ELSEVIER

Journal of Cystic Fibrosis 10 (2011) 326–332

Journal of Cystic Fibrosis

www.elsevier.com/locate/jcf

Original Article

Assessment of CFTR function in homozygous R117H-7T subjects

R.A. de Nooijer [a,*], J.M. Nobel [a], H.G.M. Arets [b], A.G. Bot [a], F. Teding van Berkhout [a], Y.B. de Rijke [c], H.R. de Jonge [a,d], I. Bronsveld [a]

[a] *Department of Pulmonology, University Medical Centre Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands*
[b] *Department of Paediatric Pulmonology, Wilhelmina Children's Hospital, University Medical Centre Utrecht, P.O. Box 85090, 3508 AB Utrecht, The Netherlands*
[c] *Department of Clinical Chemistry, Erasmus MC Sophia, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands*
[d] *Department of Biochemistry, Erasmus Medical Center, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands*

Received 2 January 2011; received in revised form 4 March 2011; accepted 22 March 2011
Available online 19 April 2011

Abstract

Background: R117H is a frequent missense mutation included in most *CFTR* mutation panels. However knowledge about the residual function of R117H-CFTR channels in cystic fibrosis-affected organs, *e.g.* airways, intestines and sweat glands is presently lacking.
Methods: We evaluated clinical CF symptoms and assessed CFTR function by sweat tests, nasal potential difference and intestinal current measurements in 2 homozygous R117H individuals (7T variant).
Results: The CFTR activity in airways and intestine was within the normal range. However both individuals presented with a borderline sweat test and the male patient was infertile.
Conclusions: The lack of impact of the R117H mutation on chloride secretion in intestine and nose contrasts with the ~80% loss of CFTR activity reported in patch clamp studies. Apparently CFTR activity is not rate-limiting for chloride secretion in both tissues at levels >20% of normal, or compensatory factors may operate that are absent in heterologous host cells *in vitro*.

Keywords: Cystic fibrosis; R117H; NPD; ICM; CFTR

1. Introduction

Since the discovery of the cystic fibrosis transmembrane conductance regulator (*CFTR*)-gene, approximately twenty years ago, over 1600 mutations have been identified. These mutations can be classified as severe (typical) or mild (atypical). Recent publications suggest a correlation between the type of *CFTR* mutations and the clinical impairment in (later) life [1,2]. This suggestion is based on genotype–phenotype associations in epidemiological studies where clinical outcome was compared with genotype. However, some atypical mutations, such as R117H, are less suitable for this classification because their phenotypical manifestations are more sensitive to variations in other genetic and epigenetic factors or environmental factors such as a certain lifestyle [3].

The R117H mutation is a relatively frequent mutation in cystic fibrosis (CF) patients worldwide [4]. It is included in most mutation panels for newborn screening and in CF carrier screening in couples seeking genetic counseling. This missense mutation has mixed conductance (class IV) and gating (class III) abnormalities which lead to severe loss of CFTR channel function [5]. R117H can occur in *cis* with either the polypyrimidine stretch T5 or T7 [6]. The T5 variant in intron 8 of the *CFTR* gene leads to improper splicing, removing exon 9 from 90% of the CFTR protein produced. Therefore only 10% of the CFTR protein produced by an allele with the 5T variant may be functional, and the combined effect of R117H and T5 on the same chromosome, with *e.g.* a F508del mutation on the other allele, results in classic CF. This splicing defect is less severe and more variable in the T7 variant and can either result in CF or in congenital bilateral absence of the vas deferens (CBAVD) [7].

Whereas much is known about the phenotypic variation among compound heterozygotes for F508del and R117H,

* Corresponding author. Tel.: +31 88 755 6180.
E-mail address: r.denooijer@umcutrecht.nl (R.A. de Nooijer).

1569-1993/$ - see front matter © 2011 European Cystic Fibrosis Society. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.jcf.2011.03.009

present information about the phenotype of the individual R117H mutation is restricted to expression studies in heterologous host cells [5,8]. Extrapolation of such data to native human tissues is questionable because tissue-specific factors may affect the open probability and even the permeability of the forskolin-activated CFTR channel *in vivo* [9]. This report describes 2 rare index cases of individuals who are homozygous for the R117H-7T *CFTR* mutation. *In vivo* and *ex vivo* assays to measure residual CFTR function in both patients, *i.e.* the sweat test, the nasal potential difference (NPD), and intestinal current measurements (ICM) in freshly excised rectal suction biopsies were applied to gain insight into the phenotype of the R117H mutation.

2. Methods

2.1. Subjects

A 35-year old male presented with infertility and appeared to have CBAVD. A 33-year old female had no clinical symptoms but was recognized by mutation analysis after her son was identified with F508del/R117H by newborn screening. Both individuals were homozygous for the R117H-7T *CFTR* mutation, diagnosed on the basis of mutation analysis at the *CFTR* locus. They were examined for clinical CF symptoms including determination of pancreatic elastase in the stool as an index for the exocrine pancreatic status, lung function tests (spirometric measurements of FVC and FEV_1), bacteriology of sputum, and a history of meconium ileus or diabetes mellitus.

