

Does extensive genotyping and nasal potential difference testing clarify the diagnosis of cystic fibrosis among patients with single-organ manifestations of cystic fibrosis?

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ABSTRACT

Background The phenotypic spectrum of cystic fibrosis (CF) has expanded to include patients affected by single-organ diseases. Extensive genotyping and nasal potential difference (NPD) testing have been proposed to assist in the diagnosis of CF when sweat testing is inconclusive. However, the diagnostic yield of extensive genotyping and NPD and the concordance between NPD and the sweat test have not been carefully evaluated.

Methods We evaluated the diagnostic outcomes of genotyping (with 122 mutations included as disease causing), sweat testing and NPD in a prospectively ascertained cohort of undiagnosed patients who presented with chronic sino-pulmonary disease (RESP), chronic/recurrent pancreatitis (PANC) or obstructive azoospermia (AZOOSP).

Results 202 patients (68 RESP, 42 PANC and 92 AZOOSP) were evaluated; 17.3%, 22.8% and 59.9% had abnormal, borderline and normal sweat chloride results, respectively. Only 17 (8.4%) patients were diagnosable as having CF by genotyping. Compared to sweat testing, NPD identified more patients as having CF (33.2%) with fewer borderline results (18.8%). The level of agreement according to kappa statistics (and the observed percentage of agreement) between sweat chloride and NPD in RESP, PANC and AZOOSP subjects was 'moderate' (65% observed agreement), 'poor' (33% observed agreement) and 'fair' (28% observed agreement), respectively. The degree of agreement only improved marginally when subjects with borderline sweat chloride results were excluded from the analysis.

Conclusions The diagnosis of CF or its exclusion is not always straightforward and may remain elusive even with comprehensive evaluation, particularly among individuals who present at an older age with single-organ manifestations suggestive of CF.

In early childhood the diagnosis of cystic fibrosis (CF) is usually straightforward in the presence of a CF phenotype and sweat chloride concentration of 60 mmol/L or greater. However, since the discovery of the CF transmembrane conductance regulator (*CFTR*) gene,¹ an expanded spectrum of conditions has been associated with *CFTR* mutations, particularly in older children and adults who present with

Key messages

What is the key question?

- Does extensive genotyping and NPD testing help clarify and improve the diagnostic yield of CF over sweat testing alone in patients with single organ manifestations of CF?

What is the bottom line?

- The diagnosis of CF may remain inconclusive despite comprehensive evaluation by sweat testing, extensive genotyping and NPD testing.

Why read on?

- Extensive *CFTR* genotyping was the least sensitive diagnostic test even when 122 additional mutations defined by *CFTR2* as disease causing were included. While NPD testing identified more patients diagnosable with CF and fewer patients with borderline results in comparison with the classic sweat test, there was considerable test discordance (sweat test normal but NPD abnormal and vice versa).

single-organ manifestations such as sino-pulmonary diseases, pancreatitis or obstructive azoospermia.^{2–9} The majority of these individuals are pancreatic sufficient (PS).

The sweat test remains the principal test for the diagnosis of CF.^{10–11} As the reference range for sweat chloride was originally defined by evaluating children presenting with classic multi-organ symptoms,^{12–15} it is not surprising this test satisfactorily discriminates the majority of pancreatic insufficient (PI) CF patients from unaffected individuals. However, sweat testing may be inconclusive in a subset of patients, especially those who present at an older age with single-organ manifestations of CF.^{4–5 8–10}

Several strategies such as extensive genotyping and nasal potential difference (NPD) testing have been proposed to reduce diagnostic dilemmas.^{10–11 16} Until recently, genotyping has been



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limited by the fact that only 23 mutations were designated as CF causing.¹⁰ In fact, most patients presenting at an older age typically carry mutations that were not listed as disease causing. The clinical and functional translation of *CFTR* (CFTR2) project recently expanded the list to 122 CF-causing mutations.¹⁷ CFTR2 also described mutations of 'varying clinical consequence', because they had been identified in individuals with and without CF. The diagnostic yield of this greatly expanded number of mutations has not been evaluated. NPD testing, which measures the bioelectric properties of the nasal epithelium, was first established for research purposes. It has also been recommended as a diagnostic tool in individuals with an uncertain diagnosis of CF. Nevertheless, NPD is limited in clinical practice by lack of availability and diagnostic reference values, and may still lead to inconclusive outcomes. According to American and European consensus recommendations, NPD is only recommended as the next test when patients have 'borderline' sweat chloride concentrations.^{10 11 16} NPD testing is thus not recommended when the sweat test is normal (<40 mmol/L) or abnormal (>60 mmol/L). This assumes that 'clear-cut' normal or abnormal sweat test results would concur with the NPD result. However, the concordance between the two tests has never been assessed.

