Journal of Cystic Fibrosis xxx (2019) xxx

Contents lists available at ScienceDirect

Journal of Cystic Fibrosis

journal homepage: www.elsevier.com/locate/jcf

Original Article

Nasal potential difference in suspected cystic fibrosis patients with 5T polymorphism

Bente L. Aalbers [a,*], Yasmin Yaakov [b], Nico Derichs [c], Nicholas J. Simmonds [d], Elke De Wachter [e], Paola Melotti [f], Kris De Boeck [g], Teresinha Leal [h], Burkhart Tümmler [i], Michael Wilschanski [b], Inez Bronsveld [a]

[a] Department of Pulmonology, University Medical Center Utrecht, Postbus 85500, 3508, GA, Utrecht, the Netherlands
[b] Pediatric Gastroenterology Unit and Cystic Fibrosis Center, Hadassah-Hebrew University Medical Center, Kiryat Hadassah, POB 12000, Jerusalem 91120, Israel
[c] CF Center, Pediatric Pulmonology and Immunology, Charité Universitätsmedizin, Charitépl. 1, 10117 Berlin, Germany
[d] Department of Cystic Fibrosis, Royal Brompton Hospital and Imperial College, Sydney Street, SW3 6NP London, United Kingdom
[e] Department of Pediatric Pneumology, UZ Brussel, Laarbeeklaan 101, B-1090 Brussels, Belgium
[f] Cystic Fibrosis Centre, Azienda Ospedaliera Universitaria Integrata. Piazzale Aristide Stefani 1, 37126 Verona, Italy
[g] Department of Pediatric Pulmonology, University Hospital Leuven, Herestraat 49, 3000 Leuven, Belgium
[h] Louvain Centre for Toxicology and Applied Pharmacology (LTAP), Institut de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain, Place de l'Université 1, B-1348 Louvain-la-Neuve, Brussels, Belgium
[i] CF Center and Clinical Research Group, Department of Pediatric Pneumology and Neonatology, OE 6710, Medical School Hannover, Carl-Neuberg-Straße 1, 30625 Hannover, Germany

ARTICLE INFO

Article history:
Received 30 March 2019
Revised 5 July 2019
Accepted 6 July 2019
Available online xxxx

Keywords:
IVS8-5T
CFTR function
Nasal potential difference
Sweat chloride
Cystic fibrosis
CFTR-related disorder

ABSTRACT

Background: 5T polymorphism is a *CFTR* mutation with unclear clinical consequences: the phenotype varies from healthy individuals to Cystic Fibrosis (CF). The aim of this study was to evaluate if nasal potential difference (NPD) and sweat testing correlate with symptoms and CF diagnosis in 5T patients.
Methods: 86 patients with 5T who had undergone NPD measurement, were included (6 homozygous (5T/5T), 41 with a PI-CF causing mutation *in trans* (5T/PI-CF), 11 with a PS-CF causing mutation *in trans* (5T/PS-CF) and 28 without a known mutation *in trans* (5T/?). Data including age, phenotype, sweat chloride and follow up were collected.
Results: 33% of the 5T/5T patients had abnormal NPD results, compared to 70% in 5T/PI-CF; 33% in 5T/PS-CF and 29% in 5T/?. The percentage of high or borderline sweat chloride was highest in 5T/PI-CF, and 5T/?, compared to 5T/5T and 5T/PS-CF (91, 96, 80, and 63%, respectively). TGm (number of TG repeats in intron 8) analysis was performed in 21 5T/PI-CF patients. TG11 was associated with lower sweat chloride, lower percentage of abnormal NPD and less progression of symptoms compared to TG12 and TG13.
Conclusion: There is much variation in clinical status among 5T patients. All patients in this study with 5T/PS CF, all patients with both normal NPD and sweat test, and most patients with TG11 were stable or improving over time. Therefore, NPD measurement and TGm status aid to assess if a patient is at high risk for developing CF or CFTR-related disease and if specific follow up in a CF center is required.