2.2. CFTR mutation analysis

Mutation detection was performed using a line probe assay (INNO-LiPA CFTR19 and CFTR17+Tn, Innogenetics, Ghent, Belgium) for simultaneous detection and identification of 36 *CFTR* sequence variants and identification of the T alleles in intron 8 of *CFTR* (5T, 7T and 9T). The presence of a mutation was confirmed using direct sequence analysis (ABI 3730xl system, Applied Biosystems) [10].

2.3. Nasal potential difference measurements (NPD)

In vivo potential-differences across the nasal epithelium were measured in principle according to Knowles et al. [11,12]. In short, an exploring catheter was positioned against the inferior turbinate in the nose, and a subcutaneous needle in the forearm, functioning as reference electrode. Both the exploring catheter and the subcutaneous needle were connected to a high-impedance voltmeter by Ag/AgCl electrodes and agar/saline-filled salt bridges, connected to a Powerlab (8/30, ADInstruments) for signal digitalization and registration using LabChart software. The superfusion solutions were applied *via* the exploring double-barreled catheter. The nasal turbinate was superfused (5 mL/min) for periods of three minutes with the following solutions (mM): Solution A (for measurements of basal PD): Custom Ringer's. 135 NaCl; 1.2 $MgCl_2$; 2.25 $CaCl_2$; 2.4 K_2HPO_4; 0.4 KH_2PO_4. Solution B (for measurements of amiloride-sensitive sodium absorption by the epithelial sodium channel, ENaC): 0.1 amiloride hydrochloride (HCl) in Ringer's solution. Solution C (for measurements of basal chloride conductance): Cl^--free solution (+amiloride):135 sodium gluconate, 1.2 $MgSO_4$, 2.2 calcium gluconate, 2.4 K_2HPO_4, 0.4 KH_2PO_4, 0.1 amiloride HCl. Solution D (for measurements of cyclic adenosine monophosphate (cAMP)-stimulated CFTR conductance): 0.01 isoproterenol HCl in solution C. Solution E (for measurements of Ca^{2+}-activated chloride conductance): 0.1 ATP in solution D. Measurements were performed in duplicate in both nostrils. The NPD tracing of the nostril with the highest Cl^- secretory response, *i.e.*, with the largest capacity to transport Cl^-, was assessed for the calculations performed in this report.

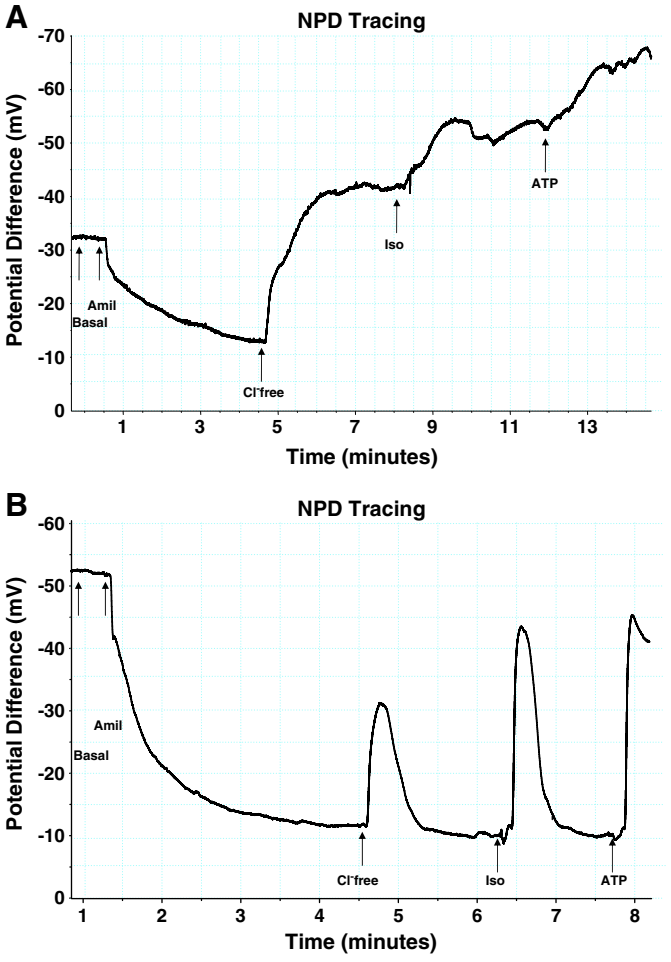

Fig. 1. Representative *in vivo* recordings of nasal potential difference (mV). (A) Non-CF control showing hyperpolarization of the nasal epithelium, *i.e.* changes towards a more negative PD, in response to a Cl^--free solution (Cl^--free), and the β-agonist isoproterenol (Iso) which induces cAMP-dependent chloride secretion. The depolarizing response to amiloride, indicating the activity of epithelial Na^+ channels (ENaC), is relatively modest. (B) F508del homozygous CF patient showing a comparatively larger amiloride-induced depolarization, and the lack of a hyperpolarizing response to Cl^--free solution and isoproterenol, indicative for ENaC hyperactivity and/or a lack of CFTR activity.