Due to the aforementioned issues, we evaluated and compared the diagnostic outcomes of sweat testing, genotyping and NPD in a prospectively ascertained cohort of undiagnosed patients who presented with single-organ manifestations suggestive of CF. Reference cohorts of non-CF and established CF subjects were also evaluated for comparison. In particular, we aimed to investigate the diagnostic yield from genotyping using CFTR2's expanded list of CF-causing mutations and to assess the contribution and concordance between sweat chloride and NPD results.

METHODS

Subjects

Two cohorts were prospectively and consecutively enrolled from the Toronto CF clinics from 1994 to 2008: undiagnosed patients (>5 years) with a single-organ manifestation of CF, and reference cohorts consisting of non-CF (healthy controls and obligate heterozygotes) and CF (CFPS and CFPI) subjects.¹⁸ All subjects were evaluated by sweat test, *CFTR* genotyping and NPD (figure 1); subjects unable to complete all three tests were excluded from analyses.

Undiagnosed patients with suspected CF included those with: idiopathic chronic sino-pulmonary disease (RESP); idiopathic recurrent acute/chronic pancreatitis (PANC); or infertile men with obstructive azoospermia (AZOOSP) (see supplementary material, available online only). Healthy controls consisted of volunteers without a past or family history of CF, pancreatic disease and male infertility. No healthy controls were excluded on the basis of age, sex or race. Heterozygotes consisted of parents/siblings of CF patients. Reference subjects with a previous diagnosis of CF based on the US Cystic Fibrosis Foundation consensus criteria had ion channel measurements derived prospectively, irrespective of previously performed test(s).¹⁰ These subjects were included in a report comparing the American and European diagnostic guidelines for CF.¹⁸

Ion channel measurements

Sweat test and NPD were performed concurrently on the same day on all subjects. Sweat chloride was interpreted as normal (<40 mmol/L), borderline (40–59 mmol/L), or abnormal

(≥60 mmol/L).¹⁰ NPD was performed as described by Knowles *et al*,¹⁹ by a single operator masked to other test results. The change in *CFTR*-mediated chloride diffusion following chloride-free and isoproterenol perfusion ($\Delta\text{Cl-free+Iso}$) was used as the primary diagnostic parameter. As previously described, the reference range for NPD was based on the range of overlap between reference non-CF and CF individuals: $\Delta\text{Cl-free+Iso}$ normal (<-12 mV), intermediate (-12 to -7.7 mV), and abnormal (>-7.7 mV).¹⁸

CFTR genotyping

All subjects, including reference CF subjects with one or two unidentified *CFTR* mutations, underwent genotyping by multiplexed heteroduplex analysis followed by sequencing of identified fragments.²⁰ Large deletions were detected using established conditions.^{21 22} Subjects who had no or one identified mutation underwent complete sequencing of all exons and adjoining introns.

Statistical analysis

Concordances between the sweat test and NPD were examined using observed agreement (calculated as the number of patients with the same diagnosis divided by the total number of patients) and weighted Cohen's kappa statistics. Kappa values range from -1 (complete disagreement) to 1 (perfect agreement), with 0 corresponding to agreement by chance alone. Because they condition on the marginal probabilities, they are considered conservative estimates of agreement. Kappa values were classified as: <0.20=poor; 0.21–0.40=fair; 0.41–0.60=moderate; 0.61–0.80=good; 0.81–<1.00=excellent.²³