1. Introduction

Cystic Fibrosis (CF) is the most common life-limiting autosomal recessive inherited disorder. It is caused by mutations in the *CFTR* gene, leading to inadequate function of the CFTR protein [1]. Over 2000 different mutations have been discovered since 1989 [2]. Different types of mutations seem to correlate with different grades of residual CFTR function, so that certain types of mutations lead to more severe disease than others [3]. Throughout this article 5T polymorphism is referred to as 5T, but in literature it is also called IVS8-5T or c.1210-12T [5]. This polymorphism occurs in the polythymidine tract in intron 8 of the CFTR gene, leading to inappropriate splicing whereby exon 9 is left out in 70–90% of the mRNA strands, resulting in a CFTR protein that will not reach the cell membrane. The other intron 8 polythymidine tract polymorphisms 7T and 9T lead to 0–50% and <5% aberrant transcript, respectively [4,5].

When combined with a CF causing mutation on the other allele and R117H *in cis*, 5T is known to cause CF by aggravating the effect of the R117H mutation [6].

If present solely on one allele and combined with a CF causing mutation *in trans* (compound heterozygous), 5T may have various consequences, from no symptoms or isolated CBAVD to pancreatic sufficient CF (PS-CF) or even pancreatic insufficient CF (PI-CF) [7,8]. The extent of correct splicing of exon 9 is further determined by the number of adjacent TG repeats (TGm), located upstream of the Tn locus of intron 8. The numbers of TG repeats *in cis* on a 5T background influences the

* Corresponding author at: UMC Utrecht, Huispostnr E03.511, Postbus 85500, 3508 GA, Utrecht, the Netherlands.
E-mail address: b.l.aalbers-2@umcutrecht.nl (B.L. Aalbers).

https://doi.org/10.1016/j.jcf.2019.07.001
1569-1993/

efficiency of splicing, hereby acting as a complex allele [9]. According to the CFTR2 database, 5T (not in a complex allele) is defined as a 'mutation with varying consequences', irrespective of the number of TG repeats; individuals who are homozygous for 5T – whether TG11, TG12 or TG13, without R117H – have all been reported across the full spectrum of CFTR disorders from healthy individuals to CF, although symptoms are usually mild in the majority [10,11].

Patients that are compound heterozygous for this mutation may be symptomatic and suffer from a mono-organ disease, e.g. CBAVD (congenital bilateral absence of vas deferens), sinusitis or bronchiectasis, and some have multi-system disease and fulfill the diagnosis for CF.

5T variant is particularly common in Ashkenazi Jews and accounts for 18% of all PS CF cases; in non-Ashkenazi Jews this is 10%. 32% of CBAVD in Ashkenazi Jews is associated with 5T polymorphism, and 36% in non-Ashkenazi Jews [12]. 5T is also widely present in other ethnic groups [13,14].

This study aims both to provide descriptive information about a large cohort of 5T patients referred for diagnostic workup, and to evaluate if NPD measurement is of added value in these patients to estimate CF severity and the need for follow up.

2. Methods

2.1. Patient characteristics and follow up data

Data was collected from patients with at least one 5T allele, who were seen in a CF center for diagnostic evaluation, in a retrospective manner. This includes patients with bronchiectasis, recurrent pancreatitis, CBAVD, or in case of a family history of CF. Tables 1.a and 1.b provide an overview of the nature and extent of symptoms leading to referral. Patients were included if they underwent at least genetic testing of both alleles (in a panel or full sequencing), NPD and sweat test. In all patients, information about age, gender and CFTR mutations on both alleles was collected. These mutations were grouped in PI and PS CF causing mutations, according to the CFTR2 database [10] (Table 2). Mutations with varying or completely unknown clinical consequences were also included in the group with PS CF causing mutations. In 38 patients information about the TGm status was available.

Other variables collected were symptoms at the time of evaluation and follow-up of these symptoms during at least one year(median follow up duration: 5,0 years, range 1,1–26,0 years), as well as the final diagnostic state after full evaluation (CF, CFTR-RD, no CF), according to the clinician. Ethical approval for the anonymous use of patient data for research was ensured in all participating centers.

2.2. Sweat chloride

Sweat test was performed in a standardized manner in certified laboratories. A minimum of 1 g/m2/min was collected for 20–30 min by iontophoresis, after application of pilocarpine on the skin. Chloride concentration was measured in the collected sweat, according to the CFF guidelines [15]. Most patients had multiple (2–4) sweat tests taken. In these cases the mean result was used for the study. In seven patients no sweat test results were available, for five patients this was due to repeatedly insufficient sweat volume obtained on multiple occasions. One patient suffered from ichthyosis, and one patient refused to undergo a repeat test after the first attempt yielded insufficient sweat volume.