Examples of crude NPD tracings obtained in a non-CF and homozygous F508del CF patient are shown in Fig. 1A and B respectively.

2.4. Intestinal current measurement (ICM)

ICM was performed on freshly excised rectal suction biopsies as outlined in detailed published protocols [13–16]. In short, electrogenic transport of ions across the intestinal epithelium was measured as a short-circuit current (Isc). The biopsy specimens were preserved in phosphate-buffered saline on ice and directly mounted in adapted micro-Ussing chambers (aperture, 1.13 mm^2). After equilibration, the following compounds were added in a standardized order to the mucosal (M) or serosal (S) side of the tissue: (a) amiloride (0.01 mM, M), to inhibit amiloride sensitive electrogenic Na^+ absorption; (b) indomethacin (0.01 mM, M+S), to reduce basal Cl^- secretion caused by endogenous production of prostaglandins; (c) carbachol (0.1 mM, S), to initiate the cholinergic Ca^{2+}- and protein kinase C-linked Cl^- secretion; (d) DIDS (0.2 mM, M), to inhibit DIDS-sensitive, non-CFTR Cl^- channels like the Ca^{2+}-dependent Cl^- channels (CaCCs); (e) histamine (0.5 mM, S), to reactivate the Ca^{2+}-dependent secretory pathway and to measure the DIDS-insensitive component of Ca^{2+}-dependent Cl^- secretion; (f) forskolin (0.01 mM, S)+8-Br-cAMP (1 mM, M+S), to further activate cAMP-dependent Cl^- channels such as CFTR and the outwardly rectifying Cl^- channel (ORCC). Four rectal biopsies were obtained in both R117H-7T homozygous individuals. The ICM tracing belonging to the biopsy with the highest Cl^- secretory response was included in the calculations in order to facilitate a comparison with historical data (Table 1). Crude Isc values (μA) were converted to μA/cm^2 based on the 0.011 cm^2 surface area of the aperture.

Table 1
Summary of electrophysiological measurements in a non-CF group versus a group of F508del homozygotes given as mean (SD).

	Control group (non-CF)	CF group
NPD[17]	ΔPD (mV)	ΔPD (mV)
	n=25	n=23
Basal PD	−24 (11)	−45 (10)
Amiloride	+10 (6)	+21 (9)
Gluconate (Cl^--free)	−15 (10)	−1 (5)
Isoproterenol	−8 (4)	−2 (3)
ATP	−1 (3)	−1 (3)
ICM[18]	ΔIsc (μA/cm^2)	ΔIsc (μA/cm^2)
	n=50	n=51
Amiloride	−8.7 (11)	−8.7 (11)
Carbachol	38.5 (23)	−5.3 (10)
Histamine	33.0 (26)	−5.0 (10)
Forskolin/cAMP	7.1 (8.2)	3.0 (2.9)
Cumulative Cl^- secretion (=Δcarbachol+Δhistamine +Δforskolin/cAMP)	81.4 (36)	2.3 (3.5)