RESULTS

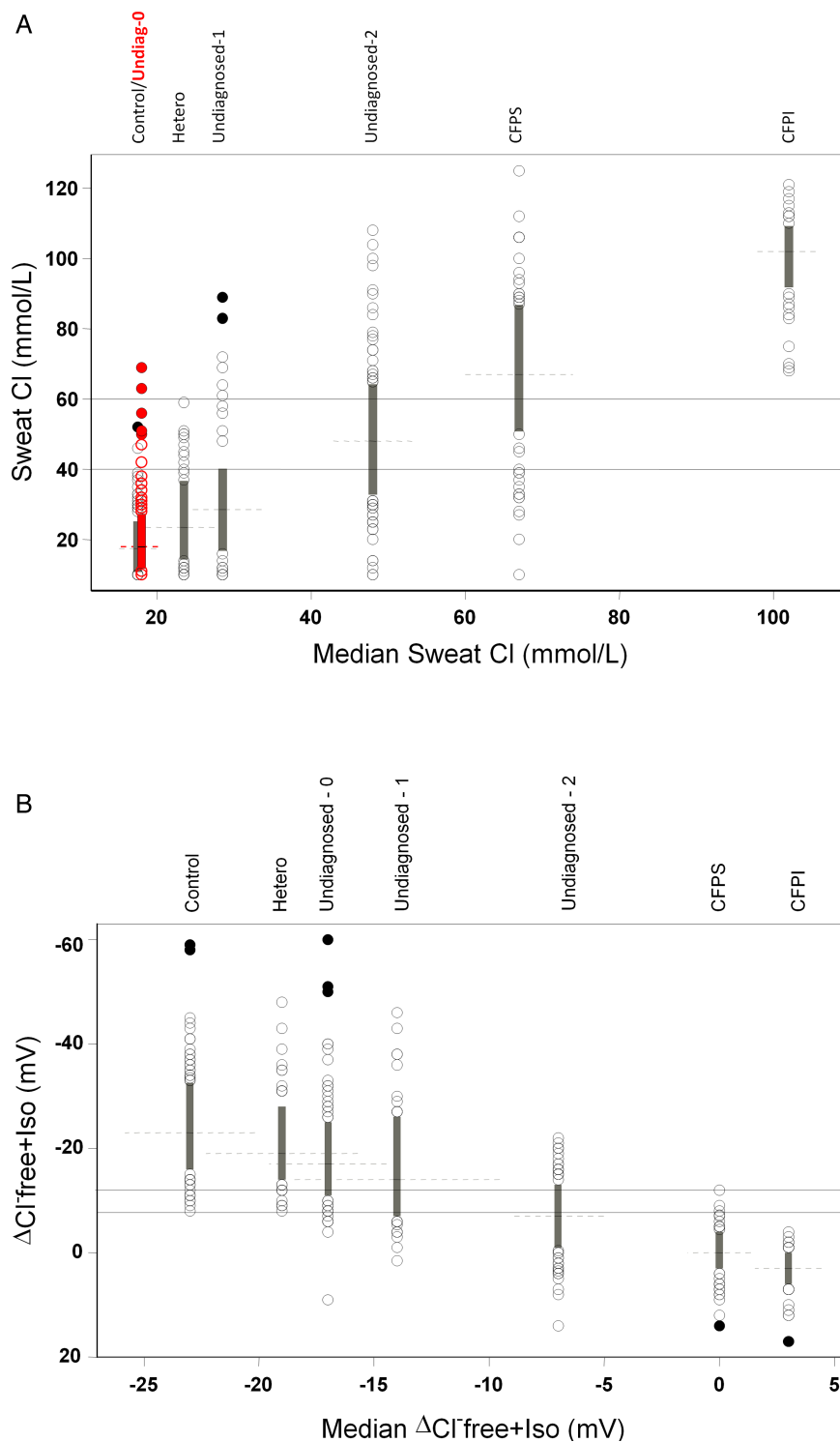
Subject characteristics

Among 208 subjects with single-organ manifestations of CF, six were excluded (four RESP and two PANC) because NPD was not successful, resulting in 202 patients (68 RESP, 42 PANC and 92 AZOOSP). Eighty-six per cent were European Caucasians (see supplementary material, available online only). The majority of RESP and PANC subjects were women. The reference group included 104 healthy controls, 52 obligate heterozygotes, 64 CFPS and 43 CFPI subjects (table 1).