Table 1.a
Frequency of symptoms contributing to referral to a CF center.

		Number of patients (%)
Respiratory	Bronchiectasis or recurrent lower respiratory tract infections	36 (42%)
	Obstructive lung disease (including astma/COPD)	15 (17%)
	Sinusitis and/or nasal polyps	21 (24%)
	Chronic cough/sputum	9 (10%)
Gastro-intestinal	Recurrent pancreatitis	10 (12%)
	Pancreatic insufficiency / chronic diarrhea / failure to thrive	11 (13%)
	PSC / liver test abnormalities / cholelithiasis	4 (5%)
CBAVD		16 (19%)[a]

[a] 19% of total, 29% of male patients.

Table 1.b
Extent of symptoms per patient.

	Number of patients (%)
No symptoms, family history led to referral	7 (8%)
Symptoms in one organ	49 (57%)
Symptoms in multiple organs	30 (35%)

2.3. Nasal Potential Difference (NPD)

NPD test was carried out in all centers according to the Standardized Operating Procedure of the ECFS [16]. Each operator had received training in the technique before the commencement of the measurements.

NPD measurement data was obtained retrospectively from multinational CF diagnostic centers (Jerusalem, Berlin, London, Leuven, Brussels, Hannover, Utrecht), where NPD measurements were performed in the patients for diagnostic purposes. Subsequently the data was anonymized and assembled in a data file. The response to amiloride (Δamil) and the response to chloride free solution added to the response to isoproterenol (ΔClfree+Δiso) was derived. From this, the exponent of (Δamil/(ΔClfree+Δiso)) was calculated as a measure for CFTR function. An $e^{(\Delta amil/(\Delta Clfree+\Delta iso))}$ of 0.7 or higher indicates a poor CFTR function as is seen in CF, being defined as an abnormal NPD result [17,18]. All centers followed the SOP as provided by the ECFS; therefore the only difference noted between the centers was the way the reference electrode was attached to the skin. In most centers a subcutaneous needle was inserted into the forearm, in a few centers the skin abrasion technique was used, where a superficial skin abrasion is made and the electrode was attached onto the skin. Both methods are described in the SOP and shown to discriminate CF from non-CF subjects in a comparable way.

2.4. Approach to description of 5T patients and subgroups

The patients were divided into four groups according to the mutation on the second allele: Group 1: Homozygous for 5T, Group 2: Mutation *in trans* known to cause PI CF (Tables 1.a, 1.b), Group 3: Mutation *in trans* known to cause PS CF or with various clinical consequences, Group 4: no second mutation found. The patients with an abnormal NPD result ($e^{(\Delta amil/(\Delta Clfree+\Delta iso))} \geq 0.7$) were compared to the patients with a normal NPD result.

Patients were also divided according to their symptom evolvement during follow up; either their symptoms resolved or remained stable

Table 2
PS-CF and PI-CF causing mutations *in trans* and their frequency in the studied group.

PS-CF causing (or varying/unknown clinical consequence) mutation ($N = 9$)	PI-CF causing mutation ($N = 43$)
D1152H (c.3454G > C) $N = 4$	F508del (c.1521_1523delCTT) $N = 28$
F1052 V (c.3154 T > G)	W1282X (c.3846G > A) N = 9
621 + 3A > G (c.489 + 3A > G)	G85E (c.254G > A) N = 2
Q799K (c.2395C > A)	G542X (c.1624G > T)
N1303I (c.3908A > T)	1259insA (c.1127_1128insA)
V562 L (c.1684G > C)	1717-1G > A (c.1585-1G > A)
	3056delGA (c.2924_2925delGA)

Legacy name is shown, with nomenclature according to HGVS (Human Genome Variation Society) between parenthesis.