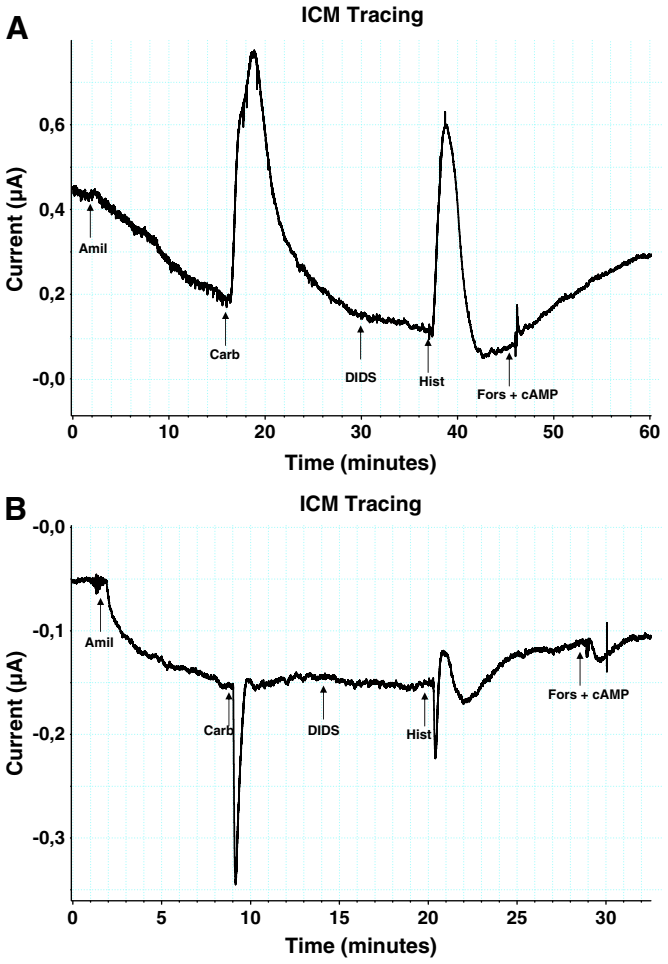

Fig. 2. Representative recordings of short circuit currents in human rectal biopsies. (A) Non-CF control showing a large increase in Isc upon addition of carbachol (carb), histamine (hist) and forskolin/cAMP (fors/cAMP) representing apical Cl^- secretion. (B) F508del homozygous CF patient showing a lack of Cl^- secretory response to carbachol, histamine and forskolin/cAMP. The reversed current observed in response to carbachol and histamine (reflecting net K^+ secretion) and the absence of a Cl^- secretory response to forskolin/cAMP are indicative of classical CF.

Examples of crude ICM tracings obtained in a non-CF control and a homozygous F508del CF patient are shown in Fig. 2A and B respectively.

The electrophysiological findings in upper airways and distal intestine were compared with that of a large cohort of non-CF individuals and F508del CF homozygotes who had been studied previously by the same protocol and by the same team of investigators (Table 1) [17,18].

2.5. Sweat test

Sweat was collected after pilocarpine iontophoresis according to Gibson and Cooke for 30 min on gauze pads [19]. Determination of the sweat sodium and chloride concentrations was accomplished using a flame photometer (IL 943, Instrumentation Laboratory) and a coulometer (Marius, Netherlands), respectively.

3. Results

3.1. CFTR mutation analysis

Both subjects were tested homozygous for the R117H-7T *CFTR* mutation by INNO-LiPA which was subsequently confirmed by direct sequence analysis.

3.2. Clinical features

Both subjects were screened for symptoms compatible with CF disease. As mentioned above, our male patient presenting with infertility was subsequently diagnosed with CBAVD which was demonstrated by physical examination and ultrasound in which no vasa deferentes were found. Our female subject, the mother of 2 children, was evidently fertile and did not show any indication of subfertility.

A lung function test was performed in our male patient and showed a FVC of 6.87 L (114% predicted) and a FEV_1 of 5.75 L (117% predicted).

Both individuals were free of nasopharyngeal or sinus problems. The pancreatic elastase values in their stools as an index for the exocrine pancreatic status were also normal (male subject: 221 μg/g; female subject: >500 μg/g). No pathogenic microorganisms were found in sputum cultures. Both individuals had a negative history for meconium ileus or a (CF-related) diabetes mellitus. In summary, the clinical features of both persons were normal and not indicative of CF disease.

3.3. NPD results

In NPD measurements, the basal and amiloride-sensitive PD can be used as a marker for the activity of epithelial sodium channels, ENaC, but is also influenced by the presence of other ion conductances in the apical membrane, in particular CFTR