CFTR genotyping

There were 70 (34.7%), 41 (20.3%) and 91 (45.0%) undiagnosed subjects with none, one and two *CFTR* mutations, respectively. The number and frequency of identified mutations varied considerably between phenotypes (table 2). Based on the original 23 CF-causing mutations,¹⁰ only seven (3.5%) were diagnosable as CF, all of whom were diagnosed by sweat testing. Using CFTR2's expanded list of 122 mutations, 10 (4.9%) more subjects became diagnosable by genotyping, giving a total of 17 (8.4%) patients. Furthermore, diagnosis by genotype varied by phenotype: none in PANC, one of 68 (1.5%) in RESP, and nine of 92 (9.8%) among AZOOSP patients. In short, genotyping could not establish or exclude the diagnosis of CF in 74 of 91 (81.3%) with two *CFTR* mutations. Forty-seven of 91 (51.7%) subjects with two *CFTR* mutations carried at least one mutation of varying clinical consequence: 44/91 (48.4%) carried a CF-causing mutation together with a mutation of varying clinical consequence, while three (3.3%) carried mutations of varying clinical consequence on both alleles. Of the 10 additional subjects with two CF-causing mutations designated by CFTR2, the diagnosis of CF could also be established by at least one abnormal ion channel measurement. Sweat testing

Figure 1 The range of each measurement and the relationship of each group with one another are demonstrated by arranging box plots for each group along the x axis according to the value of the median for: (A) sweat chloride and (B) ΔCl^- -free + Iso. Each box plot represents values within the 25th to 75th percentiles (IQR). Values outside the 25th and 75th percentile are represented by circles. Outliers, values that are more than 1.5 IQR above and below the 75th and 25th percentiles, respectively, are represented by solid circles. Horizontal dashed lines depict the medians of each group. Due to the near-superimposition between control and undiagnosed-0 box plots in Figure 1A, Undiagnosed-0 subjects are plotted in red (see online material). CF, cystic fibrosis; CFPI, pancreatic insufficient; CFPS, pancreatic sufficient; Undiagnosed-0, subjects who presented with a single organ CF phenotype and not identified with any *CFTR* mutation; Undiagnosed-1=subjects who presented with a single organ CF phenotype and one mutation; Undiagnosed-2, subjects who presented with a single organ CF phenotype and two mutations.



alone missed three of 10 patients (one normal and two borderline results) while NPD testing alone missed two patients (both borderline results).

All study subjects with no or one *CFTR* mutation (including healthy controls and heterozygotes) underwent extensive genotyping. No healthy controls or heterozygotes carried two CF-causing mutations, but a second mutation of unknown clinical consequence was identified in five obligate heterozygotes. *CFTR2* increased the number of CFPI and CFPS patients fulfilling the diagnostic criteria for CF by genotype alone from 37 (86%) to 39 (90.7%) and 18 (28.1%) to 29 (45.3%), respectively.

Sweat chloride

Figure 1A demonstrates the variability of sweat chloride concentration within each group and the relationship (similarities and differences) among the groups. Among reference subjects, there was a spectrum and contiguity of increasing sweat chloride concentration from healthy controls and heterozygotes at one extreme to CFPI at the other. Among undiagnosed symptomatic individuals there was a similar wide range of sweat chloride measurements and sweat chloride values increased according to the number of mutations.

Thirty-five (17.3%), 46 (22.8%) and 121 (59.9%) undiagnosed patients had abnormal, borderline and normal sweat

Table 1 Subject characteristics and outcomes of sweat chloride and NPD testing

Groups	N	Age, mean±SD	Gender: male, n (%)	FEV ₁ % predicted, mean±SD*	Sweat chloride (mmol/L)			NPD: ΔCl-free+Iso (mV)		
					Normal	Borderline	Abnormal	Normal	Borderline	Abnormal
RESP†	68	38.9±15.1	19 (27.9%)	80±21	46 (67.6%)	8 (11.8%)	14 (20.6%)	35 (51.5%)	13 (19.1%)	20 (29.4%)
PANC	42	24.3±12.8	16 (38.1%)	93±16	36 (85.7%)	4 (9.5%)	2 (4.8%)	24 (57.1%)	6 (14.3%)	12 (28.6%)
AZOOSP	92	34.8±5.3	92 (100%)	94±14	39 (42.4%)	34 (36.9%)	19 (20.7%)	38 (41.3%)	19 (20.7%)	35 (38.0%)
CONTROL	104	31.7±8.2	51 (49.0%)	91±10	100 (96.2%)	4 (3.8%)	0	95 (91.3%)	9 (8.7%)	0
HETERO	52	38.9±8.6	21 (40.4%)	92±13	41 (78.8%)	11 (21.2%)	0	43 (82.7%)	9 (17.3%)	0
CFPS‡	64	32.3±12.5	33 (51.6%)	72±25	12 (18.8%)	11 (17.2%)	41 (64.0%)	0	3 (4.7%)	61 (95.3%)
CFPI	43	22.5±10.8	31 (72.1%)	64±20	0	0	43 (100%)	0	0	43 (100%)

*Pulmonary function was evaluated by standard pulmonary function methods. Forced expiratory volume in 1 s was expressed as a percentage of the predicted values for height and sex (FEV₁% predicted).^{24, 25}

†See Gonska *et al*³ for additional clinical details.