Please cite this article as: B.L. Aalbers, Y. Yaakov, N. Derichs, et al., Nasal potential difference in suspected cystic fibrosis patients with 5T polymorphism, Journal of Cystic Fibrosis, https://doi.org/10.1016/j.jcf.2019.07.001

over time, or they showed progressive or recurrent symptoms during follow up. If patients suffered from bronchiectasis, sinusitis or CBAVD and had no new symptoms or recurrent infections, they were classified as stable. If there were recurrent infections or patients suffered from recurrent pancreatitis with episodes during follow up, this was noted as recurrent symptoms. Any new symptoms or worsening of existing symptoms was also noted as clinical worsening.

CBAVD, acute recurrent or chronic pancreatitis and disseminated bronchiectasis as a mono-organ disease were defined as CFTR-RD [19]. CF diagnosis was recorded if the treating physician concluded that a person had CF based on multi-organ involvement with impaired CFTR function, after full evaluation [20].

3. Results

In total, 86 patients were included, of which 6 were 5T homozygous (group 1) and 52 were compound heterozygous 41 with a PI CF causing mutation *in trans* (group 2), 11 with a PS CF or varying consequence second mutation *in trans* (group 3; Tables 1.a and 1.b). In 28 patients no second mutation was found (group 4). In addition, we tried to obtain information about the length of the TG stretch (TGm) *in cis* in all patients, since research suggests that this has a major impact on splicing in 5T. TG repeats were known in 38 patients out of 86 (TG11: $N = 7$, TG12: $N = 27$, TG13: $N = 4$).

When divided according to the mutations *in trans*, marked differences appear between the groups (Table 3). The percentage of patients with an abnormal sweat test is distinctively higher in group 2 and 4, compared to group 1 and 3 among which sweat test results were comparable.

The proportion of patients diagnosed with CF after full workup and follow up was 54% in the group with a PI CF causing mutation *in trans*, in the other groups this was 17–33%.

The percentage of males also differs between these groups. The difference may be partially due to small group size, although gender may also have an impact on CFTR function in 5T patients [21]. Males were frequently referred for diagnostic evaluation because of CBAVD as their main or only symptom, which is more common in the combination F508del/5T, accounting for 17% of the total of CBAVD patients [22]. This does not account for the full difference in this group.

When the patients with an abnormal sweat test were compared to those with a borderline and normal sweat test, those with a high sweat chloride (above 60 mEq/l) had surprisingly less worsening or recurrent symptoms (7%), compared to those with borderline (30–59 mEq/l, 31%) or low (<30 mEq/l, 33%) sweat chloride (Table 4). Patients with an abnormal sweat test were diagnosed with CF as often as patients with borderline sweat test (40% vs 43%), while none of the patients with low sweat chloride was diagnosed with CF.

When divided according to NPD results, it is noted that in the group with abnormal NPD results (Exp ≥0.7) worsening or recurrent symptoms were more often seen compared to subjects with an abnormal sweat test. There also is a larger percentage of CF diagnoses and less patients that were categorized as not having CFTR-related symptoms, compared to the group with normal NPD. Frequency of CFTR-RD is comparable between the groups; 23 versus 20%, (Table 5).

When the patients are divided according to age at presentation, no important differences appear between the groups concerning sweat chloride, NPD results or progression of symptoms.

In the group of patients referred because of isolated CBAVD or without symptoms ($N = 11$), the percentage of patients with worsening symptoms over time was very low; only 1 patient had new symptoms in follow up. 3 Of the patients were diagnosed with CFTR-RD after full workup, none was diagnosed with CF. However, follow up time in this specific group was relatively short (mean: 3,9 years, range 1,1–14), so that new symptoms might have been missed in these patients.

TGm status was known in 38 of the patients. Within the group with known TGm repeats *in cis*, the groups with a PS-CF causing (or varying consequences) mutation and without a mutation in trans, were too small to compare variables between groups with a different TGm state. In the group of patients with a PI-CF mutation in trans ($N = 26$) a cautious comparison is possible however the groups are small: TG11 $= 4$, TG12 $= 18$, TG13 $= 4$. The mutation *in trans* was F508del in all but 4 patients (1717 + 1G > A, 3056delGA, 1259insA and W1282X).

Fewer patients with TG11 had an abnormal NPD compared to those with TG12 and TG13. TG11 also was associated with lower sweat chloride, lower percentage of CF diagnoses and less worsening of symptoms over time (Table 6).