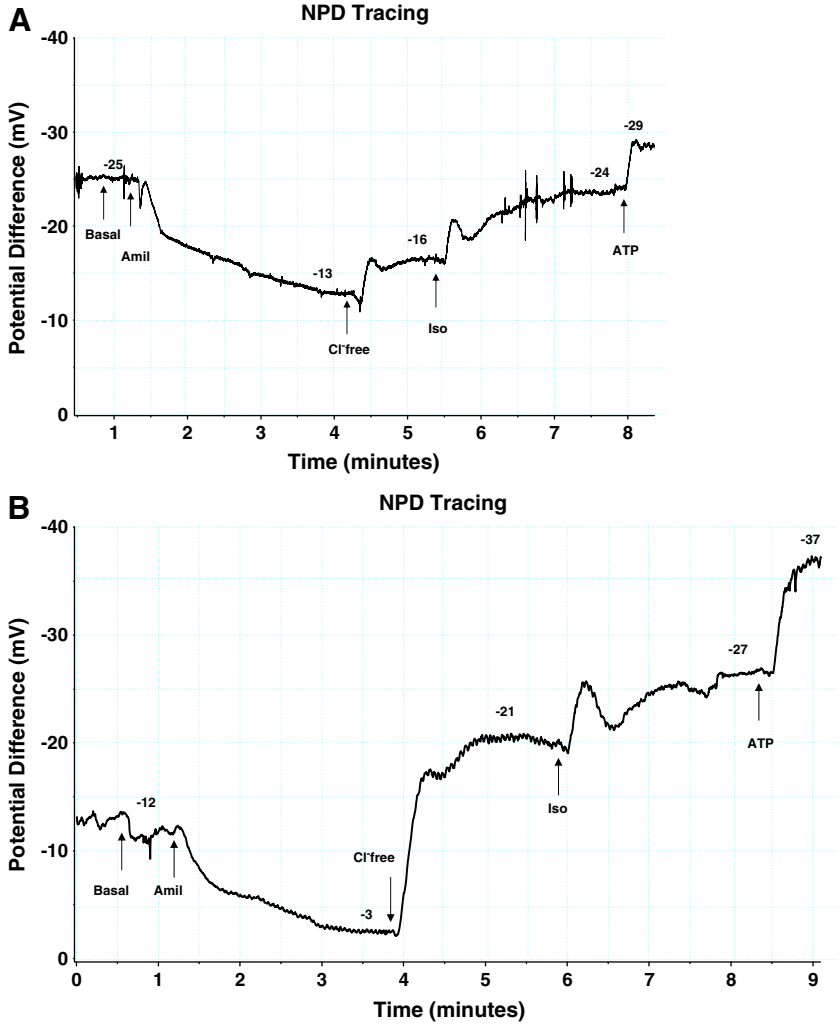

Fig. 3. NPD tracings of the R117H-7T homozygotes. (A) Male: The baseline potential difference was −25 mV which is within the normal range (see Table 1). Superfusion with the ENaC inhibitor amiloride caused a 12 mV depolarization, *i.e.* within the normal range (see Table 1). The hyperpolarizing response to zero chloride solution (−5 mV) and isoproterenol (−6 mV) was also in the normal range, indicating a normal Cl^- conductance in the nasal epithelium. (B) Female: The baseline potential difference was −12 mV and superfusion with amiloride caused a depolarization of 9 mV, both values within the normal range (see Table 1). The large hyperpolarizing response to a zero chloride solution (−18 mV) and isoproterenol (−6 mV) was indicative of a normal Cl^- conductance.

[20]. In contrast the hyperpolarizing response to Cl^--free perfusion in the presence of amiloride and in the absence or presence of a CFTR activator isoproterenol, solely reflects the activity of the CFTR chloride channel. As is clear from a comparison between the data in Fig. 3 and Table 1, none of the electrophysiological parameters measured were different from the control values obtained in non-CF patients. Therefore, both R117H-7T homozygotes showed a normal electrophysiological phenotype in their upper airways, not indicative of CF disease.

3.4. ICM results

Transepithelial chloride secretory currents in the intestine are carried mainly or exclusively by CFTR in the apical membrane of the colonocyte, as evidenced by the absence of such currents in individuals with classical CF (*cf.* Fig. 2B versus 2A). The cumulative chloride secretory response ($=\Delta Isc^{carbachol}+\Delta Isc^{forskolin/cAMP}+\Delta Isc^{histamine}$) was recently identified as the most discriminative ICM marker with a clear cut-off value of 34 μA/cm^2 between pancreatic sufficient (PS) CF patients and control subjects [21].