‡Among the 12/64 (18.8%) CFPS subjects with normal sweat test, all except one had abnormal NPD: five had previous abnormal sweat test (four with abnormal NPD, one borderline NPD), four were diagnosable by genotype as well as abnormal NPD, and three had abnormal NPD only. Of the 11/64 (17.2%) CFPS subjects with borderline sweat test, all except one had abnormal NPD also: eight had previously abnormal sweat chloride (seven had abnormal NPD, one borderline NPD) and three had abnormal NPD only.

AZOOSP, obstructive azoospermia; CF, cystic fibrosis; CFPI, pancreatic insufficient; CFPS, pancreatic sufficient; CONTROL, healthy controls; FEV₁, forced expiratory volume in 1 s; HETERO, heterozygotes; NPD, nasal potential difference; PANC, chronic/recurrent pancreatitis; RESP, chronic sino-pulmonary disease.

chloride results, respectively (table 1). Patients with AZOOSP had the highest proportion of subjects with borderline sweat chloride (36.9%), as compared to RESP (11.8%) and PANC (9.5%) subjects. Although none of the healthy controls and obligate heterozygotes had abnormal sweat chloride measurements, four (3.8%) and 11 (21.2%) had borderline results, respectively. All CFPI subjects had abnormal sweat chloride concentrations. However, only 41 (64%) CFPS subjects had abnormal sweat tests. Twelve (18.8%) and 11 (17.2%) CFPS subjects had normal and borderline sweat chloride concentrations.

Nasal potential difference

The wide spectrum of *CFTR* dysfunction noted on sweat testing was also observed with NPD (figure 1B). Similarly, all groups except CFPI had subjects with borderline NPD measurements. Among undiagnosed symptomatic patients, fewer patients had borderline NPD results than with sweat testing (18.8% vs 22.8%) (table 1). Thus, NPD could establish a diagnosis of CF in 67 (33.2%) subjects compared with 35 (17.3%) by sweat chloride.

Test concordance

According to the kappa statistic, there was a 'moderate' level of agreement between sweat chloride and NPD (table 3) among

the RESP subjects, while among PANC and AZOOSP subjects, the levels of agreement were 'poor' and 'fair', respectively. Observed agreement for RESP, PANC and AZOOSP were 65%, 55% and 44%, respectively. Subanalysis was then performed after excluding all undiagnosed subjects with borderline results for either test. This analysis reflects discordance between the sweat test and NPD in subjects with clear-cut normal or abnormal diagnostic results (sweat test normal but NPD abnormal, and vice versa). Completely discrepant diagnostic outcomes (sweat chloride vs NPD) were present in 14%, 33% and 28% of RESP, PANC and AZOOSP subjects, respectively.

In view of different lower cut-offs for borderline sweat chloride between the American and European guidelines (40 vs 30 mmol/L), concordance analysis was repeated using the 30 mmol/L cut-off. There was no difference in the concordance level between 40 versus 30 mmol/L cut-offs (see supplementary material, available online only).

Further comparisons of the diagnostic outcomes are shown in table 4 and figure 2. Among 121 undiagnosed subjects with normal sweat chloride, 24 (19.8%) had abnormal NPD results. Only 24 of 35 (68.6%) subjects with abnormal sweat chloride values had abnormal NPD results. Seven (20%) with abnormal sweat chloride values had normal NPD results.