It is notable that two patient groups do particularly well over time. In these patients, none had worsening or recurrence of symptoms over time.

1. Patients with 5T and a PS CF causing mutation or a mutation with varying clinical consequence in trans (regardless of NPD and sweat test result).
2. 5T patients with both sweat chloride below 30 mEq/l and normal NPD results (regardless of the CFTR mutation in trans).

4. Discussion

This study provides the largest cohort of 5T patients that has been examined systematically. It is an important illustration of the enormous variety in clinical consequences among patients with a 5T mutation, also in those who have the same mutation *in trans*. In addition, we demonstrate how NPD correlates with genotype, clinical outcome and diagnosis and how it can contribute to determining diagnosis and prognosis in these patients, supplementary to sweat testing.

Due to the retrospective setup of this study, it also faces relevant limitations; it is important to note that the length of follow up is variable between patients and for some patients has been quite short to accurately tell if symptoms have recurred, worsened of improved. Therefore diagnosis remained unclear in some patients.

Another limitation, as mentioned earlier, is that data on TG repeats is not available for all study subjects, especially as in Israel determination

Table 3
5T patient characteristics according to mutations *in trans*.

Division according to mutations	Group 1: 5T/5T $N = 6$	Group 2: 5T/PI CF $N = 43$	Group 3: 5T/PS CF $N = 9$	Group 4: 5T/? $N = 28$
Mean age in years (range) n.s.	32 (16–63)	26 (1–69)	35 (8–76)	23 (7–65)
Gender (% male) n.s.	50%	74%	89%	61%
High sweat test	20%	17%	38%	29%
Borderline sweat test	60%	74%	25%	76%
Percentage abnormal NPD	33%	70%*	33%	29%*
Worsening/recurrent symptoms n.s.	17%	21%	0%	4%
CF diagnosis	17%*	54%*	33%	17%*
CFTR-RD diagnosis n.s.	17%	27%	22%	21%

n.s.: no significant differences between any of the groups.
Percentage abnormal NPD: significant differences between group 2 and 4 (indicated with *, P = .004).
CF diagnosis: significant differences between group 1, 2 and 4 (indicated with *, P = .016).
CF, CFTR-RD: diagnosis defined by the CF center according to sweat test, NPD, mutation and clinical picture.

Please cite this article as: B.L. Aalbers, Y. Yaakov, N. Derichs, et al., Nasal potential difference in suspected cystic fibrosis patients with 5T polymorphism, Journal of Cystic Fibrosis, https://doi.org/10.1016/j.jcf.2019.07.001

Table 4
5T patient characteristics according to sweat test results.

Division according to sweat test		Sweat chloride <30 mEq/l $N = 9$	Sweat chloride 30–59 mEq/l $N = 52$	Sweat chloride >60 mEq/l $N = 18$
Mean age in years (range) n.s.		35 (9–66)	27 (1–76)	23 (6–64)
Gender (% male) n.s.		75%	67%	88%
Percentage abnormal NPD n.s.		50%	54%	59%
Worsening/ recurrent symptoms n.s.		33%	31%	7%
Diagnosis n.s.	CF	0%	43%	40%
	CFTR-RD	0%	28%	7%
	None	100%	29%	53%

n.s.: no significant differences between any of the groups.

of TG repeats was not part of standard care. This explains why in 48 patients TG status is missing. As a result of this, the groups after division according to TG repeats are too small to achieve a significant conclusion about the difference between these groups. This limitation is important as TGm is earlier described as an important influence on splicing in 5T [9]. In addition, in the group of 28 patients in whom no second mutation was found (5T/?), there are 18 patients who only underwent panel testing of the most frequent mutations, but had no sequencing. This means that in those patients, a second mutation may have been missed, to that this group cannot be viewed as carriers.

This study reflects on patients with 5T who were referred for diagnostic evaluation, therefore the patients described are not a representation of the entire group of persons with a 5T allele; it is likely that there are many persons carrying a 5T allele, with or without a CFTR mutation in trans, who do not experience symptoms at all and thus are never referred to a CF center for evaluation. For our study, we confined our scope to only the subgroup of 5T patients in whom CF or CFTR-RD diagnosis is considered, as these can pose relevant dilemmas in CF diagnostic center.