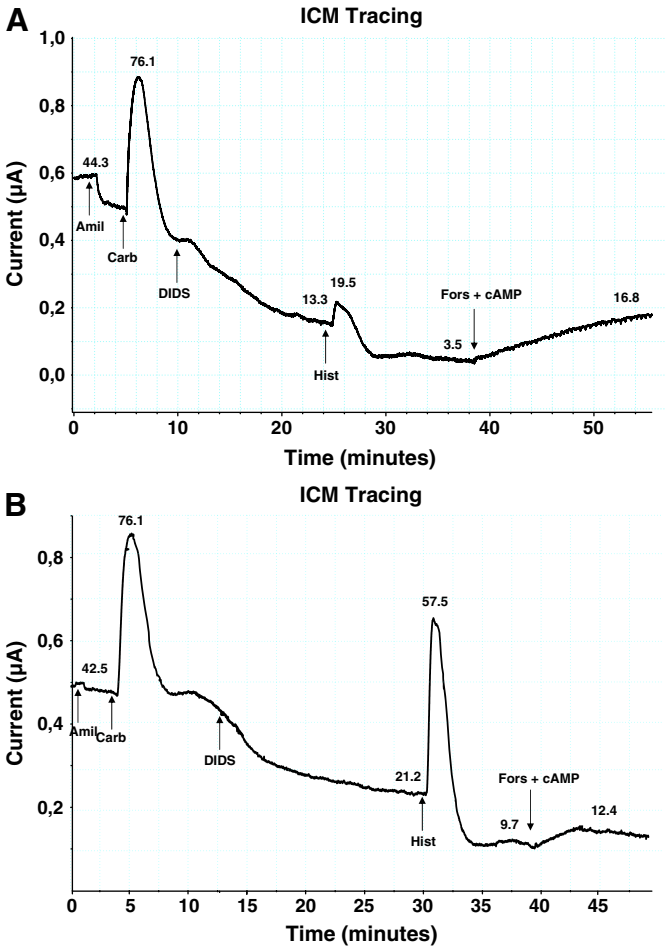

Fig. 4. ICM tracing of the R117H-7T homozygotes. Numbers above the crude tracing indicate normalized Isc values (μA/cm^2). (A) Male: The cumulative Cl^- secretion defined as a summation of the Isc response (in μA/cm^2) to carbachol (31.9), histamine (6.2), and forskolin/cAMP (13.3), amounted to 51.4, *i.e.* fell into the normal range (see Table 1). (B) Female: The cumulative Cl^- secretion (72.6 μA/cm^2) calculated from the Isc response to carbachol (33.6), histamine (36.3), and forskolin/cAMP (2.7) was likewise in the normal range. The unusually large Isc response to histamine was not observed in the three other biopsies taken from this individual.

In both R117H-7T individuals the Isc responses to secretagogues (Fig. 4), and the cumulative value of the Cl^- secretory responses ($=\Delta Isc^{carbachol}+\Delta Isc^{forskolin/cAMP}+\Delta Isc^{histamine}$) were normal and far above the CF range (Fig. 4, legend; Table 1). According to the new criterium [21], both R117H homozygotes would therefore belong to the "CF unlikely" group.

3.5. Sweat test results

The sweat test of our male patient was Cl^- 34 mmol/L and Na^+ 57 mmol/L. Our female subject showed a Cl^- value of 42 mmol/L and Na^+ of 50 mmol/L.

Both sweat tests are in the borderline range with a Cl^- value between 30 and 60 mmol/L and different from most non-CF healthy controls. This indicates that CFTR activity is reduced but not absent in at least one tissue, the sweat duct, in line with the substantial loss of CFTR conductance of the R117H mutant (70–85%) reported in heterologous expression systems *in vitro* [5,8].

4. Discussion

In this study we describe both clinical and electrophysiological findings in two R117H-7T homozygous subjects. Based on their electrophysiological signature in both NPD and ICM, these two individuals were not distinguishable from non-CF controls. Because these assays measure the basic defect in CF, *i.e.* abnormalities in CFTR-mediated chloride transport in epithelial tissues, there is a clear discrepancy between the apparently normal CFTR chloride channel function in airways and intestine reported here and the findings in patch clamp studies of the R117H CFTR channel in heterologous host cells *in vitro*, showing a loss of Cl^- conductance of ~70–85% [5,8]. This loss of function results for a minor part from a small reduction in pore conductance for Cl^- (14%) but is mainly due to a strong reduction in channel open probability (~72%), indicating that the R117H mutation affects both the pore properties and the gating of the CFTR channel, *i.e.* it has mixed class III and class IV properties [5]. The intracellular processing of the R117H channel, and its trafficking to the cell surface are not affected by the mutation, ensuring normal levels of mature CFTR protein in the apical membrane of the epithelial tissues.

In addition, the normal bioelectrical phenotype in the nasal epithelium of the R117H homozygous subjects contrasts with the elevated sodium absorption and minimal Cl^- conductance reported in NPD measurements for CF patients carrying the A455E mutation [22]. This mutant channel has normal Cl^- conducting and regulatory properties but is severely misprocessed (class II), resulting in an ~90% loss of Cl^- current in heterologous epithelial cells [5]. These combined data show that two different mutations in *CFTR*, both resulting in an ~85–90% loss of conductive Cl^- channel transport in heterologous cell types, and both associated with absent or mild pulmonary disease [23], pancreatic sufficiency, borderline sweat tests and

CBAVD in males [24], have distinct effects on the bioelectrical phenotype of the airways, ranging from normal (R117H) to severe (A455E). This comparison illustrates that the loss of function observed for rare *CFTR* missense mutations in heterologous epithelial cells is not a reliable predictor for the residual CFTR function in native airway epithelium and is only of limited prognostic value [25].

Why the *in vitro* and *in vivo* phenotypes do not match is not clear but several mechanisms could be involved: first, rescue mechanisms may operate in native epithelium which are completely or partially lacking in the heterologous host cells *in vitro*. For example, functional rescue of a class III regulatory mutant including R117H may depend on the expression of stimulating co-factors such as the IRBIT (Inositol 1,4,5-triphosphate receptor-binding protein released with Inositol 1,4,5-triphosphate) that reduces channel mean close time or the NHERF1 (Na^+/H^+ exchange regulatory factor 1) scaffolding protein that drives CFTR dimerization and is known to increase the open probability of the CFTR channel [26,27]. Secondly, other CFTR-dependent Cl^- conductances such as the recently identified SLC26-A9 chloride channel [28] or the ORCC [29] may be expressed in nasal epithelium but not in cultured cell models. Thirdly, compensatory overexpression of a mutant CFTR protein with some residual function may occur in some individuals and rescue the CF phenotype.