Table 2 Breakdown of *CFTR* mutations in all subjects

Groups	N	No of <i>CFTR</i> mutations*			Two CF-causing mutations†		
		0	1	2	Consensus	CFTR2‡	Both§
RESP	68	37 (54.4%)	15 (22.1%)	16 (23.5%)	3 (4.4%)	1 (1.5%)	4 (5.9%)
PANC	42	21 (50%)	9 (21.4%)	12 (28.6%)	2 (4.8%)	0	2 (4.8%)
AZOOSP	92	12 (13%)	17 (18.5%)	63 (68.5%)	2 (2.2%)	9 (9.8%)	11 (12%)
All undiagnosed symptomatic subjects	202	70 (34.7%)	41 (20.3%)	91 (45%)	7 (3.5%)	10 (4.9%)	17 (8.4%)
CONTROL	104	84 (80.8%)	18 (17.3%)	2 (1.9%)	0	0	0
HETERO	52	0	47 (90.4%)	5 (9.6%)	0	0	0
CFPS	64	1 (1.5%)	9 (14.1%)	54 (84.4%)	18 (28.1%)	11 (17.2%)	29 (45.3%)
CFPI	43	1 (2.3%)	1 (2.3%)	41 (95.4%)	37 (86%)	2 (4.7%)	39 (90.7%)

*Subjects with none, one or two *CFTR* mutations.

†Subjects with CF-causing mutations on both alleles.

‡Additional subjects with two CF-causing mutations following expansion of the list of CF-causing mutations from 23 (consensus) to 122 (CFTR2) mutations.

§Subjects with two CF-causing mutations based upon consensus plus CFTR2.

AZOOSP, obstructive azoospermia; CF, cystic fibrosis; CFPI, pancreatic insufficient; CFPS, pancreatic sufficient; CONTROL, healthy controls; HETERO, heterozygotes; PANC, chronic/recurrent pancreatitis; RESP, chronic sino-pulmonary disease.

Table 3 Concordance analysis between sweat chloride and NPD in all subjects and after excluding subjects with borderline results

Group	All subjects			Subjects with borderline sweat test and/or NPD results excluded		
	N	Observed agreement	Kappa (95% CI)	N	Observed agreement	Kappa (95% CI)
RESP	68	0.65	0.48 (0.29 to 0.66)	49	0.86	0.67 (0.46 to 0.89)
PANC	42	0.55	0.05 (−0.15 to 0.26)	33	0.67	0.06 (−0.18 to 0.30)
AZOOSP	92	0.44	0.23 (0.07 to 0.38)	46	0.72	0.40 (0.13 to 0.67)

AZOOSP, obstructive azoospermia; NPD, nasal potential difference; PANC, chronic/recurrent pancreatitis; RESP, chronic sino-pulmonary disease.

DISCUSSION

The diagnosis of CF should be made with a high degree of certainty because it carries significant medical, financial and psychosocial implications. While the diagnosis of CF is usually established by the traditional sweat test alone in the majority of infants and young children, older children and adults presenting with single-organ manifestations suggestive of CF exhibit a wide overlapping spectrum of *CFTR* dysfunction. Therefore, the diagnosis of CF may be difficult to establish or exclude¹⁸ in those who fall within the 'grey zone' of the *CFTR* spectrum. This underlies the importance of having insight into the utility and limitations of diagnostic algorithms, various diagnostic tests, as well as knowledge of the spectrum of clinical manifestations at different ages.

In this study, the sweat test reliably discriminated individuals at the extreme ends of the *CFTR* spectrum, as evidenced by sweat chloride concentrations among healthy controls and CFPI. However, sweat chloride is limited by the wide overlapping values between non-CF and CF subjects. Approximately 20% each of obligate heterozygotes and subjects with single-organ manifestations of CF had borderline sweat chloride results. This is not surprising considering the upper limit of sweat chloride concentration among healthy non-CF individuals over 10 years old has been observed to overlap into the borderline range, with the upper limit for ages 10–14, 15–19 and over 20 years being 47, 51 and 56 mmol/L, respectively.²⁶

Although several consensus reports offer varied opinions concerning their emphasis and role, genotyping and alternative ion channel measurements of the nasal and rectal epithelium have been proposed to clarify the diagnosis of CF in individuals with borderline sweat tests.^{10 11 16 27} While genotyping had previously been limited by the fact that only 23 of the more than

1900 *CFTR* mutations were designated as CF causing, this study shows that despite the expansion of the number of disease-causing mutations with *CFTR2*, genotyping still provided the lowest diagnostic yield when compared to the sweat test and NPD. Furthermore, all subjects with single-organ manifestations of CF diagnosable by genotyping had at least one ion channel measurement (sweat test and/or NPD) within the diagnostic range. In contrast to RESP and PANC patients, a large subset (68.5%) of AZOOSP subjects was identified with two *CFTR* mutations and was associated with the highest diagnostic yield from the list of 122 CF-causing mutations. A possible explanation is that among all the *CFTR*-affected organs, the vas deferens is most dependent on *CFTR* for ion transport and fluid secretion.²⁸

The NPD measurements also demonstrated a wide spectrum of *CFTR* dysfunction with an overlapping 'grey zone' between non-CF and CF individuals. Among the three diagnostic tests, NPD identified the largest number of patients diagnosable with CF with fewer borderline outcomes. However, considerable discordance between the sweat test and NPD results is very concerning particularly when discordance remained after subjects with borderline results on sweat testing and/or NPD were excluded from the analysis. Discordance was lowest in RESP and highest in PANC subjects.

