separations

MDPI

Supplementary material

3D printed skin-wash sampler for sweat sampling in cystic fibrosis diagnosis using capillary electrophoretic ion ratio analysis.

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Study population characteristics

We enrolled 69 subjects in the study. Groups of cystic fibrosis patients (CF), healthy carriers (carriers) of one CFTR mutation, and healthy subjects without any CFTR mutation (healthy) were preselected and invited for testing with classical Macroduct sweat test, skin wipe test and skin wash test.

In the CF group, both CFTR mutations for every subject were known. The genetic analysis was done in early childhood when the diagnose was established.

The genetic status of carriers and healthy was confirmed by blood draw and CFTR gene sequencing during the study. We chose a massively parallel sequencing (MPS) approach to determine the sequence of the CFTR gene. A commercially available kit, the Devyser CFTR kit (amplicon library), was used to prepare the library. The sequencing reaction was performed on a MiSeq System (Illumina). Two sequencing runs were performed using MiSeq Reagent Micro Kit, V2 sequencing chemistry (300 cycles).

The obtained data were analyzed using Amplicon Suite software (SmartSeq). We evaluated the pathogenicity of the found variants according to the CFTR2 database [1] (CF-causing category, etc.). If a variant was not in this database, we assessed the pathogenicity with the ACMG-AMP Variant-Interpretation Guidelines [2]. We provide mutation legacy names if known.

Of 69 subject enrolled, 65 finished, 4 failed. Three subjects were excluded from the study because Macroduct sweat test failed (two due to dry and eczematous skin, one due to low age and prolonged time to collect enough sweat), which illustrates the limitations of the classical method. Sweat sampling with skin wipe and skin wash tests were performed on the subjects. Skin wash testing failed in one subject who did not remain calm during the sampling which led to degradation of the sample.

We evaluated results from 65 subjects, 32 women, 33 men, the group healthy (21 subjects, mean age 15 ±6 years), the group carriers (22 subjects, mean age 11 ±5 years), the group CF (22 subjects, mean age 11 ±5 years). Details of Macroduct sweat test results and CFTR gene mutations are in the Tables T1-T3.

Table S1. Healthy subjects without CF-causing CFTR mutations.

Healthy females	Age (years)	Sweat chlorides (mmol/l)	CFTR gene mutation	Healthy males	Age (years)	Sweat chlorides (mmol/l)	CFTR gene mutation
1	5	8	None	**1**	6	8	None
2	8	21	None	**2**	8	11	None
3	9	5	None	**3**	11	12	None
4	12	3	None	**4**	12	16	None
5	12	27	None	**5**	14	6	None
6	16	24	None	**6**	16	29	None
7	17	11	None	**7**	16	29	None
8	18	10	None	**8**	17	10	None

9	23	22	None	**9**	18	17	None
10	26	11	None	**10**	23	26	None
-	-	-	-	**11**	25	34	None

Table S2. CF carriers – subjects with CF-causing CFTR mutation in one allele.

CF carrier females	Age (years)	Sweat chlorides (mmol/l)	CFTR gene mutation	CF carrier males	Age (years)	Sweat chlorides (mmol/l)	CFTR gene mutation
1	2	5	F508del	**1**	7	11	3272-26A->G
2	5	3	D1152H	**2**	7	10	F508del
3	6	7	3659delC	**3**	7	10	G542X
4	7	10	2184insA	**4**	7	8	F508del
5	7	28	3849+10kbC->T	**5**	13	16	F508del
6	8	16	F508del	**6**	15	34	F508del
7	12	8	F508del	**7**	15	17	F508del
8	13	19	3272-26A->G	**8**	15	13	F508del
9	13	13	F508del	**9**	16	15	F508del
10	14	25	F508del	**10**	16	38	F508del
-	-	-	-	**11**	17	29	F508del
-	-	-	-	**12**	23	24	F508del

Table S3. a: Cystic fibrosis female patients – subjects with CF-causing CFTR mutation in two alleles.