The lack of an intestinal bioelectrical phenotype in both R117H homozygous individuals may likewise result from intestine-specific rescue mechanisms but is also readily explained by the known insensitivity of the intestinal current measurements to a partial loss of CFTR function [30–32]. Western blot analysis of CFTR in intestinal membranes combined with ICM analysis of the Cl^- secretory current in CF mouse models and CF patients (*e.g.* homozygous for the 3272-26A>G splice mutation) that express variable amounts of fully functional CFTR protein in the apical membrane showed that the CFTR conductance is no longer rate-limiting for transepithelial Cl^- transport at CFTR protein levels above ~20% of non-CF controls (H.R. de Jonge, unpublished observations). Consequently, *CFTR* mutations associated with less than ~80% loss of CFTR expression or function in the colon would escape detection by the ICM technique. The R117H (and the A455E) CFTR mutants may therefore behave as borderline cases in which the homozygous expression is associated with a normal ICM pattern (≥20% residual CFTR conductance) whereas compound heterozygotes carrying a second more severe mutation (*e.g.* F508del) show a more variable residual CFTR Cl^- current in the intestine ranging from normal to severely reduced, but not absent [24,33].

Still another explanation can be given for the lack of pancreatic insufficiency noted in both R117H homozygotes and compound heterozygotes for this mutation. Despite the severe loss of Cl^- channel function of the R117H mutant CFTR, its bicarbonate transport function is not impaired [8] or even enhanced [34], in clear contrast to all known mutations associated with pancreatic insufficiency [8]. The finding of pancreatic sufficiency in both R117H homozygous subjects therefore confirms the notion that the loss of HCO_3^- transport function is of more importance for the pathogenesis of CF in the pancreas than the loss of Cl^- transport function.

In conclusion, the only CFTR-associated abnormalities found in the R117H-7T homozygous subjects in this study were a slightly elevated sweat Cl^- and CBAVD in the male individual. The latter confirms the extreme susceptibility of the epididymis to defective Cl^- transport, resulting in early regression of the mesonephric duct [24]. The abnormal sweat chloride illustrates the high sensitivity of the sweat test to detect partial loss of CFTR function in patients in which the NPD and ICM bioelectrical assays fail to monitor any abnormalities in CFTR chloride transport function.

Acknowledgments

The authors would like to thank the patients and family for their cooperation. The valuable contributions of Dr. Hennie T. Brüggenwirth and Dr. Karen M.K. de Vooght are gratefully acknowledged. The authors thank C.M (Kees) Ligtvoet for technical support.

References

[1] Castellani C, Cuppens H, Macek Jr M, et al. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J Cyst Fibros 2008;7:179–96.
[2] Farrell PM, Rosenstein BJ, White TB, et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. J Pediatr 2008 Aug;153(2):S4–S14.
[3] Peckham D, Conway SP, Morton A, Jones A, Webb K. Delayed diagnosis of cystic fibrosis associated with R117H on a background of 7T polythymidine tract at intron 8. J Cyst Fibros 2006;5:63–5.
[4] http://www.who.int/genomics.
[5] Sheppard DN, Rich DP, Ostedgaard LS, Gregory RJ, Smith AE, Welsh MJ. Mutations in CFTR associated with mild-disease-form Cl^- channels with altered pore properties. Nature 1993 Mar 11;362(6416):160–4.
[6] Kiesewetter S, Macek Jr M, Davis C, et al. A mutation in CFTR produces different phenotypes depending on chromosomal background. Nat Genet 1993;5:274–8.
[7] Cuppens H, Lin W, Jaspers M, et al. Polyvariant mutant cystic fibrosis transmembrane conductance regulator genes. The polymorphic (Tg)m locus explains the partial penetrance of the T5 polymorphism as a disease mutation. J Clin Invest 1998;101:487–96.
[8] Choi JY, Muallem D, Kiselyov K, Lee MG, Thomas PJ, Muallem S. Aberrant CFTR-dependent HCO3-transport in mutations associated with cystic fibrosis. Nature 2001;410:94–7.
[9] Park HW, Nam JH, Kim JY, et al. Dynamic regulation of CFTR bicarbonate permeability by $[Cl^-]_i$ and its role in pancreatic bicarbonate secretion. Gastroenterology 2010;139:620–31.
[10] Dequeker E, Stuhrmann M, Morris MA, et al. Best practice guidelines for molecular genetic diagnosis of cystic fibrosis and CFTR-related disorders—updated European recommendations. Eur J Hum Genet 2009 Jan;17(1):51–65.
[11] Knowles MR, Carson JL, Collier AM, et al. Measurements of nasal transepithelial electrical potential differences in normal human subjects *in vivo*. Am Rev Respir Dis 1981;124:484–90.
[12] Knowles MR, Paradiso AM, Boucher RC. *In vivo* nasal potential difference: techniques and protocols for assessing efficacy of gene transfer in cystic fibrosis. Hum Gene Ther 1995;6:445–55.
[13] Veeze HJ, Sinaasappel M, Bijman J, Bouquet J, de Jonge HR. Ion transport abnormalities in rectal suction biopsies from children with cystic fibrosis. Gastroenterology 1991;101:398–403.
[14] De Jonge HR, Ballmann M, Veeze HJ, et al. *Ex vivo* CF diagnosis by intestinal current measurements (ICM) in small aperture, circulating Ussing chambers. J Cyst Fibros 2004;3:159–63.

