2 tests for SPD (2.9 mV, p=0.4) \( (C) \) or sweat [Cl⁻] \( (2.2 \text{ mmol/l}, p=0.8) \) \( (D) \) and no proportional bias between 2 tests for SPD (Spearman \( r = 0.05, p=0.8 \)) or sweat [Cl⁻] (Spearman \( r = 0.1, p=0.6 \)). The 95% confidence limits were -18.6 to 24.5 mV for SPD and -16 to 20.3 mmol/l for sweat [Cl⁻], respectively.
Figure 5: **Sweat gland potential difference (SPD) and sweat [Cl⁻] following sequential cholinergic/β-adrenergic sweat stimulation**

Each subject underwent contemporaneous sweat [Cl⁻] and two SPD measurements. One SPD was measured after cholinergic (chol) alone and another SPD after sequential cholinergic/β-adrenergic (β-adren) stimulation (cf. Methods). Connecting lines indicate sequential measurements of SPD and sweat [Cl⁻] in the same subject. SPD values remained relatively constant for healthy controls and heterozygotes, but depolarized markedly in all CF patients (A). The sweat [Cl⁻] values were not detectably altered in any group (B). Box plots show medians and IQR of the difference between chol and β-adren induced SPD responses (ΔSPD_{chol/β-adren}). Not only are the ΔSPD_{chol/β-adren} changes highly statistically significantly different for patients vs. control groups (* p<0.001) (C), but the amount of depolarization is statistically significantly less in CFPS patients than in CFPI patients (**p=0.04) (D). Groups are defined as in figure 2.
REFERENCES


Figure 1

A

B

 SPD (mV) vs. Iontophoresis current (µA/cm²)

Sweat volume (µL) vs. Intophoresis current (µA/cm²)
Figure 2
Figure 4

Receiver-Operator Curves

A

Correlation

B

Sweat [Cl]\(^{-}\) (mmol/L)

SPD (mV)

Bland Altmann Plots

C

SPD

Difference between 2 SPD tests

Average of 2 SPD tests

D

Sweat Cl\(^{-}\)

Difference between 2 sweat Cl\(^{-}\) tests

Average of 2 sweat Cl\(^{-}\) tests
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