While the reasons for this discordance are unclear it may reflect variation in the penetrance of ion channel abnormalities in different CF-affected organs. Furthermore, ion channel measurements across different epithelia may be variably influenced by non-*CFTR* genetic and environmental factors. It seems plausible that discordant results will also be present between other ion channel measurements, for example, intestinal current measurement and β -adrenergic sweat secretion.^{27 29}

There are several important questions. Is it appropriate to attribute a final diagnostic outcome based on sequential interpretation of diagnostic tests, which forms the basis of current European and American guidelines?^{10 11} As subjects can have a normal sweat test but abnormal NPD and vice versa, symptomatic individuals with a normal (or abnormal) sweat test may receive a spurious diagnosis because in such circumstances an NPD test is neither routinely recommended nor performed in clinical practice. In particular, do individuals with a normal sweat test plus abnormal NPD, or alternatively those with an abnormal sweat test but normal NPD have CF or not? In addition, as previously reported, a large number of the undiagnosed subjects in this study fulfil the criteria for a 'CFTR-related disorder'.¹⁸

Despite the recent increased number of designated CF-causing mutations by *CFTR2*, genotyping continues to have limited diagnostic utility in challenging cases such as those presenting with a single-organ CF-like phenotype at a later age. There are several plausible explanations. The majority of newly

Table 4 Comparison of sweat chloride and NPD outcomes

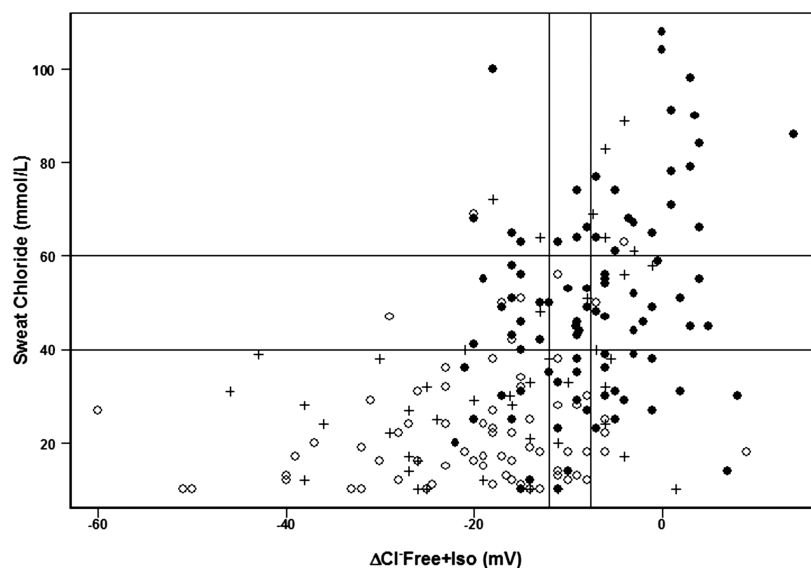
		NPD		
		Normal (n=97)	Borderline (n=38)	Abnormal (n=67)
Sweat chloride	Normal (n=121)	73 (60%)	24 (20%)	24 (20%)
	Borderline (n=46)	17 (37%)	10 (22%)	19 (41%)
	Abnormal (n=35)	7 (20%)*	4 (11%)	24 (69%)†

*The seven subjects include one RESP, one PANC and five AZOOSP.

†14/24 (58%) were not identified with two CF-causing mutations: 12 carried one CF-causing mutation (DF508/−(x5); DF508/5 T (x3); DF508/D1152H; R75X/V456A; 1717-1G>A/Q1291H; 1717-1G>A/5 T) and two had no CF-causing mutation (D579G/D579G; −/−).