[15] Bronsveld I, Mekus F, Bijman J, et al. Residual chloride secretion in intestinal tissue of ΔF508 homozygous twins and siblings with cystic fibrosis. Gastroenterology 2000;119:32–40.
[16] Bronsveld I, Mekus F, Bijman J, et al. Chloride conductance and genetic background modulate the cystic fibrosis phenotype of ΔF508 homozygous twins and siblings. J Clin Invest 2001;108:1705–15.
[17] Bronsveld I. Modifying factors of cystic fibrosis disease: residual chloride secretion, genetic background and epigenetics. PhD thesis, Erasmus University Rotterdam, The Netherlands; 2000.
[18] Bronsveld I, Bijman J, Mekus F, et al. Clinical presentation of exclusive cystic fibrosis lung disease. Thorax 1999;54:278–81.
[19] Gibson LE, Cooke RE. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis. nPediatrics 1959;23:545–9.
[20] Chen J-H, Stoltz DA, Karp PH, et al. Loss of anion transport without increased sodium absorption characterizes newborn porcine cystic fibrosis airway epithelia of anion transport without increased sodium absorption characterizes newborn porcine cystic fibrosis airway epithelia. Cell 2010;143:911–23.
[21] Derichs N, Sanz J, von Kaenel T, et al. Intestinal current measurement for diagnostic classification of patients with questionable cystic fibrosis: validation and reference data. Thorax 2010;65:594–9.
[22] Walker LC, Venglarik CJ, Weatherly MR, et al. Relationship between airway ion transport and a mild pulmonary disease mutation in CFTR. Am J Respir Crit Care Med 1997;155:1684–9.
[23] Gan KH, Veeze HJ, van den Ouweland AM, et al. A cystic fibrosis mutation associated with mild lung disease. N Engl J Med 1995;333:95–9.
[24] Dohle GR, Veeze HJ, Overbeek SE, et al. The complex relationship between cystic fibrosis and congenital bilateral absence of the vas deferens: clinical, electrophysiological and genetic data. Human Reproduction 1999;14:371–4.
[25] Stanke F, Ballmann M, Bronsveld I, et al. Diversity of the basic defect of homozygous CFTR mutation genotypes in humans. J Med Genet 2008;45:47–54.
[26] Yang D, Shcheynikov N, Zeng W, et al. IRBIT coordinates epithelial fluid and HCO_3^- secretion by stimulating the transporters pNBC1 and CFTR in the murine pancreatic duct. J Clin Invest 2009;119:193–202.
[27] Broere N, Hillesheim J, Tuo B, et al. CFTR activation is reduced in the small intestine of Na^+/H^+ exchanger 3 regulatory factor 1 (NHERF-1)-but not NHERF-2-deficient mice. J Biol Chem 2007;282:37575–84.
[28] Bertrand CA, Zhang R, Pilewski JM, Frizzell RA. SLC26A9 is a constitutively active, CFTR-regulated anion conductance in human bronchial epithelia. J Gen Physiol 2009;133:421–38.
[29] Fulmer SB, Schwiebert EM, Morales MM, Guggino WB, Cutting GR. Two cystic fibrosis transmembrane conductance regulator mutations have different effects on both pulmonary phenotype and regulation of outwardly rectified chloride currents. Proc Natl Acad Sci USA 1995 Jul 18;92(15):6832–6.
[30] De Jonge HR, Bijvelds M, Wilke M, et al. Intestinal and pancreatic biomarkers for CF. Ped Pulmonol Suppl 2006;29:148–9.
[31] Charizopoulou N, Wilke M, Dorsch M, et al. Spontaneous rescue from cystic fibrosis in a mouse model. BMC Genet 2006;7:18–27.
[32] De Boeck K, Derichs N, Fajac I, et al. New clinical diagnostic procedures fo cystic fibrosis in Europe. J Cyst Fibros 2010;9(Suppl 3):S44–57.
[33] Veezde HJ, Halley DJJ, Bijman J, de Jongste JC, de Jonge HR, Sinaasappel M. Determinants of mild symptoms in cystic fibrosis patients: residual chloride secretion measured in rectal biopsies in relation to the genotype. J Clin Invest 1994;93:461–6.
[34] Reddy MM, Quinton PM. Control of dynamic CFTR selectivity by glutamate and ATP in epithelial cells. Nature 2003;423:756–60.