According to our results, NPD has shown to be useful in addition to the clinical picture to evaluate if the patient is at risk for worsening of symptoms over time, as it correlates better with outcomes compared to sweat test or genotype alone. There is very limited availability of earlier published research about NPD results in 5T patients. Kerem et al. evaluated sweat tests in 5T patients [7]. Segal et al. describe 2 patients with 5T (with W1282X and D1152H *in trans*) and recurrent pancreatitis who had borderline sweat tests and in whom the NPD results were also abnormal [23].

Noone et al. describe another 2 patients; one 5T homozygous, with borderline sweat test and.one F508del/5T with abnormal sweat test. NPD was performed with methods not comparable to our measurements, but is described as indicative for reduced CFTR function [24]. In another study by Sharer et al., only one of the included 5T patients (F508del/5T) underwent NPD. Calculated $e^{(\Delta amil/(\Delta Clfree+\Delta iso))}$ in this patient was normal at 0.58, sweat chloride was 34 mEq/l [25]. A few other studies involving 5T patients used NPD testing, however the 5T patients' results were not mentioned separately from the group they were included in [26,27].

These reports show that in patients with 5T mutations, genotype alone does not accurately define CF-diagnostic state. Similarly, in all four different genotype groups in our study, the clinical picture varies from CF to asymptomatic individuals.

The fact that the percentage of patients with an abnormal sweat test is distinctively higher in group 2, compared to group 1 and 3, stresses the importance of the effect of the mutation on the second allele to the CFTR residual function. There is no definite explanation for the high percentage of abnormal sweat test result in group 4, it is possible however that in this group based on genotype, an abnormal sweat test may have been the main reason to still refer the patient for further diagnostic evaluation.

In some cases, genotype does provide some prediction of symptom change over time: patients with a PS or varying clinical consequence second mutation all were stable or improved during follow up. In all 3 other genotype groups, clinical outcome could not be predicted based on genotype.

The finding that variation is large within groups with the same genotype should not surprise as this is also seen in patient groups with CF genotypes not involving 5T (for example F508del/F508del), caused by previously established factors such as modifier genes and environmental factors like infections in early life [6].

In the patient groups with known TG repeats, we found a correlation between lower TGm and lower sweat chloride as well as more frequent normal NPDs. This however was not statistically significant, as would be expected based on the limited group size. It is in line with the earlier findings that 5T-TG11 preserves more CFTR function than TG12 and TG13 [5,28,29].

However, patients with 5T-TG11 may develop CF as demonstrated in our study. This is in accordance with the findings of Magne et al. who described a patient homozygous for 5T-TG11 without any other CFTR mutation, who deteriorated around the age of 50 and recovered partially after starting ivacaftor [30]. This highlights the limitation of genotype and TGm state alone, as it is not always predictive of CF diagnosis or phenotype. Moreover, the fact that there are mutation-specific

Table 5
5T patient characteristics according to NPD results.

Division according to NPD results		Normal NPD (exp < 0.7), $N = 44$	Abnormal NPD (exp ≥ 0.7), $N = 42$
Mean age in years (range) n.s.		30 (3–69)	23 (1–76)
Gender (% male) n.s.		62%	79%
Mean sweat chloride (mEq/l, SD) n.s.		50 (18)	53 (16)
Group	1: 5T / 5T	4 (9%)	2 (5%)
	2: 5T / PI CF	13 (30%)*	30 (71%)*
	3: 5T / PS CF	6 (16%)	3 (7%)
	4: 5T /?	21 (48%)*	7 (17%)*
Worsening/recurrent symptoms n.s.		10%*	31%*
Diagnosis	CF (PS or PI)	18%*	62%*
	CFTR-RD	20%	23%
	Not CFTR related	64%*	15%*

N.s.: no significant differences between the groups.
SD: standard deviation.
Second mutations: significant difference of frequency of 5T/PI CF and 5T/?, (indicated with *, P = .008).
Diagnosis: significant difference of frequency of CF and not CFTR related symptoms, (indicated with *, P = .000).
Worsening/recurrent symptoms: Significant difference of frequency between the groups, (indicated with *, P = 0,018).

Table 6
5T patients with a PI CF mutation in trans divided by number of TG repeats.