CF females	Age (years)	Sweat chlorides (mmol/l)	CFTR gene mutation 1	CFTR gene mutation 2
1	2	100	F508del	F508del
2	4	72	F508del	3849+10kbC->T
3	5	104	F508del	1774delCT
4	5	100	G542X	2184insA
5	5	99	G542X	3272-26A->G
6	11	87	F508del	2184insA
7	*13*	*106*	F508del	F508del
8	13	97	F508del	1898+1G->A
9	13	86	F508del	F508del
10	17	105	c.1394C->T	3140-26A->G
11	20	102	F508del	F508del
12	21	100	F508del	3141del19

Table S3. b: Cystic fibrosis male patients – subjects with CF-causing CFTR mutation in two alleles.

CF males	Age (years)	Sweat chlorides (mmol/l)	CFTR gene mutation 1	CFTR gene mutation 2
1	1	102	F508del	N1303K
2	6	111	F508del	G542X
3	7	109	G542X	N1303K
4	11	53	F508del	S955P
5	11	116	F508del	N1303K
6	13	112	F508del	F508del
7	14	104	F508del	F508del
8	14	94	F508del	F508del
9	14	93	F508del	F508del

10	18	102	F508del	F508del

Table S4. Calibration slopes and regression coefficients for chloride, potassium and sodium ions.

Ion	Cl^-	K^+	Na^+
Slope	0.0485	0.0322	0.0320
R^2	0.9981	0.9987	0.9980

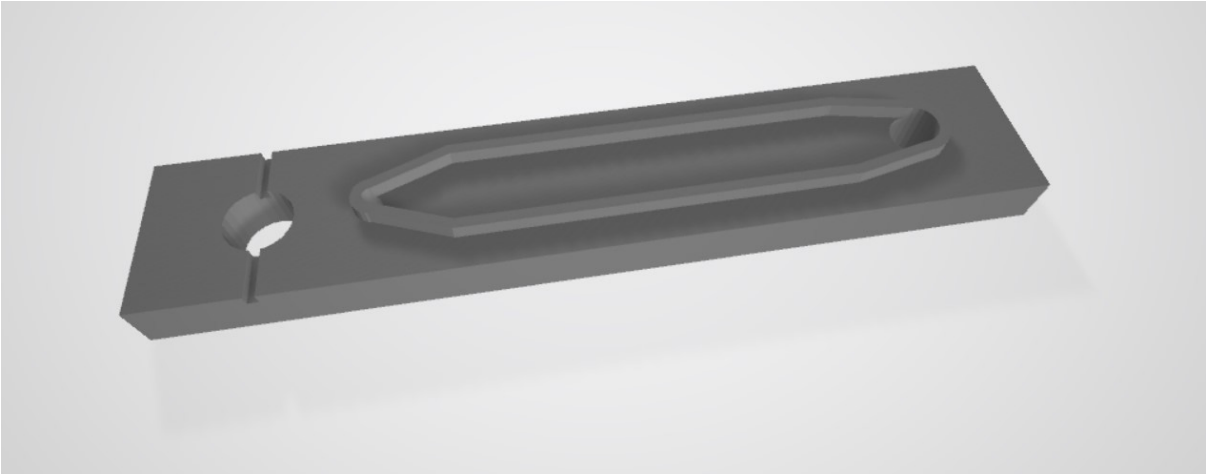

Figure S1. A schematic of the 3D-printed device.