AZOOSP, obstructive azoospermia; CF, cystic fibrosis; NPD, nasal potential difference; PANC, chronic/recurrent pancreatitis; RESP, chronic sino-pulmonary disease.

Figure 2 Sweat chloride measurements plotted against NPD ($\Delta\text{Cl-free+Iso}$) for individual subjects with a single-organ manifestation of CF. The reference ranges for both tests are illustrated to identify patients with normal, borderline or abnormal values for each test, and demonstrate the discordance in diagnostic test outcomes (eg, bottom left: normal sweat chloride and NPD values; top right: abnormal sweat chloride and NPD; bottom right: normal sweat test but abnormal NPD; top left: abnormal sweat test but normal NPD. CF, cystic fibrosis; cross (+), subjects who presented with a single organ CF phenotype and one mutation; NPD, nasal potential difference; open circle (o), subjects who presented with a single organ CF phenotype and not identified with any *CFTR* mutation; solid circle (•), subjects who presented with a single organ CF phenotype and two mutations.



identified disease-causing mutations by *CFTR2* have severe phenotypic consequences, which are associated with clearly abnormal sweat chloride concentrations and confer the PI phenotype. Hence, most patients carrying these mutations could be identified by a simple sweat test alone. Second, half of the subjects with at least one or two identified *CFTR* alleles carried mutations designated by *CFTR2* as having ‘varying clinical consequences’. As the majority of these mutations are associated with milder dysfunction, it is unsurprising that a large subset of patients with single-organ manifestations of CF were found to carry these mutations. Third, as most of the mutations reported in the CF mutation database (<http://www.genet.sickkids.on.ca>) are missense mutations, *CFTR2* was neither able to assign a disease-causing nor a benign designation. Genotype interpretation may also be limited in non-European Caucasian groups, as common mutations in these groups have not been assessed by *CFTR2*. Nonetheless, genotyping may play an important role in situations in which sweat testing cannot be performed (eg, unavailability or prenatal diagnosis) or in situations in which disease causing mutations are associated with a normal or borderline sweat test or NPD. Nevertheless, the clinical utility of genotyping is expected to improve as more information regarding a greater number of specific mutations is obtained.

The role of in silico tools to predict the functional consequences of rare *CFTR* mutations has also been evaluated.^{30–31} While in silico tools may provide insight into the potential pathogenicity of rare mutations, they were shown to predict the clinical severity of missense mutations with known clinical consequences poorly, raising considerable doubt over their diagnostic role in mutations with variable or unknown clinical consequences.³²

A diagnosis of CF in RESP patients has important clinical implications. However, follow-up should also be offered to PANC and AZOOSP patients with *CFTR* dysfunction due to the risk of disease development in other organs (eg, pulmonary disease). Subclinical pulmonary disease has been reported in AZOOSP men with two *CFTR* mutations and *CFTR* dysfunction, but due to a lack of longitudinal studies long-term pulmonary outcomes are unknown.³³ Respiratory disease at/after CF

diagnosis has also been reported in patients with pancreatitis.³⁴ In addition, there may be a future role for *CFTR*-assist therapies in patients with pancreatitis.³ The female preponderance among RESP and PANC subjects is consistent with previous reports.^{2, 35}

This study has several strengths. We prospectively ascertained a large number of well-characterised older subjects from three single-organ phenotypes and who represented a wide spectrum of *CFTR* dysfunction. Furthermore, the findings on discordance between the sweat test and NPD were based on concurrent testing. The major limitations include the lack of longitudinal clinical monitoring and repeat ion channel testing. For practical and analytical reasons (ie, avoiding the need for separate visits and the complications of incomplete recall for a second test) we opted to perform each test concurrently on a single occasion. To obtain meaningful insight into the natural history of disease among older subjects with single-organ manifestations, prospective monitoring of a large sample over several decades is essential. Although approximately 5% of mutations may be missed by the genotyping methods used, we are confident that all 122 mutations listed by *CFTR2* would have been identified.

In conclusion, the diagnosis of CF or its exclusion is not always straightforward and may remain elusive even with comprehensive evaluation, particularly among individuals who present at an older age with single-organ manifestations of CF. Considerable diagnostic uncertainty remains because many of these patients have ‘borderline’ and/or overlapping *CFTR*-mediated ion channel measurements, and there is considerable discordance between sweat testing and NPD. Finally, the diagnostic yield of *CFTR2*’s expanded list of disease-causing mutations remains somewhat limited.

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