Division according to TG repeats		5T-TG11 ($N = 4$)	5T-TG12 ($N = 18$)	5T-TG13 ($N = 4$)
Mean age (range) n.s.		46 (18–66)	27 (3–69)	20 (1–36)
Gender (% male) n.s.		3 (75%)	14 (78%)	4 (100%)
Sweat chloride (mEq/l, SD) n.s.		37 (8)	49 (12)	55 (15)
Abnormal NPD N n.s.		2 (50%)	13 (72%)	3 (75%)
Worsening/recurrent symptoms n.s.		1 (25%)	5 (28%)	1 (25%)
Diagnosis	CF n.s.	25%	59%	33%
	CFTR-RD n.s.	75%	18%	67%
	None n.s.	0%	24%	0%

N.s.: no significant differences between any of the groups.

Please cite this article as: B.L. Aalbers, Y. Yaakov, N. Derichs, et al., Nasal potential difference in suspected cystic fibrosis patients with 5T polymorphism, Journal of Cystic Fibrosis, https://doi.org/10.1016/j.jcf.2019.07.001

treatments for CF and drugs specifically for 5T may become available in the near future, underlines the importance of a better characterization of this group of patients.

Interestingly, sweat chloride did not correlate well with clinical outcomes in the studied group. A group of 17 5T patients was described by Kerem et al., of which 15 had sweat test results. A wide range of sweat test results was seen between the patients with no clear correlation with diagnosis, which is in line with our findings [7]. However, in the subgroup with a normal sweat chloride (<30 mEq/l) in our study, none of the patients were diagnosed with CF or CFTR-RD. This suggests that a sweat test is a valid diagnostic test to rule out CF in this particular group, taking into account the lower cut-off value to rule out CF.

5. Conclusion

There is much variation in clinical status among 5T patients. TGm state and NPD measurement help to define the clinical impact in these patients better than genotype and sweat test alone.

Although not fully discriminative between patients who will remain stable and patients who will experience worsening or recurrent symptoms, our data obtained from this group of 5T patients suggest that NPD measurement can help in estimating if a patient is at high risk for worsening of symptoms, and could thus aid the decision if the patient should attend a CF center.

All patients with a PS-CF mutation *in trans* and all patients who have a combination of normal sweat test and normal NPD were stable or improving over time. Therefore we suggest that these patients may not require follow up in a CF center. In all other 5T patients who are referred for diagnostic workup, and do not have a 5T/PS-CF genotype or a combination or normal sweat chloride and normal NPD, follow up should be considered by the physician after extensive diagnostic work up.

Acknowledgements

The start of this study in Israel has been supported by a NCFS young investigator travel grant.

I. Bronsveld and M. Wilschanski have operated both as senior authors in this project with equal contributions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcf.2019.07.001.