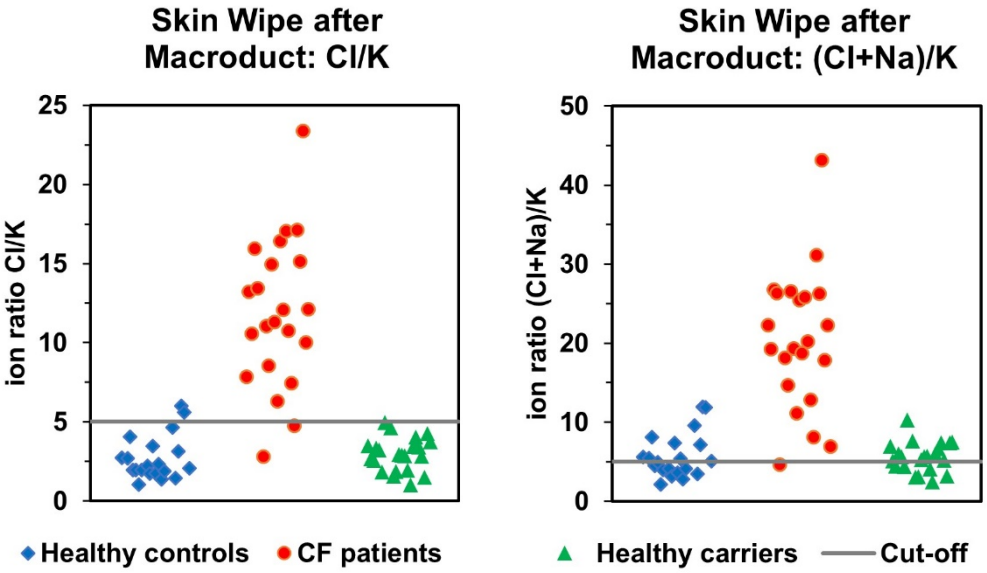

Figure S2. Comparison of Cl^-/K^+ and $((Cl^- + Na^+)/K^+)$ ion ratios from skin-wipe samples after stimulation of sweating by pilocarpine. Both datasets were obtained from the same persons.

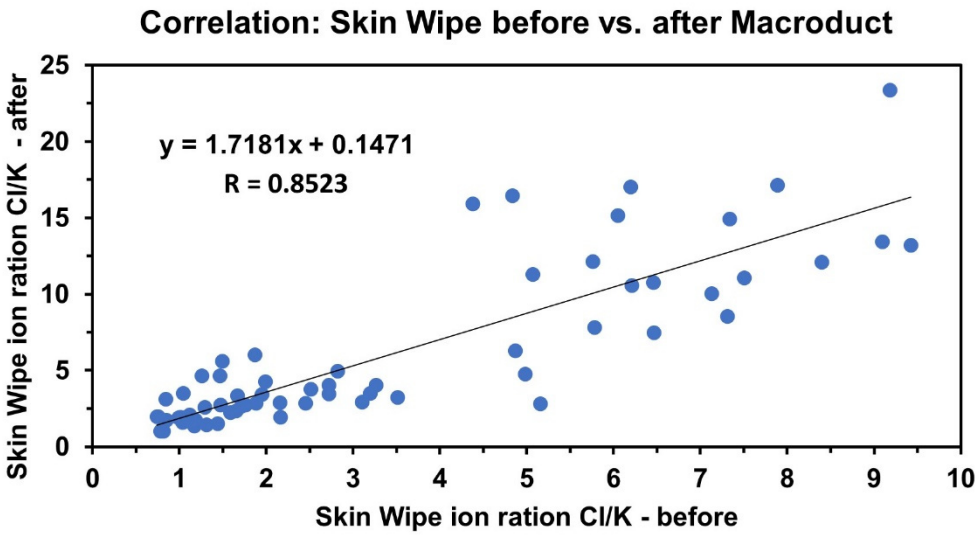

Figure S3. Correlation of data obtained from skin-wipe sample before and after sweat stimulation with pilocarpine.

References

[1] CFTR2 Database. Clinical and Functional Translation of CFTR. Available online: https://cftr2.org/ (accessed on December 1, 2021)

[2] Richards, S.; Nazneen, A.; Bale, S.; et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* **2015,** 17, 405-424.