References

[1] Welsh MJ, Smith AE. Cystic fibrosis. Sci Am 1995;273:52–9.
[2] Farrell PM, Rosenstein BL, et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: cystic fibrosis foundation consensus report. J Pediatr 2008 Aug;153(2):4–14.
[3] Hirtz S, Gonska T, et al. CFTR cl channel function in native human colon correlates with the genotype and phenotype in cystic fibrosis. Gastroenterology 2004;127:1085–95.
[4] Noone PG, Pue CA, et al. Lung disease associated with the IVS8 5T allele of the CFTR gene. Am J Respir Crit Care Med 2000;162:1919–24.
[5] Chu CS, Trapnell BC, et al. Extensive posttranscriptional deletion of the coding sequences for part of nucleotide-binding fold 1 in respiratory epithelial mRNA transcripts of the cystic fibrosis transmembrane conductance regulator gene is not associated with the clinical manifestations of cystic fibrosis. J Clin Invest 1992;90:785–90.
[6] Kiesewetter S, Macek Jr M, et al. A mutation in CFTR produces different phenotypes depending on chromosomal background. Nat Gentet 1993;5:274–8.
[7] Kerem E, Reve-Harel N, et al. A cystic fibrosis transmembrane conductor regulator splice variant with partial penetrance associated with variable cystic fibrosis presentations. Am J Respir Crit Care Med 1997;155:1914–20.
[8] Friedman KJ, Heim RA, et al. Rapid characterization of the variable length polythimidine tract in the cystic fibrosis (CFTR) gene: association of the 5T allele with selected CFTR mutations and its incidence in atypical sinopulmonary disease. Hum Mutat 1997;10(2):108–15.
[9] Groman JD, Hefferon TW, et al. Variation in a repeat sequence determines whether a common variant of the cystic fibrosis transmembrane conductance regulator gene is pathogenic or benign. Am J Hum Genet 2004;74:176–9.
[10] CFTR2 database. 2019 https://www.cftr2.org
[11] Cottin V, Thibout Y, et al. Late CF caused by homozygous IVS8-5T polymorphism. Thorax 2005;60(11):974–5.
[12] Kerem B, Chiba-Falek O, et al. Cystic fibrosis in Jews: frequency and mutation distribution. Genet Test 1997;1(1):35–9.
[13] Bouyada M, Fredj SH, et al. Cystic fibrosis transmembrane conductance regulator mutations and patients from North Africa. Ann Hum Biol 2012;39(1):76–9.
[14] Ni WH, Jiang L, et al. The CFTR polymorphisms poly-T, TG-repeats and M470V in chinese males with congenital bilateral absence of the vas deferens. Asian J Androl 2012;14:687–90.
[15] Green A, Kirk J. Guidelines for the performance of the sweat test for the diagnosis of cystic fibrosis. Ann Clin Biochem 2007;44:25–34.
[16] Solomon GM, Bronsveld I. Standardized Measurement of Nasal Membrane Transepithelial Potential Difference (NPD). J Vis Exp 2018 Sep 13:139.
[17] Tridello G, Menin L, et al. Nasal potential difference outcomes support diagnostic decisions in cystic fibrosis. JCF 2016;15:579–82.
[18] Wilschanski M, Famini H, et al. Nasal potential difference measurements in patients with atypical cystic fibrosis. Eur Respir J 2001 Jun;17(6):1208–15.
[19] Bombieri C, Claustres M, et al. Recommendations for the classification of diseases as CFTR-related disorders. JCF 2011;10(2):86–102.
[20] Farrell PM, White TB, et al. Diagnosis of cystic fibrosis: consensus guidelines from the cystic fibrosis foundation. J Pediatr 2017 Feb;181S:S4–S15.
[21] Castellani C, Bonizzato A, et al. Evidence of mild respiratory disease in men with congenital absence of the vas deferens. Respir Med 1999;93:869–75.
[22] Morea A, Cameran M, et al. Gender-sensitive association of CFTR gene mutations and 5T allele emerging from a large survey on infertility. Mol Hum Reprod 2005;11(8):607–14.
[23] Segal I, Yaakov Y, et al. Cystic fibrosis transmembrane conductance regulator Ion channel function testing in recurrent acute pancreatitis. J Clin Gastroenterol 2008;42(7):810–4.
[24] Noone PG, Pue CA, et al. Lung disease associated with the IVS8 5T allele of the CFTR gene. Am J Respir Crit Care Med 2000;162:1919–24.
[25] Sharer N, Schwarz M, Malone G, et al. Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. NEJM 1998;339(10):645–52.
[26] Werlin S, Konikoff FM, et al. Genetic and electrophysiological characteristics of recurrent acute pancreatitis. JPGN 2015;60:675–9.
[27] Yu J, Chen Z, et al. CFTR mutations in men with congenital bilateral absence of the vas deferens (CBAVD): a systemic review and meta-analysis. Hum Reprod 2012;27(1):25–35.
[28] Rave-Harel N, Kerem E, et al. The molecular basis of partial penetrance of splicing mutations in cystic fibrosis. Am J Hum Genet 1997;60:87–94.
[29] Cuppens H, Lin W, 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.
[30] Magne F, Durupt S, et al. Therapeutic benefit of ivacaftor in late cystic fibrosis caused by homozygous IVS8-5T CFTR polymorphism. J Cyst Fibros 2017;16:89–90.

Please cite this article as: B.L. Aalbers, Y. Yaakov, N. Derichs, et al., Nasal potential difference in suspected cystic fibrosis patients with 5T polymorphism, Journal of Cystic Fibrosis, https://doi.org/10.1016/j.jcf.2019.07.001